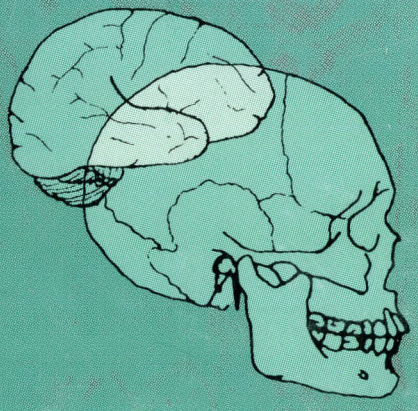
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EDITORIAL

Lectori salutem!

The new Editors greet all readers and contributors of Neurobiology. Three years have passed since the first issue of this Journal appeared, a period of time long enough to draw the conclusions of the past and to think of the future. Neurobiology, thanks to the efforts of the former Editors and the interest of authors in submitting manuscripts, has successfully survived the critical 'newborn age', and is now ready to develop into adulthood. This, of course, makes necessary to reshape editorial policy in order to respond to current challenges. The scientific background of the Journal has become fortunately widened by a recent decision of the Hungarian Neuroscience Society and the Hungarian IBRO Committee to accept Neurobiology as their mouthpiece. Together with our international Editorial Board, this is thought to help us in approaching our ultimate goal: to offer the scientific community an internationally established critical forum of good research, stimulating ideas and fruitful discussions embracing the possibly broadest range of experimental and theoretical neurosciences. Expansion of research in these fields tends to continue and the more traditional Journals seem to be unable to give room for all valuable manuscripts submitted. In this situation, recently launched periodicals may fill a gap provided they are aware of their responsibility in maintaining standard and competitiveness. With these principles in mind we invite on the first place original *research reports* which will be reviewed by two expert referees. In addition, the Editors would like to give way to rapid, *preliminary notes.* These will be reviewed by a single expert referee, possibly an Editor. A section will be opened for *views* and *reviews.* Reviews will be commissioned from leading scientists of the field, while views and comments relevant to the reviewed topic will be published in two subsequent issues. Editorial policy also includes the occasional publication of *theses* either in full or abbreviated. We feel it our obligation to provide *information* on and to publish *abstracts* of national neuroscience meetings primarily in the Central- and Eastern-European region less highlighted by the Journals of the big international societies.

So please, stay with us!

The Editors

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Neurobiology; 4 (1996) 1-11

Review article

GENE ACTIVATION PATHWAYS OF NERVE GROWTH FACTOR SIGNALING: A MINIREVIEW

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Department of Biology, University Medical School of Pécs, Hungary

Summary: PC12 rat pheochromocytoma cells provide an excellent model system to study the mechanism of nerve growth factor-induced neuronal differentation. PC12 cells expressing a dominant inhibitory Ras protein are being used to elucidate the role of this key signal transduction protein in gene activation events accompanying morphological differentiation of these cells. The possible involvement of Ras-dependent protein phosphorylation cascades in NGF-induced gene expression are discussed in this paper.
Keywords: nerve growth factor (NGF)*, PC12 cells, Ras proteins, protein kinase cascades, gene expression, neuronal differentiation

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The material of this review was presented at the 3rd Annual Meeting on Cell and Developmental Biology (organized by the Hungarian Biological Society, January 23-25, 1995, Pécs, Hungary)

*Abbreviations used: NGF, nerve growth factor; NGFR, nerve growth factor receptor; ERG, early-response gene; LRG, late-response gene; SRE, serum response element; SRF, serum response factor; TCF, ternary complex factor; CRE, cAMP response element; CREB, CRE-binding protein; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; TNFα, tumor necrosis factor α, EGF, epidermal growth factor; JNK/SAPK, c-Jun N-terminus kinase/stress-activated protein kinase; SEK, SAPK/ERK kinase; MEKK, MEK kinase; CREBK, CREB kinase; FRK, cFos-regulating kinase.

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NGF-signaling pathways are differentially mediated by proto-oncogenic Ras proteins

The rat PC12 pheochromocytoma cell line provides an excellent model system to analyze biochemical events accompanying nerve growth factor (NGF)-induced neuronal differentiation (for a review see Szeberényi and Erhardt, 1994): these cells are easy to culture, survive in the absence of NGF and produce easily scorable morphological alterations (e.g. neurite outgrowth) upon NGF treatment.

Major signal transduction components in PC12 cells can be arranged in a linear order (Fig. 1) NGF binds to its high affinity cell surface receptor (NGFR), the protooncogenic TrkA protein (Barhacid, 1993). The signal is passed on to several proteins tyrosine-phosphorylated and recruited by the activated TrkA protein. Some of these signaling proteins activate Ras proteins by loading it with GTP, that in turn recruit and activate cytoplasmic protein kinases (Roberts, 1992; Blenis, 1993). The protein kinase cascade rapidly induces a subset of genes (early-response genes, ERGs) believed to regulate another class of genes (late-response genes, LRGs) via their transcription factor products (Karin and Smeal, 1992). LRGs code for proteins thought to be directly involved in producing phenotypic alterations leading to morphological differentiation (e.g. the metalloprotease transin, neurofilament proteins, etc.).

This linear interpretation of signaling events, however, is an obvious oversimplification of the situation in PC12 cells. In reality, signal transduction molecules form a complex, parallel but frequently interlocking network of pathways. An example for such complexity was uncovered by studying the role of Ras proteins in NGF-signaling: using an interfering Ras-mutant (Ha-Ras Asn-17; Feig and Cooper, 1988; Cai et al., 1990), we were able to identify highly Ras-dependent (e.g. neurite outgrowth, transin induction), weakly Ras-dependent (e.g. induction of *c-fos, cjun)* and Ras-independent (e.g. stimulation of phosphoinositide turnover) events in NGF-signaling (Szeberényi et al., 1990, 1992; Troppmair et al., 1992). In PC12 cell lines expressing different levels of the inhibitory Ras protein (called M17-subclones in Table I) NGF-induction of ERGs, LRGs, and morphological alterations were differentially affected: complete inhibition of the latter could be achieved without significantly reducing the activation of several ERGs. However, the

signal transduction mechanisms mediating the differential expression of genes in PC12 cells are still poorly understood.

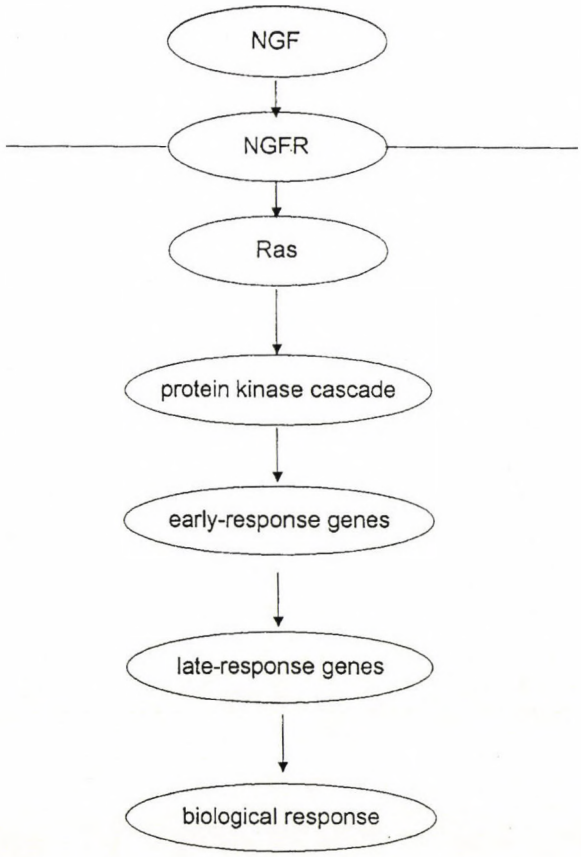


Fig. 1: Main components of NGF-signaling

A paradigm of complex gene regulation: the *c-fos* promoter

Detailed maps of regulatory sequences have already been constructed for a number of promoter regions (McMahon and Monroe, 1992). The best-studied example is the promoter of the ERG *c-fos*. This proto-oncogene codes for a

component of the transcription factor AP-1 involved in the regulation of a large number of genes.

Table I: The role of Ras proteins in mediating NGF-induction of gene expression and morphological differentiation (+++, normal level of NGF-induction, +, reduced NGF-induction,-no NGF-induction)

	M 17-SUBCLONES	
THE EFFECT OF NGF	weak Ras-Asnl7 expressor	strong Ras-Asnl7 expressor
Induction of early-response genes (*c-fos, c-jun,* ziJ268)	+++	+
Induction of late-response genes (transin, SCG10)	—	—
Biological response (neurite outgrowth)	—	—

At least three major enhancer elements have been identified in the *c-fos* promoter region. The *serum-response element* (SRE) is recognized by the serum-response factor (SRF) and the ternary complex factor (TCF), is activated by serum and polypeptide growth factors and is believed to be the major Ras-responsive element of the *c-fos* promoter. The AP-l site is activated by Fos/Jun heterodimers (AP-1 factor) and was originally identified as a phorbol ester-inducible element. However, it can be activated by NGF in a Ras-dependent and protein kinase C-independent manner indicating that the regulation of this enhancer element is more complex than imagined earlier (Szeberényi et al., 1992). The cAMP-response element (CRE) is activated by the binding of CREB (CRE-binding protein) that is stimulated by phosphorylation by protein kinase A (cAMP pathway), calmodulin-dependent kinases (Ca++ pathway) and CREB kinase (NGF-stimulated, Ras-dependent pathway) (Ginty et al., 1994). The example of the *c-fos* promoter

demonstrates that signal transduction pathways diverging from a single signaling protein (e.g. Ras) may regulate various elements of the same promoter (e.g. SRE, AP-1, CRE), or converging pathways might land on the same enhancer element (e.g. the regulation of CRE). This sophisticated network of signaling routes provides versatility in regulation, but makes sorting out the pathways extremely difficult.

Major Ras-dependent transcytoplasmic protein kinase cascades in PC12 cells

The last three years have brought extremely rapid progress in the identification of signaling components linking growth factor receptors to nuclear events. Efforts were mainly concentrated on studies on cytoplasmic protein kinase systems (for a recent review see Marshall, 1995). These signaling networks consist of parallel but cross-talking cascades of protein kinases; most elements of these pathways were identifed in PC12 cells as well. An important common feature of these signal transduction pathways is that a member of a growing family of protein kinases called mitogen-activated protein kinases (MAPKs) takes a central position in them. MAPK isozymes show significant sequence homology and, having a Ser/Thr-Pro motif phosphorylation site in the target protein, belong to the class of proline-directed protein kinases (Hill and Treisman, 1995). Three major types of MAPKs have been implicated in NGF-signaling: ERKs (extracellular signal-regulated kinases), JNKs (c-Jun N-terminus kinases) and CREBK (CREB kinase) (Fig. 2).

The *Ras/Raf1/EK/ERK* pathway was described first and is considered to be the major mediator of mitogenic and differentiation signaling (Roberts, 1992; Blenis, 1993). Following stimulation of the NGF receptor by its ligand, Ras becomes activated by binding GTP and attracts members of the Raf serine/threonine kinase family. In PC12 cells the BRat protein appears to participate in differentiation signaling (Jaiswal et al., 1994). Activated B-Raf stimulates MEK1, a specific member of the MEK (MAPK/ERK kinase) family (Rosen et al., 1994). This enzyme, as the whole pathway, is critical for NGF-signaling: a constitutively active MEK1 mutant induces neuritogenesis, while a dominant negative MEK1 mutant blocks NGF-induced differentiation of PC12 cells (Cowley et al. 1994). MEKs are dual specificity kinases: they activate ERKs by phosphorylating specific threonine and tyrosine residues.

ERKs phosphorylate a number of target proteins including transcription factors and other protein kinases (e.g. Rsk). Upon NGF-stimulation, ERKs translocate into the nucleus; this step is thought to be critical for the differentiation response (Marshall, 1995).

Painstaking studies on the mechanism of c-Jun phosphorylation led to the identification and characterization of another transcytoplasmic protein kinase cascade, *Ras/MEKK/SEK/JNK* (reviewed in Marshall, 1995; Hill and Treisman, 1995). JNKs located on the distal end of this pathway are

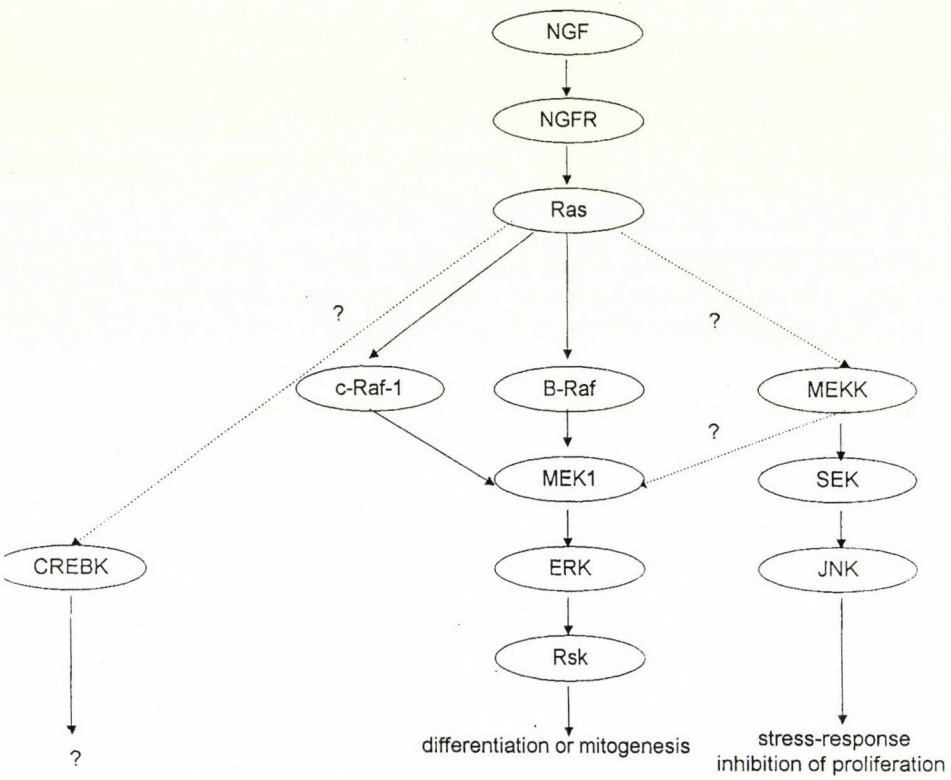


Fig. 2: NGF-stimulated protein kinase cascades. (Solid and dotted arrows indicate well and poorly characterized connections between signaling proteins, respectively)

ubiquitously expressed enzymes capable of activating c-Jun proteins by site-specific serine-phosphorylation. JNKs are strongly activated during the stress-

response of cells evoked by TNFα UV-irradiation, heat or osmotic shock (therefore they are also called stress-activated protein kinases or SAPKs). In PCl2 cells NGF-, EGF- or UV-activation of JNKs are Ras- dependent, while stimulation by TNFα is mediated by Ras-independent mechanisms. The activators of JNKs, SEKs (SAPK/ERK kinase) are close relatives of MEK enzymes. As dual-specificity protein kinases, they activate JNKs by phosphorylating threonine and tyrosine residues. The most proximal member of this cascade is MEKK (MEK kinase). This protein is expressed in PCl2 cells at a high level; its constitutive activation strongly inhibits proliferation. All components of the Ras/MEKK/SEK/JNK pathway are expressed in PC12 cells, but its role in NGF-signaling remains unclear.

A recently described member of the MAPK family is *CREBK* (Ginty et al., 1994). This enzyme is expressed in PC12 cells, is stimulated by NGF-treatment via Ras-dependent mechanisms and phosphorylates the same serine residue on CREB as protein kinase A or calmodulin-dependent protein kinase. Its significance in NGF-signaling is underlined by observations that the CRE enhancer is necessary (but not sufficient) for the NGF-induction of the *c-fos* promoter, and that CREs are present in the promoters of NGF-inducible early response genes (e.g. *zif268, nur77,* etc.) and late-response genes (e.g. transin, tyrosine hydroxylase, peripherin etc.).

Other members of the MAPK family (e.g. FRK tc-Fos-regulating kinase, Deng and Karin, 1994, see Fig. 3) are also intensively studied, but data supporting their participation in NGF-signaling are not available.

Regulation of AP-1 activity

The picture is very complicated, and, with the discovery of additional components and elucidation of further details, is expected to become even more complex. To demonstrate this, a signal transduction network regulating the activity of a single transcription factor, AP-1, is shown in Fig. 3. AP-1 is a dimer formed from members of the Fos and Jun families of proto-oncogenic proteins and is responsible for the regulation of a number of genes containing a specific enhancer element in their promoter regions. The low AP-1 activity of quiescent cells can be stimulated by growth factor treatment, at both transcriptional and posttranscriptional levels. Upon growth factor treatment,

Ras-dependent induction of the *c-fos* and *c-jun* genes takes place, mediated by ERK → TCF, CREBK → CREB, and JNK → c-Jun phosphorylation events. (In resting cells AP-1 consists of Jun homodimers, therefore activation of FRK does not have a role at this phase.) Newly synthesized Fos and Jun proteins appear in the cells and can be further stimulated by phosphorylation by FRK and JNK. These changes lead to a marked increase in AP-1 activity that, in

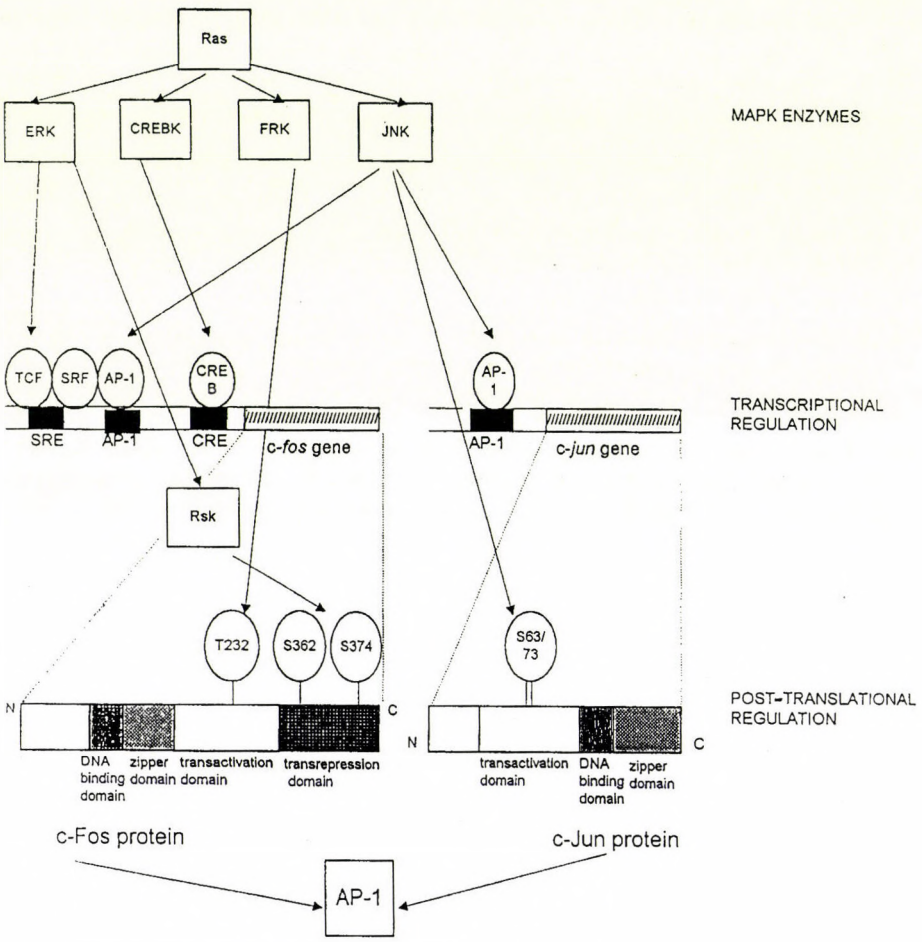


Fig 3: Regulation of the level and activity of AP-1 factor

turn, triggers further gene expression events. Stimulation of AP-1 is influenced by several other factors: tissue specific expression of protein kinases and their targets, generation of second messenger molecules able to modulate transcription factor activities (e.g. cAMP → PK A → CREB → diacylglycerol → PKC → AP-1) inhibitory phosphorylation by certain protein kinases (e.g. ERK → c-Jun), the antagonistic effects of phosphoprotein phosphatases, etc.

A model system to study parallel NGF-signaling pathways

Selective inhibition of specific pathways is a useful approach to dissect complex signal transduction networks. Besides other possibilities (e.g. the use of inhibitors, expression of antisense oligonucleotides, microinjection of specific antibodies), introduction of dominant inhibitory proteins into the cells is a powerful technique to study the function of individual signaling molecules. The advantage of this approach is that stably transfected cell lines expressing the mutant protein can be isolated and characterized by detailed biochemical analysis.

Our PC12 subclones expressing a dominant negative Ras protein can be used to study the role of Ras in neuronal differentiation. Ras-dependent and -independent pathways can be selectively analyzed (see Table I). Our present work is concentrated on the connections between expression of early- and late-response genes and the biological response to NGF. Preliminary observations indicate that NGF-induced synthesis and phosphorylation of c-Fos and Zif268 ERG-products are not sufficient to activate late-response gene expression and neurite outgrowth (Szeberényi et al., unpublished observations). In another set of experiments, we are currently looking for strongly and weakly Ras-dependent, NGF-inducible genes using the differential display technique (Liang and Pardee, 1992): characterization of differentially expressed genes would give useful information regarding the role of gene expression in neuronal differentiation.

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REFERENCES

Barhacid, M. (1993) Nerve growth factor: a tale of two receptors. Oncogene, 8: 2033-2042.

Blenis, J. (1993) Signal transduction via the MAP kinases: Proceed at your own RSK. Proc. Natl. Acad. Sci. USA, 90, 5889-5892.

Cai, H., Szeberényi, J. and Cooper, G.M. (1990) Effect of a dominant inhibitory Ha-ras mutation on mitogenic signal transduction in NIH 3T3 cells. Mol. Cell. Biol., 10, 5314-5323.

Cowley, S., Paterson, H., Kemp, P.- and Marshall, C.J. (1994) Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. Cell, 77, 841-852.

Deng, T. and Karin, M. (1994) c-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK. Nature, 371, 171-175.

Feig, L.A. and Cooper, G.M. (1988) Inhibition of NIH 3T3 cell proliferation by a mutant Ras protein with preferential affinity for GDP. Mol. Cell. Biol., 8, 3235-3243.

Ginty, D.D., Bonni, A., and Greenberg, M.E. (1994) Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-fos transcription via phosphorylation of CREB. Cell, 77, 713-725.

Hill, C.S. and Treisman, R. (1995) Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell, 80, 199-211.

Jaiswal, R.K., Moodie, S.A., Wolfman, A. and Landreth, G.E. (1994) The mitogen-activated protein kinase cascade is activated by B-Raf in response to nerve growth factor through interaction with $p21^{ras}$. MCB 14, 6944-6953.

Karin, M. and Smeal, T. (1992) Control of transcription factors by signal transduction pathways: the beginning of the end. Trends Biochem. Sci., 17, 418-422.

Liang, P. and Pardee, A.B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science, 257, 967-971.

Marshall, C.J. (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell, 80, 179-185.

McMahon,S.B. and Monroe, J.G. (1992) Role of primary response genes in generating cellular responses to growth factors. FASEB J. 6, 2707-2715.

Roberts, T.M. (1992) A signal chain of events. Nature, 360, 534-535.

Rosen, L.B., Ginty, D.D., Weber, M.J. and Greenberg, M.E. (1994) Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron, 12, 1207-1221.

Szeberényi, J., Cai, H. and Cooper, G.M. (1990) Effect of a dominant inhibitory Ha-ras mutation on neuronal differentiation of PC12 Cells. Mol. Cell. Biol., 10, 5324-5332.

Szeberényi, J., Erhardt, P., Cai, H. and Cooper, G.M. (1992) Role of Ras in signal transduction from the nerve growth factor receptor: relationship to protein kinase C, calcium and cyclic AMP. Oncogene, 7, 215-2113.

Szeberényi, J. and Erhardt, P. (1994) Cellular components of nerve growth factor signaling. Biochim. Biophys. Acta, 1222, 187-202.

Troppmair, J., Bruder, J.T., App, H., Cai, H., Szeberényi, J., Cooper, G.M. and Rapp, U.R. (1992) Ras controls coupling of growth factor receptors and protein kinase C in the membrane to Raf-1 and B-Raf protein serine kinases in the cytosol. Oncogene, 7, 1867-1873.

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Research report

A COMPUTER MODEL OF THE VISUAL CORTEX

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Summary: A three-layered simulation of the visual cortex was constructed, receiving inputs from 252 retinal photoreceptors. Lateral geniculate body was taken as a simple relay station. In the cortical module afferent neurons with circular receptive fields, orientation selective simple cells, tuned complex cells, excentricity neurons and different kinds of inhibitory neurons were included. The model reproduces neuronal and network responses recorded in experiments and some sensory illusions, of which one is here demonstrated.
Keywords: neuron model, network model, visual cortex, sensory illusions

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INTRODUCTION

The neuronal models play an increasing role in understanding the nervous system. Since our knowledge about neural structure and function is rather imperfect, neuronal models are extremely useful in completing and integrating it and in trying to build up a holistic picture of the system. Present models of the nervous system are integrating a tremendous body of knowledge but are defective in telling anything about the wiring scheme which is performing the actual functions.

The traditional effort of neurobiology and recently of neuronal modeling is directed to answer the question: how the well-known functions of the nervous system are implemented by its structure. Therefore, instead of searching analogues of nervous functions, it seems much more promising to look for connections and correlations between structures and the functions realized by them. At present one cannot hope to be able to build up models of large neural structures, such as cerebellum or hippocampus; modeling should take aim at smaller, but relatively well discernible sub-systems, whose function was previously studied from morphological, physiological and biochemical points of view (Douglas and Martin, 1991a, b). When taking aim at building of as realistic simulations of nervous sub-structures as possible, one has to fulfill some basic requirements.

First of all, neural models must be built up of elements, that correspond to neurons. Smaller units, such as intrinsic sub-cellular mechanisms, membrane processes need not be included, because their final output is the discharge of the cell, as the only event that is relevant for the function of a network. Summation, facilitation leading to discharge, or inhibition preventing it, can be simulated in a far simpler way, without being involved into not well understood intracellular mechanisms.

Secondly, the functional capacity of the neuron-analogues must not exceed that of the real ones. One must not give way for the temptation to replace neurons with miniature, all knowing automata, because this structure will stand far away from reality. Also their connections have to analogize synapses as true as possible.

A third requirement of realistic simulations is, that assemblies of neurons separable anatomically as layers, nuclei or other structures, must be dealt with separately, taking in view their special cellular constitution. This is the feeblest point of the existing models. The outcome of a structure-true

simulation should be substantiated by stimulus patterns specific to the actual inputs and being comparable to physiological and/or behavioral records collected from the existing neural sub-systems.

The question can now be raised, what is the reason for such a strictly wired neuronal net, in which everything happens as it was designed by the author. It could be told, that such a system is simulating much more the author and not the system. However, one must consider, that in a network, consisting of several hundreds or thousands of neurons, the consequences of a stimulus pattern can be followed up only with the aid of a computer. For representation of consecutive states in large neuronal masses neurobiology has no adequate method. Moreover, simulated networks will be not absolutely deterministic, because the parameters of the constituents may be continuously modified by the ongoing processes and their actual state is the resultant of all previous happenings. In this respect neural models give good simulations of the nervous system.

Neurophysiology is especially in need of system modeling. Three groups of recording methods stand at her disposal: the single electrode, the multi-electrode (including also encephaloscopy, PET and magnetoencephalography) and behavioural methods. Methods belonging to the first group give information about activity of one or several neurons, but tell practically nothing about the system. The other two give an outline about distribution of excitation and inhibition on the whole, with some hints for the details, but far from yielding any insight into events at the cellular level. At present neurophysiology is in need of methods which could account about the activity of at least a sub-system and of its elements, in temporal contiguity. The need is fulfilled by neuronal modeling, which, based on physiological data informing about cellular events and mass phenomena, helps to build up networks, gives opportunity to verify or reject theories and raises ideas for further experimentation. Thus computer modeling of neural structures has become an indispensable tool for neurobiological investigation.

RESULTS

The network

The elements of the network are taken as neuron models. They are substantiated by four neighbouring memory addresses, each occupying one word.

The first of them quantifies the state of the neuron on a continuous scale extending from zero to 127 units. Level 48 represents the resting state, below it inhibition, above it excitation takes place. Impulses impinging upon the neuron modify this parameter: inhibitory pulses decrease, excitatory ones elevate it. Their summation is taken as linear. No special segments (soma, dendrite, axon) are defined within the neuron. After each stimulus series the resting state becomes restored with a time constant of 4.4 ms (Fig. 1).

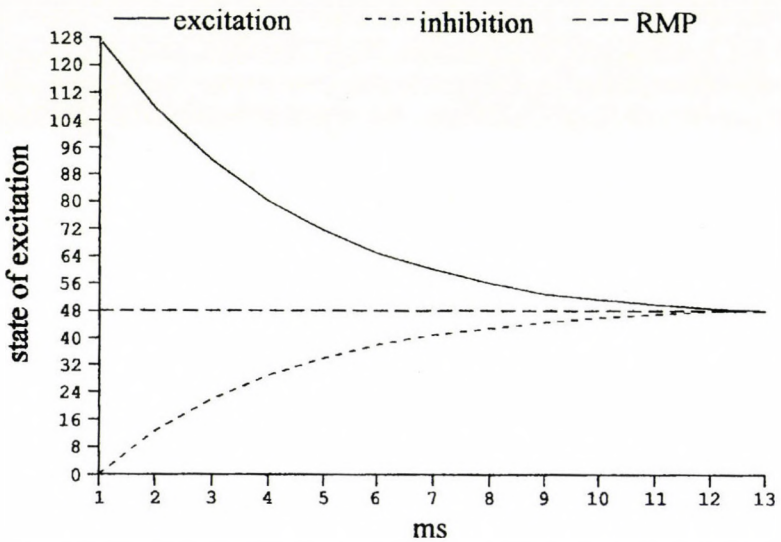


Fig. 1: Relaxation curves of state of excitation departing from a depolarized (above) and an inhibited state (bottom). Ordinate: state of excitation in arbitrary units, RMP: resting state. Abscissa: time in ms. Time constant is at 4.4 ms

When running the model on the computer, the state of the neurons is visualized by color coding. Neurons in resting state are not colored. Inhibited

cells from zero to 43 units appear grey, from 43 to 47 units in blue, above 49 to 94 the color changes by every three units (because of technical difficulties, color coding is here replaced by black-white tints).

The second address represents the threshold. This is situated with several units above the resting state, at different levels in each neuron type or at special neurons. In some neurons modifications in stage of excitation may modify the threshold. In this way Hebb-type neurons can be simulated.

The third address counts the spike potentials discharged by the cell. Action potentials are generated each time, when excitation reaches or exceeds threshold. As long as it is over threshold, at each step one or more action potentials are sent out toward synaptically coupled cells. At each impulse the counter is set higher with unity. The amplitude of action potentials (from 2 to 9) becomes added to or (in case of inhibition) subtracted from the state of excitation of the innervated cell. A flowchart diagram of functioning of a model neuron is represented in Fig. 2.

The program includes optional number and direction of synapses and thus networks of any type and structure can be built up. Summation, facilitation, convergence, divergence, feedbacks and other basic neurophysiological processes can readily be simulated. The fourth memory address contains the serial number of the neuron. In addition to graphic representation neurons are listed including their actual functional parameters (state of excitation, threshold, number of discharged spike potentials) and serial number. The synaptic strength is determined by the height of action potentials and threshold of the recipient cell. Both can be varied optionally at each synapse and thus individual relations in every synaptic linkage can be formed. Networks built up of neurons and synapses of this type may lack any uniformity and are able to simulate a wide variety of neuronal sub-systems. State dependent modification of the synaptic strength may underlie the simulation of Hebb-type synapses.

The sequence of events is laid down in the main program which operates the system in discrete time steps. This corresponds to roughly one millisecond in the functioning nervous tissue. Initiation of each step may be governed by hand or by program. The main program written in Turbo Pascal is complemented with a graphic program visualizing the processes going on in the system.

Retinal "on" and "off" elements, although graphically not denoted on the screen, are distinguished in the written scheme. Every "on" element is surrounded by four "off" cells and *vice versa.*

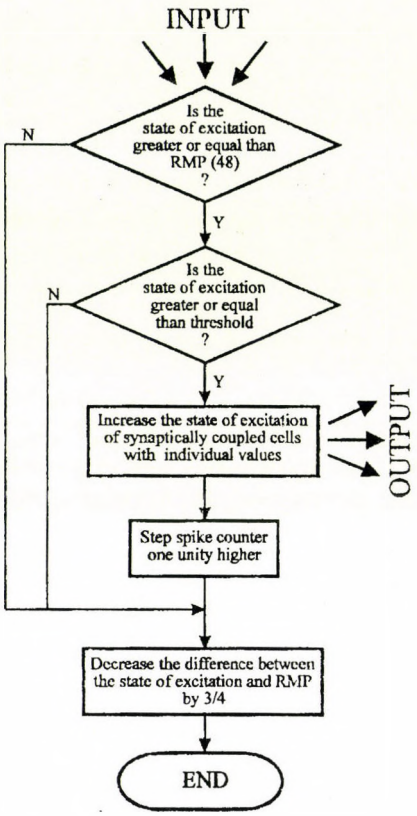


Fig 2: Flowchart diagram of a model neuron

3-4 neighbouring simple orientation selective cells activate a complex neuron (Figs 5 and 6), whose receptive field is larger and therefore orientation selectivity looser They exert some collateral and "end" inhibition to one another via a special sort of inhibitory cells of the "gi" neurons. The counts of complex cells are, as follows: at 0 degree 18, at 30 degree 14, at 60 degree 18, at 90 degree 15, at 120 degree 18, at 150 degree 15, 98 orientation

selective complex cells altogether. An equal number of "gi" neurons cooperates in sharpening boundaries around them.

Groups of "a" neurons (fed by W type retinal ganglion cells) consisting of 2x4 neighbouring cells activate "n" neurons. These have larger receptive fields and lack orientation selectivity. They are called "excentricity" neurons because their excitation reflects the extent of confluent patches, around the geometric center of the retina. This is situated around "a" neurons N° 111 and 122. They are members of the small group converging upon the "nO" cell. Around it 30 "n" neurons are situated arranged in concentric circles. Their function is to measure distances from the middle of the visual field, to give information about dimensions of objects and distances among them. Assumedly they play some role also in motion perception. Excitation of "n" neurons is facilitated by simple cells.

Model of the visual system

The mode of simulation detailed above has been applied for modeling the primary visual cortex of the cat, based upon physiological data (Douglas et al., 1991; Berman et al., 1991). According to the principles of structure-true modeling, the known cell types of the cortex have been included: simple cells without orientation selectivity, simple cells with preferred orientation (towards six directions of the plane, separated by 30 degrees from one another), complex cells, excentricity neurons, and different types of inhibitory neurons (see later).

The input of the system is provided by the retina, containing 252 photoreceptors arranged in hexagonal array. This forms the basis of the graphic representation. Their activation is transmitted immediately to the cortex. Insertion of retinal transmission and lateral geniculate body (LGB) did not seem to contribute essentially to modeling of the visual cortex. However, the structure of the model can readily be completed with sub-programs representing retina and/or LGB.

The input is arranged so, that one impulse impinging upon a photoreceptor excites one input (a-type) cell in the cortex, usually sub-threshold. The retinal matrix, consisting of 12x11+12xl0 : 252 photoreceptors is suitable to perceive a lot of complex stimuli and forward them to the cortex. The retinal elements (arranged in a hexagonal array) become colored as they are selected to be

stimulated by the operator and remain so as long as the stimulatory situation holds.

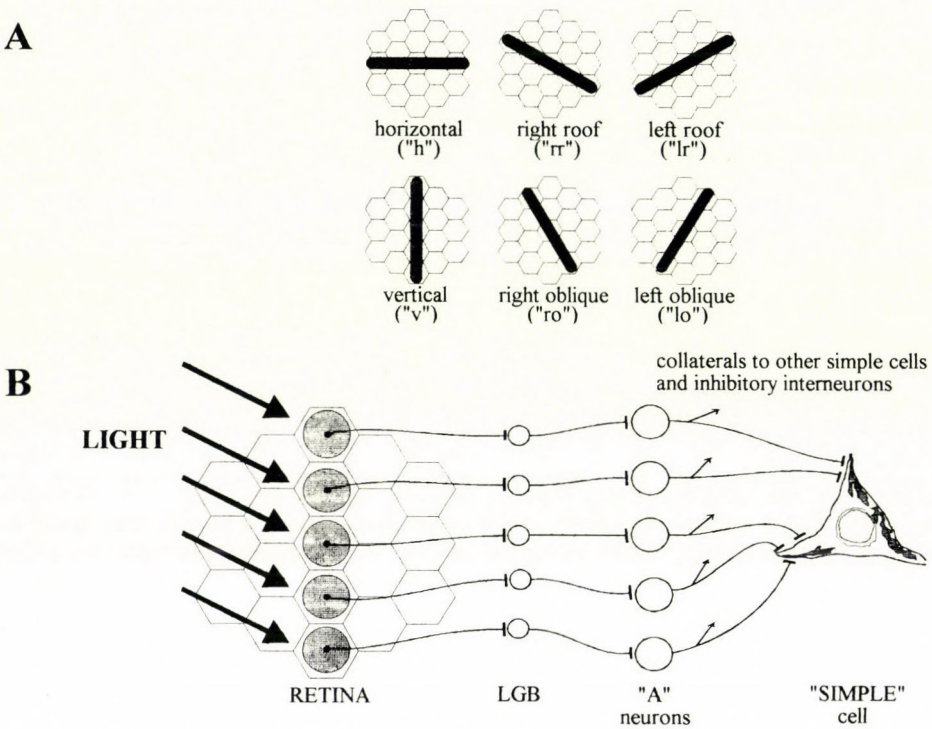


Fig. 3: A: Orientation selective simple cells arranged in six directions of the plane with their special notation (further details see in the text). B: Activation of a single, in this case "v" type cell, by retinal afferents excited by a vertical bar. LGB is taken as a simple relay station. Five "a" cells excite a single "v" neuron. The collaterals of these same "a" cells activate inhibitory "g" neurons (each one) enhancing orientation selectivity (see next Figure)

The input elements of the visual cortex are the "a" neurons, excited immediately by the photoreceptors, each only by one. The "a" neurons represent interneurons in layer IVCβ of the cat visual cortex, having circular receptive fields (Lund et al., 1979). Their number in the model equals with that of photoreceptors, i.e. 252. The activated photoreceptors, distinguished by coloring, signalize also the similarly numbered "a" neurons in the cortex. "a" axons ascend to layers II and III and innervate different sorts of orientation

selective simple cells. Bundles consisting of axons of 3-7 neurons, aligned in some direction, converge on a simple cell, whose orientation selectivity is determined by the line, on which the retinal receptors lie. Six sorts of orientation selective simple cells are distinguished, separated by 30 degrees from one another. They are at 0 degree "h" neurons (72 in number), at 30 degree "Ir" neurons (40 in number), at 60 degree "Io" neurons (69 in number), at 90 degree "v" neurons (63 in number), at 120 degree "ro" neurons (69 in number) and at 150 degree "rr" neurons (41 in number), 352 simple cells altogether (Fig. 3). Their specificity is secured by inhibitory "g" neurons, activated synchronously by "a" cells. Each "g" cell prevents the excitation of all simple cells excited by that very "a" cell, leaving active only that one, which gets impulses from other "a" cells, too. The number of "g" cells of this kind is 252. Other inhibitory neurons play role at complex cells (Fig. 4).

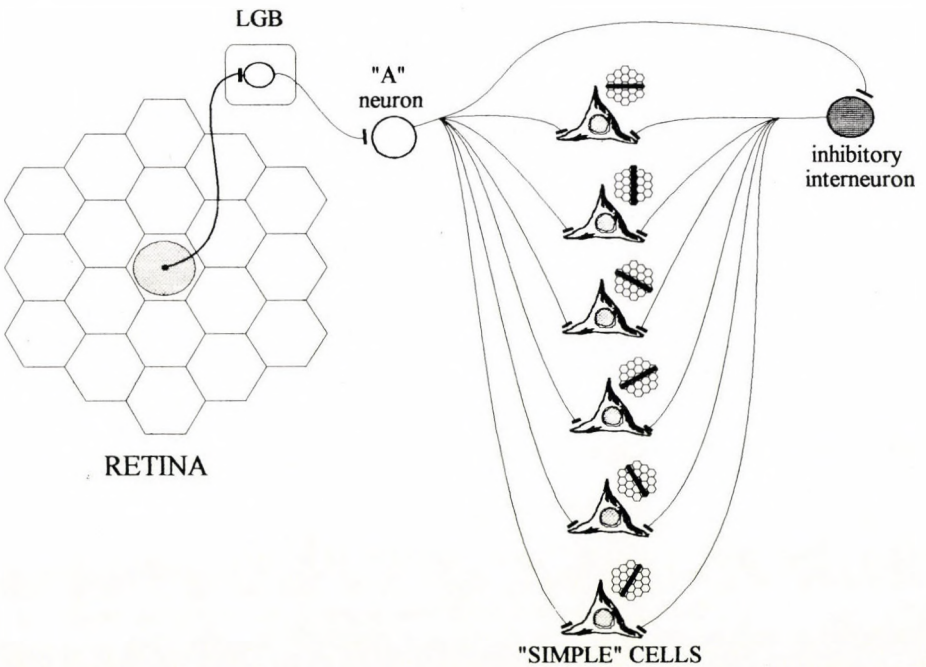


Fig. 4: Structure of collateral inhibition by "g" cells. A "g" cell activated by an "a" neuron, inhibits all the simple cells receiving impulse from that very "a" cell, thus emphasizing the activity of that simple cell which is activated by other "a" cells obtaining herewith orientation selectivity

The operation of the program

After having given a stimulus pattern to the retina, the excitation of different sorts of simple, complex and "n" cells can be visualized on the screen, in the succession as they were characterized above. Pushing a button, "v" cells appear with their color coded stage of excitation, projected upon the hexagonal array of photoreceptors. Then the other sorts of simple cells and "n" cells follow and finally all of the activated or inhibited cells appear on the same screen (Fig. 6B). Inhibitory cells are not visualized. Complex cells appear in their color coded state on another parallel hexagonal array. The operator can select the number of steps to be performed by the system, having opportunity to have a look upon the actual state of the neurons.

In the following several examples are given which demonstrate the functioning and some achievements of the model.

In Fig 5 three groups of horizontally tuned simple cells are projected to the hexagonal lattice, after 10 stimuli at left, and the complex neurons excited by

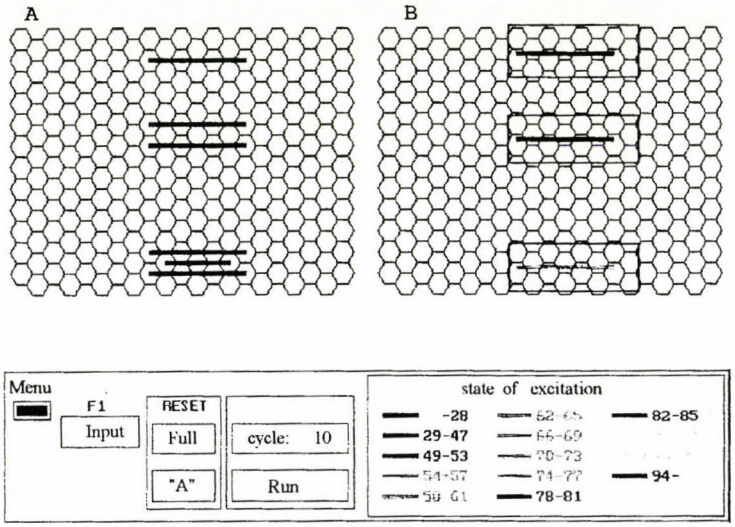


Fig. 5: Activation of three groups of horizontally tuned simple cells, projected to the hexagonal lattice of photoreceptors. A: simple cells in groups, containing 1, 2, and 3 units after 10 cycles of stimulation. B: complex cells, excited by the three groups of simple cells. Their state of excitation is different, according to the number of simple cells innervating them. (Because of technical difficulties, color coding is here replaced by black-white tints)

them, at right. It seems obviously, that the complex cells exhibit different states of excitation according to differences in intensity of stimulation by the simple cell groups.

In Fig 6A a triangle is projected to the retina and in 6B all the simple and excentricity cells, being at different states of excitation are visible in the hexagonal lattice. In Fig 6C the complex cells appear, emphasizing the most essential features of the excitation pattern on the left.

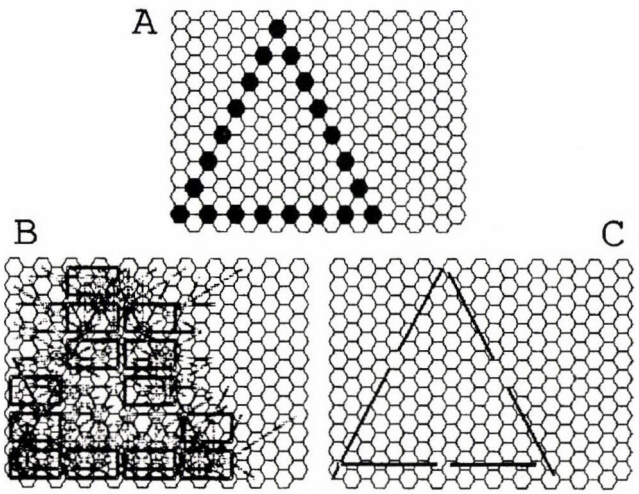


Fig. 6: A: Activation of "a" cells by projecting a triangle to the retina. B: Record of simple and excentricity cells simultaneously activated by the triangle. C: Activity of complex cells, which extract the dominant pattem from the overall excitation of the simple cell population

In Fig 7 the role of excentricity neurons is exemplified. In Fig 7A, in the Müller-Lyer illusion, the horizontal line of the lower figurine seems to be longer, than that of the upper one. After 10 series of stimulation (Fig. 7B) the excitation pattern of the excentricity neurons, corresponding to the lower horizontal line is much longer, than the upper one, underlying the impression, gained at the inspection of the figurines in Fig 7A. Thus it appears, that although excentricity neurons do not transmit immediate visual reality, underlie to background (subjective) impressions, motivating the perception as participants of the psychological image formation.

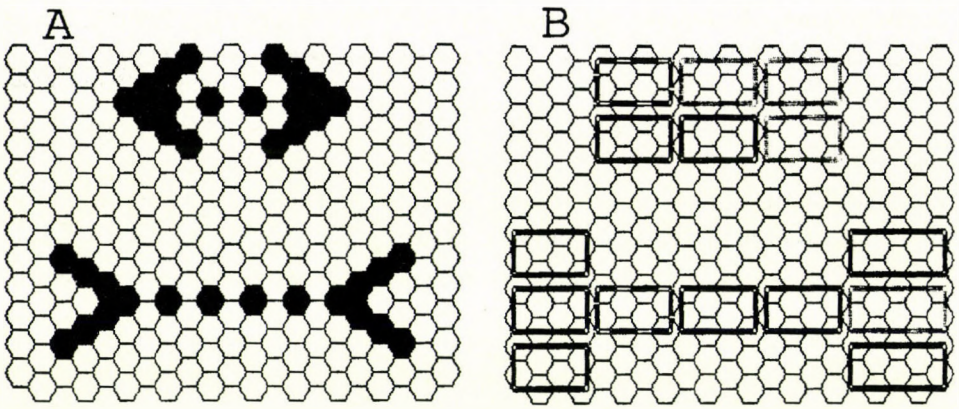


Fig. 7: A: Müller-Lyer illusion. The horizontal line of the lower figurine seems to be longer, than that of the upper one. B: Activity of the "n" neurons (rectangles) after 20 cycles. It is obvious, that the horizontal line of the lower figurine activates a longer row of "n" neurons, causing the impression of being longer

DISCUSSION

In this chapter several features of the model will be discussed, which are alike or different from other similar models, e.g. that of Wehmeier et al. (1989).

Our model does not include retina and LGB. Although the retinal photoreceptors are regarded as input channels of the system in an area, where the relation of photoreceptors and ganglion cells is 1:1, the details of transmission and modifications of excitation in the retina are not dealt with. The impulses of ganglion cells are thought to be transmitted through the LGB as a simple relay nucleus and arrive to layer IVCβ of the visual cortex in linear conformity with intensity and geometric properties of the retinal image.

The cells of layer IVCβ receiving the afferent impulses are our type "a" neurons, which have circular monocular receptive fields and lack any orientation selectivity (Lund et al., 1979). Such neurons amount to about 10 percent of the population in the cat and much more in the monkey (Hubel and Wiesel, 1968, 1972). In the retina "on" and "off" units are introduced, innervating different sorts of neurons, without any kind of interaction with each other. In view of the modeled functions this is of no importance.

Among neural elements of the retina X and W type ganglion cells are taken into account. The former have small receptive fields and are capable of sustained excitation. W type neurons are supposed to feed our "n" type excentricity neurons. They have broader receptive fields and amount to about 40-50 percent of the ganglion cells, being present in the fovea and at the periphery, as well.

In view of the physiological properties of the neurons a compromise was sought between the two state neurons of Hopfield (1984) and the more sophisticated neuronal structure of Wehmeier et al. (1989). There was no reason to simulate the neuronal membrane as an array of conductances, capacitances and batteries because excitatory and inhibitory synaptic inputs become summated linearly in it. Linear summation and the resulting action potential (the only relevant event for the network) can be simulated in a simpler way, requiring less computational efforts. Our neurones, like those of others (Hopfield, 1984; Wehmeier et al., 1989; Douglas and Martin, 1991) are not compartmentalized, their action potentials are binary events, their amplitude is fixed in the program (but may be individual for every cell and synapse). This amplitude is added to or subtracted from the state of excitation of the postsynaptic neuron, thus becoming one of the determinants of the synaptic strength. In an "integrate and fire" neuron, which has a time constant, for the network it is indifferent, in which way the neuron arrives at discharging an action potential. Taking conduction times into account does not seem to contribute too much to a realistic picture, as well.

The inhibition, thought to be indispensable for orientation tuning is the most disputed issue among modelers of the visual cortex. This has been implemented in our model as follows: a thalamocortical axon activates an "a" cell in layer IVCβ, which in turn excites an inhibitory neuron, depressing all simple cells, deriving their activity from that very neuron. Thus a mild tonic inhibition becomes dominating over the simple cell population, without preventing their activation by continuous rows of receptors in the retina, but braking non-optimal stimuli to come over threshold. It appears as a weak inhibition, without large conductance changes, around the resting membrane potential and observed in visual cortical cells by Berman et al. (1991).

Introduction of random background activity did not seem to contribute to the efficiency of the model, therefore, from the final formulation it was omitted.

REFERENCES

Berman, N. J., Douglas, R. J., Martin, K. A. C., Whitteridge, D. (1991) Mechanisms of inhibition in cat visual cortex. J. Physiol. (London) 440, 687-722.

Douglas, R. J., Martin, K. A. C. (1991a) A functional microcircuit for cat visual cortex. J. Physiol. (London) 440, 735-769.

Douglas, R. J., Martin, K. A. C. (1991b) Opening the grey box. Trends in Neurosciences 14, 286-293.

Douglas, R. J., Martin, K. A. C., Whitteridge, D. (1991) An intracellular analysis of the visual responses of neurones in the cat visual cortex. J. Physiol. (London) 440, 659-696.

Hopfield, J. J. (1984) Neurons with graded response have collective computational properties like those of two state neurons. Proc. Natl. Acad. Sci. USA 81, 3088-3092.

Hubel, D. H., Wiesel, T. N. (1968) Receptive fields and functional architecture of monkey striate cortex. J. Physiol. (London) 195, 215-243.

Hubel, D. H., Wiesel, T. N. (1972) Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. J. Comp. Neurol. 146, 421-450.

Lund, J. S., Henry, G. H., MacQueen, C. L., Harvey, A. R. (1979) Anatomical organization of the primary visual cortex (area 17) of the cat. A comparison with area 17 of the macaque monkey. J. Comp. Neurol. 184, 599-618.

Wehmeier, U., Dawei Dong, Koch, C., van Essen, D. (1989) Modeling the mammalian visual system. in: Methods in Neuronal Modeling (Eds: C. Koch and I. Segev), MIT Press, pp. 335-359.

Note: On request the Turbo Pascal prograrn can be placed at disposal.

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Research report

A COMPUTER MODEL OF PROCESSES OF BINOCULAR VISION IN THE CEREBRAL CORTEX

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Summary: Fundamentals and options of modeling cortical processes of binocular vision are dealt with in this paper. Physiologically characterized cell types of the visual cortex are modelled and used as basis of a network implementing a simulation of the binocular vision. An attempt is made to establish correlations between typical activity patterns of visual cortical cells and the network incorporating them.
Keywords: binocular cell, binocular vision, computer modeling

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INTRODUCTION

The investigation of stereopsis has a past of more than 100 years. In course of physiological, psychophysiological, cytological research a large amount of knowledge has been accumulated without giving insight into the cellular organization of the network involved in binocular vision. Since the capacity of the physiological methods in analyzing networks is very restricted, only theoretical assumptions can be raised, which although relying on experimental data, require verification. This can be satisfied by designing new experiments on the one hand, and building theoretical (mathematical, computational or physical) models aiming at simulation of the system, on the other hand. This paper presents a special kind of approach saving up mathematical analysis and concentrating on building up a system of connections which, at least in its mode of function, can be matched with the visual cortex Such a simulation enables one to follow up subsequent states of the whole system resulting from a specific input, together with those of each element. If the model is correct, the output, assessed at the output elements must be the same or analogous as recorded in physiological experiments.

MATERLALS AND METHODS

The structure and function of the model has been described in a previous paper. Shortly, it is built up of neurons, whose parameters are contained in four neighbouring memory addresses as follows: state of excitation, threshold, number of discharged spike potentials, and serial number of the neuron. Each time as state of excitation surpasses threshold, an action potential is sent out, which enhances or depresses the state of excitation in the innervated neuron in proportionality with the synaptic strength. Thus excitatory and inhibitory synaptic actions are simulated. Meanwhile the spike counter is set with one unity higher. The neurons have their own time constants of restoring their resting state either from excitation or from inhibition. Summation, facilitation, convergence, divergence, feedback and other types of connections can readily be included.

The input of the system in the retinal network of receptors and lateral geniculate body is supposed to forward retinal impulses without considerable modification. For further details see preceding paper.

RESULTS

Binocular stereoscopic vision requires that the image of an object be projected to topologically identical sites of the retina. In most cases some details of a given object do not fall to identical points and disparities of various degree occur. This is not valid for details which are situated in the horopter of the fixated distance. That part of the binocular visual field, whose details projected to the two eyes are compounded to image sensation, is called Panum's area of fusion, with the horopter in the centrum. Departing from the horopter towards the eyes or adversely, increasing disparities appear which can be classified in two groups such as near or crossed and far or uncrossed disparities. In these horizontal disparities the information referring to the depth is coded (Fig. 1)

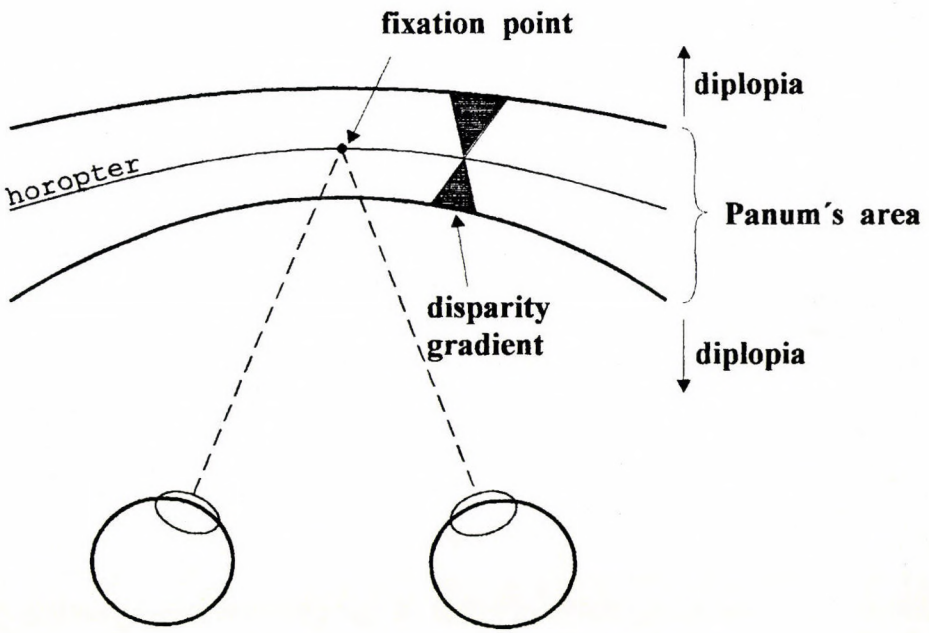


Fig. 1: Mechanism of binocular image fusion. If the object is lying on the horopter, disparity is zero. Departing from the horopter in any direction (nearer or farther) increasing disparities appear, causing diplopia. Panum's area is the range in which image fusion is still possible

Neurons, called binocular, have been demonstrated in cat and monkey visual cortex, which receive impulses from both retinas and are even optimally active only when excited simultaneously from both eyes. Characteristically most binocular neurons exhibit orientation selectivity and maximal activity in some range of disparity. In this model three types of neurons, detected in experiments of Poggio and Talbot (1981), are included such as "tuned excitatory", "near" and "far" neurons, playing role mainly in the static stereopsis. "Tuned excitatory" neurons show maximal activity in a narrow disparity range, within a few minutes of arc around zero degree, both at crossed or uncrossed disparities. "Near" and "far" cells may give responses also at quite great disparities, amounting to 1 degree; their optimal disparity however varies in a wide range. These cells respond only to disparities, specific for them, thus "near" cells respond to crossed, "far" cells to uncrossed disparities. "Tuned excitatory" cells play probably the main role in fine stereopsis, while "near" and "far" cells suffice for a coarse one.

The array of binocular cells was attached to the network built up previously and detailed in the preceding paper. The simple and complex orientation tuned cells of the two retinas converge upon binocular cells in various combinations (Fig. 2).

Six groups of orientation tuned complex cells, separated by 30 degrees in view of their preferred orientation, converge on six corresponding groups of binocular neurons from either retinas. Since the visual field of monocular complex cells is about four times wider than that of simple cells, the binocular cells innervated by two such complex cells have sufficiently wide visual field to record disparities on a large scale.

In Fig. 3A the geometric structure of the binocular vision is sketched. The segment of the Vieth-Muller circle, falling into the binocular visual field is the experimentally determined horopter. The visual fields of the monocular complex cells, projected upon this system, occupy smaller segments and help to define the innervation of the binocular neurons fed by them. The two monocular cells in the two retinas, taking place at identical points, follow the horopter with their common visual fields, thus independently of whether an object is beyond or this side, both complex cells will be excited (Fig. 3B). Therefore if a binocular neuron becomes innervated by two complex monocular cells, having identical receptive fields, it will behave as "tuned excitatory" cell. In case of "near" and "far" cells the innervation has an identical course. In correspondence with the experimental data, in this case objects lying outside the horopter activate nerve fibers which lead to "near" or

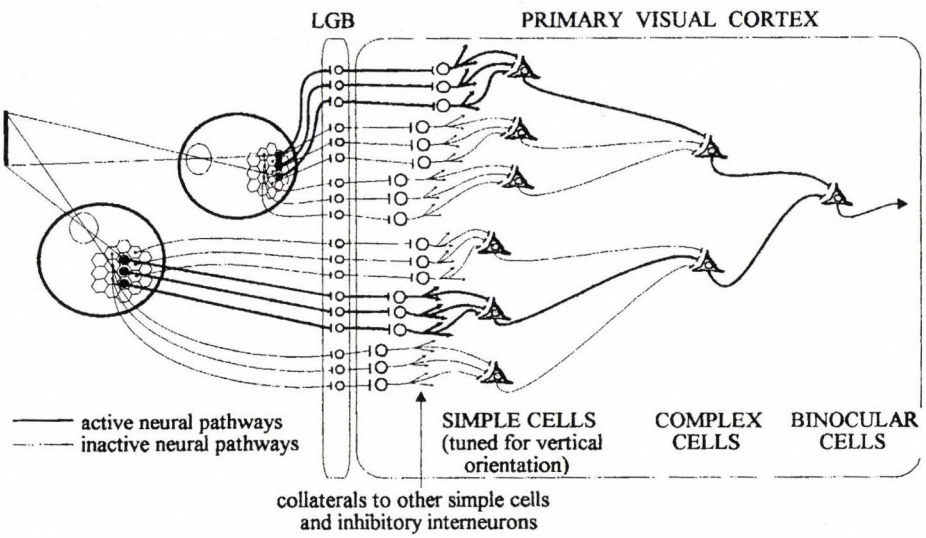


Fig. 2: Structure, and mode of functioning of the model network. A vertical line is viewed by the two eyes. In both retinas 3 photoreceptors, arranged in vertical row, become activated. Nerve fibers from the ganglion cells, excited by them, run to the LGB and are switched over to the cortex, making synapses with "a" type interneurons (small circles, without notion). Each of them excites six simple cells, having orientation selectivity with 30 degree distance from one another. 3-4 simple cells of the same orientation tuning converge on one complex cell, which has therefore larger receptive field and looser orientation selectivity. Finally, monocular complex cells, of the same tuning converge on binocular neurons, whose excitation substantiates the binocular image formation. Inhibitory interneurons contribute to the selectivity of orientation tuning

"far" cells. In Fig. 3C the receptive fields of "near" cells are hatched, while in Fig. 3D the same of "far" cells are denoted by filling. In the figures that part of the receptive field is designated, which is projected to complex cells having not identical visual fields. Both "near" and "far" cells have been classified in two groups: the first group is innervated by complex cells having neighbouring receptive fields (lightly hatched area), while at the second group the receptive fields of the converging complex cells are separated by a resting zone (heavily hatched area). Thus different sub-groups of binocular cells can be simulated.

In order to test the functional capability of the network, one cell of each category was selected for examination under the following conditions: 20 stimuli were given to the retinas and the activity of simple, complex and binocular cells was recorded from 0.2 degree crossed to 0.2 degree uncrossed

disparity (Fig. 4). Stimulation was performed with vertical lines of 1 minute of arc width, projected to both retinas. According to the orientation selectivity of the system only vertically tuned cells became excited. In Fig. 4A the responses of the simple cells are presented (in the upper row the left side, in the lower row the right side), in Fig. 4B the complex cell responses, in Fig. 4C the

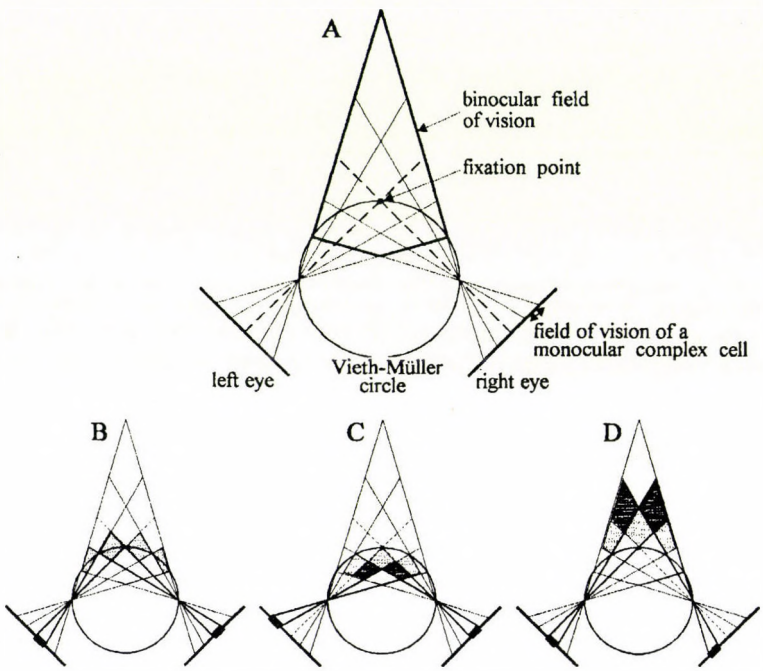


Fig. 3: A: Simplified geometry of stereopsis. Binocular visual field is bounded by thick line. The Vieth-Muller circle is built up by the geometrically possible binocular fixation points. The segment of this circle falling into the binocular visual field is the experimentally determined horopter. B: Receptive fields of "tuned excitatory" neurons. The area, bounded by thick line is viewed by cells having identical visual fields. C: Receptive fields of "near" cells. Lightly hatched areas denote receptive fields of neurons whose optimal disparity is near to the horopter, while dark areas belong to cells of larger optimal disparities. The area bounded by thick line denotes the receptive field of a characteristic "near" cell. Objects taking place in this space, excite complex cells which do not have identical visual fields. D: Receptive fields of the "far" cells. Similarly to those drawn in C lightly hatched areas mean receptive fields neighbouring to the horopter, while dark areas belong to cells having disparity optima at larger distance from the horopter

binocular cell responses are plotted. In spite of simplicity of the network (252 cones in each retina), the responses of the binocular cells reproduce truly the reactions of the neurons observed experimentally.

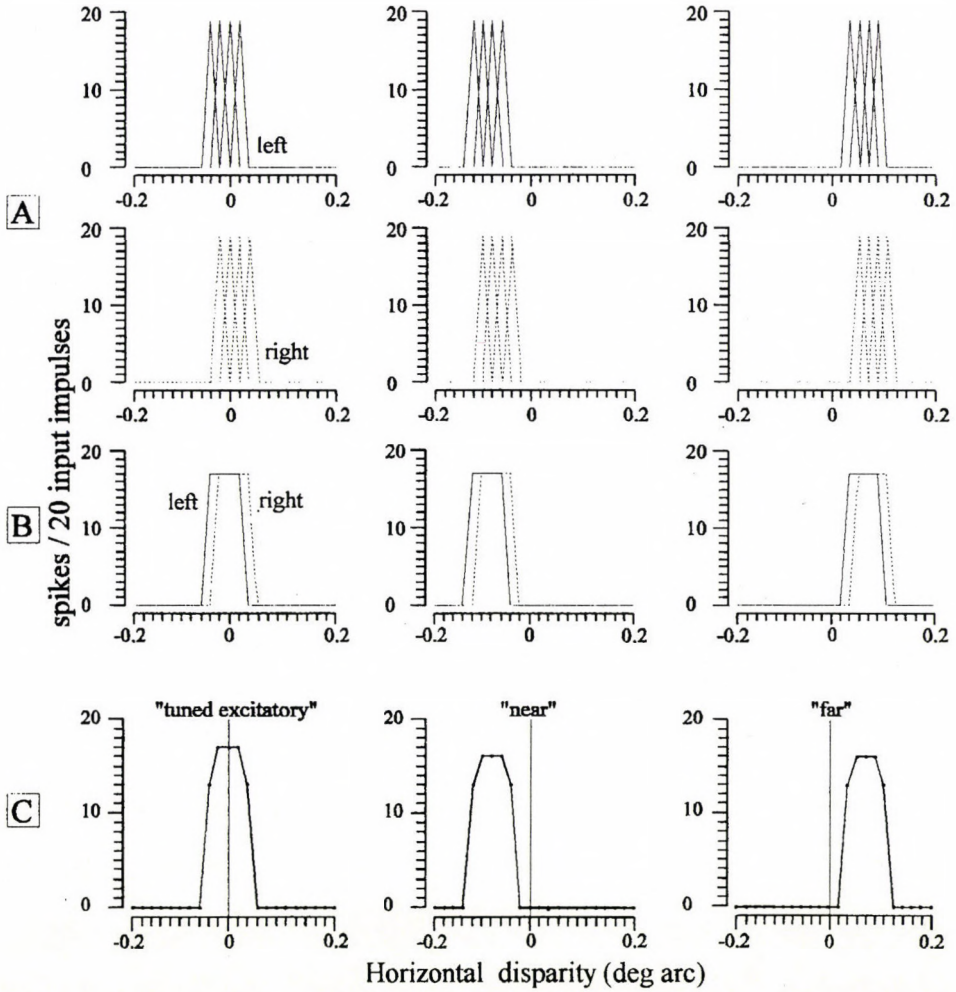


Fig. 4: A: Responses of four simple cells on the left (top) and right (bottom) side to stimuli of different disparities, scaled on the abscissa in deg. of arc plotted, as number of action potentials discharged during 20 cycles. B: Responses of monocular complex cells excited by the same groups of simple cells, receiving the same stimuli as in A. Continuous line: left side, dotted line: right side. C: Response curves of binocular neurons innervated by "tuned excitatory", "near" and "far" complex cells, respectively

binocular cell responses are plotted. In spite of simplicity of the network (252 cones in each retina), the responses of the binocular cells reproduce truly the reactions of the neurons observed experimentally.

DISCUSSION

The model detailed above reflects only a minute part of the visual cortex. The elements of the model network are in analogy also per se with the binocular cells, observed experimentally (Poggio and Talbot, 1981). The differences between the model and the simulated structure consist mainly in simplifications, being indispensable, when attempting to extract functional principles, without immersing into complexities of the natural subject. It can be ascribed to the smallness of the network, that while the operation range of the "tuned excitatory" cells is near to that of the real ones, out of "near" and "far" cells only those, having small disparities (maximally 0.1 deg. of arc) could be included. In experiments of Poggio and Talbot (1981), cells tolerating disparities between 0.2 and 0.6 deg. were recorded.

Another simplification concerns the entire system: no background activity was introduced. Consequently, the model cannot reproduce the mild inhibition of background activity of "near" and "far" cells, when they are exposed to stimuli which are not in accordance with their type: e.g. if they are crossed, in place of being uncrossed. The achievements of the model obviously do not suffer from this lack. Present simulation can be enriched by introducing complexities of activation of binocular cells by simple cells or even by geniculocortical afferents, simultaneously with the usual one, coming from complex cells. In spite of the simplicity of these models as compared with the tremendous complexity of the nervous system, they represent a useful tool in hands of neurobiologists.

REFERENCE

Poggio, G. F. and Talbot, W. H. (1981) Mechanisms of static and dynamic stereopsis in foveal cortex of the rhesus monkey. J. Physiol. 315, 469-492.

Note: On request the Turbo Pascal program can be placed at disposal.

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Research report

CHRONIC ALUMINUM TREATMENT RESULTS IN ALUMINUM DEPOSITS AND AFFECTS M_1 MUSCARINIC RECEPTORS IN RAT BRAIN

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Summary: The effects of chronic aluminum (Al) administration on the deposition of the metal and on the receptor binding characteristics of the Ml muscarinic acetylcholine receptors (M_1AChR) were studied in selected rat brain areas. Animals were injected intraperitoneally with an $AlCl_3$ solution of 1.0 mg/ml/100 g of body weight for 5 weeks, 5 days a week. Al accumulation was detected by solochrome azurine histochemistry in the brain, where the metal could be visualized in capillaries, endothelial cells and surrounding brain tissues. Changes in the binding properties of the M_1AChR after chronic Al treatment were determined with the use of selective and nonselective muscarinic antagonists. Significantly decreased number of maximal M_1AChR binding sites (B_{max}) as measured by the equilibrium binding of [^{3}H]pirenzepine, were detected in all of the brain areas examined. While the nonselective antagonist [^{3}H] (-)QNB displayed a generally decreased B_{max}, value, it reached the level of significance only in the striatum. These results provide a further indication that chronic Al treatment results in the accumulation of Al in the brain and consequently affects the cholinergic neurotransmission.

Keywords: Aluminum, M_1 muscarinic acetylcholine receptors, rat brain, neurotoxicity, solochrome azurine

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INTRODUCTION

The neurotoxic effects of aluminum (Al) might be important, either as a cause or as a consequence, in the pathogenic process of many neurodegenerative disorders. Elevated Al levels have been reported in autopsied brain samples of patients with certain neurologic diseases, such as parkinsonism (Garruto et al., 1984), amyotrophic lateral sclerosis (Perl et al., 1982) or Alzheimer's disease (Perl and Brody, 1980). Although the precise molecular mechanism of the neurotoxic action of Al is still largely unknown, a multitude of *in vivo* and *in vitro* studies have demonstrated that Al affects normal neuronal functioning (Banks and Kastin, 1985; Connor et al., 1988; Szerdahelyi and Kása, 1988; Gulya et al., 1990, 1993, 1995; Pákáski et al., 1993). For example, our previous *in vivo* animal studies (Gulya et al., 1990) revealed that chronic, intraperitoneal (i.p.) $AlCl_3$ administration elicited reductions in choline acetyltransferase (ChAT; EC 3.2.1.6) activity and in the numbers of nicotinic and muscarinic acetylcholine receptors (mAChR) in the rat brain after chronic (5-week long) treatment. Chronic Al intoxication has been shown to elevate the Al content significantly in the brain of rats (Costantini et al., 1989; Gulya et al., 1990, 1993) and mice (Sahin et al., 1994). Moreover, a single intracerebroventricular (Szerdahelyi and Kása, 1988) or intracisternal (Yates et al., 1980) injection of Al increased the metal contents of certain brain areas as measured by atomic absorption spectrophotometry.

Chronic Al administration has been shown previously by ourselves (Gulya et al., 1990) to elicit specific cholinergic changes after 5 weeks of intoxication. An identical treatment was used in the present study, and thereafter solochrome azurine histochemistry (Pearse, 1957; Kása et al., *in press*) was employed to visualize Al accumulation in selected rat brain areas. Although muscarinic receptor binding studies involving the use of the nonselective antagonist (-)Quinuclidinyl(phenyl)-4-[^{3}H]benzilate ([^{3}H](-) QNB) after chronic Al intoxication have previously been published (Gulya et al., 1990) we have further investigated the effects of such treatment on the M_1AChR subclass using a selective M_1 receptor antagonist, [N-methyl^{3}H] pirenzepine ([^{3}H]PZ).

MATERIALS AND METHODS

Seventy two male Sprague-Dawley rats (120-140 g), divided into two groups (36 control and 36 treated animals), were caged and kept on a normal diet and

tap water *ad libitum,* in an air-conditioned room, under a 12-h daylight cycle (lights on at 07.00). $AlCl_3$ was diluted in physiological saline (pH 6.5), and daily doses of 1.0 mg/ml/100 g body weight were injected i.p. 5 days a week for 5 weeks (25 days) (Gulya et al., 1990, 1993, 1995). Al solutions were prepared freshly to prevent the formation of larger hydrated Al aggregations in solution (Sharp and Rosenberry, 1985).

Solochrome azurine histochemistry, which has previously been described in detail (Pearse, 1957; Kása et al., *in press*) was employed to visualize Al accumulation in the brain. In brief, rats (6 animals per group) were deeply anaesthetized with 6% Na-pentobarbital and perfused transcardially with a 50 ml prerinse of heparinized physiological saline, followed by 300 ml of fixative containing 4% paraformaldehyde (extra pure(®), Merck, Darmstadt, Germany; metal content < 0.001%). In general, the use of Al-containing materials and instruments were avoided throughout the entire histochemical procedure in order to prevent Al contamination of the brain tissues. Brains were cryoprotected overnight in 30%, sucrose, buffered with 0.1 M Na-phosphate buffer. Twenty-µm thick coronal cryostat sections were cut and collected in phosphate-buffered saline (0.01 M, pH 7.4). After rinsing several times in distilled water (5-10 min each), sections were incubated in 0.2% solochrome azurine solution (BDH, "Gurr"; in distilled water, pH 6.8-6.9) for 30 min. Sections were then rinsed in distilled water, immediately mounted on glass slides, air-dried at room temperature and enclosed in Entellan (Merck). Frontal and parietal cortical as well as hippocampal sections were investigated with a Microphot FXA (Nikon, Japan) microscope.

For receptor binding experiments, animals were decapitated, and the brain regions of interest (frontal and parietal cortices, hippocampus and striatum) were quickly dissected. Muscarinic radioligand binding assays were described in detail elsewhere (Yamamura and Snyder, 1974; Watson et al., 1982, 1986). Briefly, membranes were incubated for l20 or 90 min at 25 °C in a total volume of 1 ml of 10 or 50 mM Na/K-phosphate buffer (pH 7.4) containing [^{3}H](-)QNB (0.05-0.2nM, 47 Ci/mmol, NEN, Boston, MA, U.S.A.) or [^{3}H]PZ (0.05-5 nM, 84.2 Ci/mmol, NEN, Boston, MA, U.S.A.), respectively, in the presence or absence of 1 µM atropine sulphate. The final protein concentration was 0.1 or 1 mg/ml for [^{3}H](-)QNB and [^{3}H]PZ binding assays, respectively. Bound and free ligands were separated by rapid filtration of the incubation mixture under vacuum through Whatman GF/C glass fillters, which were then washed by 3 x 5 ml ice-cold physiological saline. The radioactivity on the filters was counted in a liquid scintillation counter at an

efficiency of 44%. Receptor binding parameters (dissociation constant, K_d, and maximal number of binding sites, B_{max}) were analyzed by a non-linear regression computer program for the IBM PC (Leatherbarrow, 1992). The protein contents of the samples were measured by the method of Lowry et al. (1951), with bovine serum albumin as standard. Statistical analyses were performed by Student's t-test (Excel 5.0, Microsoft Co., Redmond, WA, U.S.A.). A p value less than 0.05 was considered significant.

RESULTS

Solochrome azurine histochemistry visualized Al accumulation in microvessels, endothelial cells and surrounding brain tissues of all brain areas examined (Fig. 1). Al containing plasma precipitates were also observed in capillaries, as they remained in the lumen of microvessels during the fixation procedure (Fig. 2). Chronic Al intoxication resulted in a significant decrease in the B_{max} value of M_1AChR in all brain areas examined ($p < 0.05$, Table 1), as

Table 1. Changes in specific [^{3}H]PZ and [^{3}H](-)QNB binding to rat cortical, hippocampal and striatal membranes after chronic Al intoxication

	B_{max} (fmol/mg protein)		K_d (nM)	
	Control	Chronic $AlCl_3$ treatment	Control	Chronic $AlCl_3$ treatment
[^{3}H]PZ				
Frontal cortex	637.60 ± 208.0	324.21 ± 130.4**	2.48 ± 0.8	1.29 ± 0.8
Parietal cortex	558.411 ± 126.0	384.22 ± 98.4**	2.07 ± 0.4	1.92 ± 0.2
Hippocampus	423.25 ± 145.9	245.38 ± 58.5**	2.75 ± 0.4	2.08 ± 0.5
Striatum	733.68 ± 97.7	549.62 ± 81.7*	3.01 ± 0.5	2.98 ± 0.6
[^{3}H](-)QNB				
Frontal cortex	297.78 ± 32.6	297.68 ± 56.6	0.037 ± 0.01	0.035 ± 0.01
Parietal cortex	196.54 ± 53.5	194.51 ± 19.3	0.057 ± 0.02	0.053 ± 0.01
Hippocampus	152.25 ± 18.3	143.89 ± 11.0	0.031 ± 0.01	0.028 ± 0.01
Striatum	329.53 ± 60.2	247.06 ± 35.6*	0.043 ± 0.02	0.043 ± 0.01

Changes in the parameters of the specific [^{3}H]PZ and [^{3}H](-)QNB binding (B_{max} , and K_d) after chronic Al intoxication. Frontal and parietal cortical, hippocampal and striatal membranes were prepared from control and chronically $AlCl_3$-injected rats (1.0 mg/ml/100 g of body weight). Each value is the mean ± S.D. of the results of at least 8 independent measurements carried out in duplicate. * $p < 0.05$; ** $p < 0.01$ (Student's t-test), as compared with the respective control group. Binding parameters were calculated by using a non-linear regression analysis computer program (Leatherbarrow, 1992)

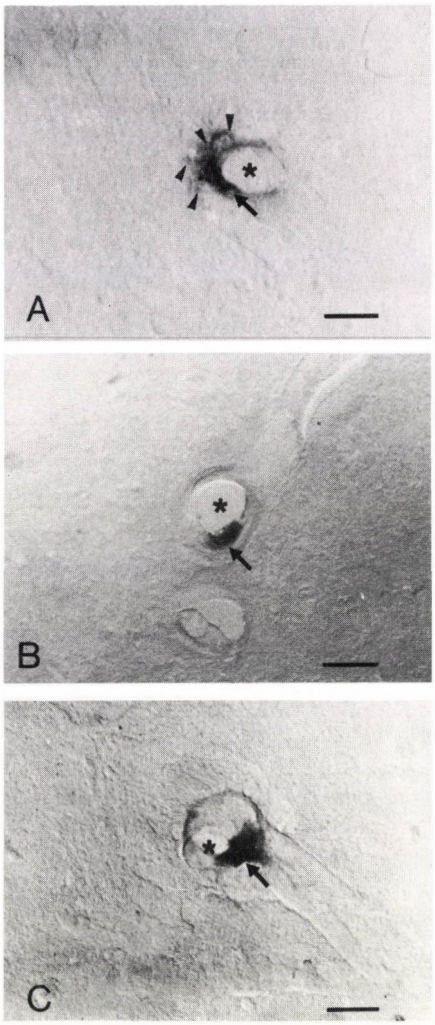


Fig. 1. Representative bright-field photomicrographs depicting Al deposits visualized by solochrome azurine staining in cortical and hippocampal microvessels and surrounding brain tissue of rats treated chronically with an $AlCl_3$ solution of 1.0 mg/ml/100 g of body weight for 5 weeks, 5 days a week. Figure A shows a microvessel in which endothelial cells (arrow) accumulate Al in the rat parietal cortex. Further, detectable amounts of Al were visualized in brain tissues surrounding the microvessel (arrowheads). Figures B and C depict microvessels filled with Al-containing plasma precipitates (arrows) from the frontal cortex and hippocampus. The asterisk denotes the lumen of the microvessel. Nomarski optics, scale bar = 50 μm (A-C)

measured via the specific binding of the M_1-selective antagonist [^{3}H]PZ. Moreover, chronic $AlCl_3$ administration elicited a slight decrease in the mAChR B_{max} value, as revealed by analysis of the binding isotherms of the nonselective antagonist [^{3}H](-)QNB, although this change reached the level of significance ($p < 0.05$) only in the striatum (Table 1). The dissociation constants (K_d) of both antagonists remained unchanged after chronic Al treatment.

DISCUSSION

It is a firmly established notion that Al exerts toxic effects on neurons. We have previously reported (Gulya et al., 1990) that Al affects cholinergic function involving such profound changes as a marked decrease of ChAT activity and that of the B_{max} of mAChR. In our earlier studies (Gulya et al., 1990, 1993, 1995) a chronic 5-week long $AlCl_3$ administration was found to be predominantly effective in a daily dose of 1.0 mg/ml/100 g of body weight.

Al deposition has been visualized in high concentrations (above 20 µg/mg wet weight of tissue; Pearse, 1957) by solochrome azurine staining in brain tissues surrounding microvessels in all brain areas examined. This accumulation may lead to elevated concentrations of Al in the brain after treatment for 5 weeks, in accordance with our previous study, in which elevated Al levels could be detected in different rat brain areas after chronic Al administration, measured by atomic absorption spectrophotometry (Gulya et al., 1990).

Since [^{3}H]PZ binding revealed a significant loss (25-49% of the control value) of M1AChR, while [^{3}H](-)QNB binding showed a significant change only in the striatum, an area known to contain the highest quantities of mAChRs, we assume that chronic Al intoxication preferentially affects M1AChR, the predominant form of mAChR (M_1-M_5) in the rat brain (Wei et al., 1994). On the basis of the broad distribution of M_1AChR in the rat brain, the loss may parallel the altered functioning of the central cholinergic system after chronic Al treatment.

In conclusion, the present histochemical and receptor binding studies provide a further indication that Al accumulates in the brain after chronic Al intoxication, and reinforce the notion that Al may directly affect normal neuronal function.

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REFERENCES

Banks, W.A. and Kastin, A.J. (1985) Aluminum alters the permeability of the blood-brain barrier to some neuropeptides. Neuropharmacology 24, 407-412.

Connor, D.J., Jope, R.S. and Harrell, L.E. (1988) Chronic, oral aluminum administration to rats: cognition and cholinergic parameters. Pharmac. Biochem. Behav. 31, 467-474.

Costantini, S., Giordano, R., Ioppolo, A., Mantovani, A., Ballanti, P., Mocetti, P. and Bonucci, E. (1989) Distribution of aluminum following intraperitoneal injection of aluminum lactate in the rat. Pharmac. Toxic. 64, 47-50.

Ganuto, R.M., Fukatsu, R., Yanagihara, R., Gajdusek, D.C., Hook, G. and Fiori, C.E. (1984) Imaging of calcium and aluminum neurofibrillary tangle-bearing neurons in parkinsonism-dementia of Guam. Proc. Natl. Acad. Sci. USA 81, 1975-1979.

Gulya, K., Rakonczay, Z. and Kása, P. (1990) Cholinotoxic effects of aluminum in rat brain. J. Neurochem. 54, 1020-1026.

Gulya, K., Szikra, J., Lengyel, Z., Harkány, T. and Kása, P. (1993) Interaction between the endogenous opioid system and the aluminum content in rat striatum. In: Recent Advances in Aging Science (eds. E. Beregi, I.A. Gergely, K. Rajczi), pp. 631-635. Monduzzi, Bologna.

Gulya, K., Szikra, J. and Kása, P. (1995) [D-Pen^2, D-Pen^5]Enkephalin, a μ opioid agonist, reduces endogenous aluminum content in the rat central nervous system. Neuroscience 66, 499-506.

Kása, P., Szerdahelyi, P. and Wisniewski, H.M. Lack of topographical relationship between sites of aluminum deposition and senile plaques in the Alzheimer's disease brain. Acta Neuropathol., *in press*

Leatherbarrow, R.J. (1992) *GraFit* Version 3.0. Erithacus Software Ltd., Staines, U.K.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measuretment with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Pákáski, M., Hlavati, I. and Kása, P. (1993) Short exposure to aluminum induces neurofilament phosphorylation in *in vitro* rat brain cultures. Adv. Biosci. 87, 265-266.

Pearse, A.G.E. (1957) Solochrome dyes in histochemistry with particular reference to nuclear staining. Acta Histochem. 4, 95-101.

Perl, D.P. and Brody, A.R. (1980) Alzheimer's disease: x-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science 208, 297-299.

Perl, D.P., Gajdusek, D.C., Garruto, R.M., Yanagihara, R.T. and Gibbs, C.J. (1982) Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Science 217, 1053-1055.

Sahin, G., Varol, I., Temizer, A., Benli, K., Demirdamar, R. and Duru, S. (1994) Determination of aluminum levels in the kidney, liver, and brain of mice treated with aluminum hydroxide. Biol. Trace Element Res. 41, 129-135.

Sharp, T.R. and Rosenberry, T.L. (1985) Ionic strength dependence of the inhibition ol acetylcholinesterase activity by Al^{3+}. Biophys. Chem. 21, 261-264.

Szerdahelyi, P. and Kása, P. (1988) Intraventricular administration of the cholinotoxin AF64A increases the accumulation of aluminum in the rat parietal cortex and hippocampus, but not in the frontal cortex. Brain. Res. 444, 356-360.

Watson, M., Roeske, W.R. and Yamamura, H.I. (1982) [^{3}H]pirenzepine selectively identifies a high affinity population of muscarinic cholinergic receptors in the rat cerebral cortex. Life Sci. 31, 2019-2023.

Watson, M., Roeske, W.R. and Yamamura, H.I. (1986) [^{3}H]Pirenzepine and [^{3}H](-)quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic cholinergic sites. II. Characterization and regulation of antagonist binding to putative muscarinic subtypes. J. Pharmac. Exp. Ther. 237, 419-427.

Wei, J., Walton, A., Milici, A. and Buccafusco, J.J. (1994) *ml-m5* muscarinic receptor distribution in rat CNS by RT-PCR and HPLC. J. Neurochem. 63, 815-821 .

Yamamura, H.I. and Snyder, S.H. (1974) Muscarinic cholinergic binding in rat brain. Proc. Natl. Acad. Sci. USA 71, 1725-1729.

Yates, C.M., Simpson, J., Russell, D. and Gordon, A. (1980) Cholinergic enzymes in neurofibrillary degeneration produced by aluminum. Brain Res. 197, 269-274.

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Research report

MODULATION BY THYROID HORMONES OF THE DEVELOPMENT OF EXTERNAL PLEXIFORM LAYER IN THE RAT OLFACTORY BULB

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Summary: Width of the external plexiform layer in olfactory bulbs and mean area of mitral and granule cell dendritic and glial processes were measured of normal, hypo- and hyperthyroid rat pups at the age of 24 days. Hypothyroidism was induced by treating the rats with a reversible goitrogen 6-n-propyl-2-thiouracil dissolved in their drinking water, while the hyperthyroid group was given water containing thyroxine. The 6-n-propyl-2-thiouracil treatment was begun on gestational day 18 and on the day of birth. Thyroxine treatment started on the day of birth. Both treatments were continued till the day of sacrifice. A significant decrease in the width of the external plexiform layer of the olfactory bulb in the prenatally 6-n-propyl-2-thiouracil treated group and a significant increase in the width of the external plexiform layer of the hyperthyroid group was shown by the Student's paired t-test. The areas of neuronal and glial processes were measured at electron microscopic level by using an IBAS image analysing system. A significant decrease was found by the Kruskal-Wallis test and Dunn's range test in the mean area of (1) mitral cell dendrites in the prenatal 6-n-propyl-2-thiouracil treated group, (2) granule cell dendrites in both the postnatally 6-n-propyl-2-thiouracil treated and in the hyperthyroid groups and (3) glial processes in the thyroxine treated group comparing to controls.
Keywords: Olfactory bulb, external plexiform layer, hypothyroidism, hyperthyroidism, morphometry

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INTRODUCTION

It is widely accepted, that thyroid hormones acting via the T3 nuclear receptor, which is in fact a thyroid hormone-dependent transcription factor (Thompson et al., 1987; Izumo and Mahdavi, 1988; Samuels et al., 1988; Nakai et al., 1990) have rather diffuse effects. They influence skeletal growth and maturation, exert metabolic changes (Schwartz, 1983) and also affect the central nervous system (Nunez, 1988; Timiras and Nzekwe, 1989). Most of the works concerning the deleterious consequences of hypo- and hyperthyroidism focuses on the cerebellum. In this brain area markedly abnormal Purkinje cells with disorganised dendrites, abnormal synapses and damaged cell migration caused by hypothyroidism have been reported (Hajós et al., 1973; Legrand, 1982-1983; Benjamin et al., 1988; Silva and Rudas, 1990; Nunez et al., 1992). Another seriously affected area is the olfactory system (Beard and Mackay-Sim, 1987), where a considerable decrease in the olfactory epithelial surface area and total number of mature olfactory receptor neurons have been observed in the hypothyroid rat (Paternostro and Meisami, 1989, 1991; Sendera and Meisami, 1995). Within the olfactory bulb (OB) all main histological layers and cell types survive these treatments. Earlier studies reported, however, that there is a significant reduction in the total number of mitral cells and a substantial decrease in the thickness of the external plexiform layer (EPL) in the hypothyroid bulbs, while there is no change in the total number of glomeruli (Meisami, 1979; Sendera and Meisami, 1995). The effects of elevated thyroxine level are less frequently studied and neonatal hyperthyroidism is believed not to affect the maturation of e.g. the granule cells in the OB (Brunjes et al., 1982). In our experiments morphometrical consequences of both hypo- and hyperthyroidism, induced in the critical period of neuronal differentiation were examined in the OB of the rat at light and electron microscopic level.

EXPERIMENTAL PROCEDURES

Animals and treatment

Pregnant CFY rats were divided into four groups: a control, drinking tap water; a hypothyroid, having 6-n-propyl-2-thiouracil (PTU, Sigma) in their drinking water (6 mM) from day 18 of gestation; another hypothyroid,

receiving the same PTU treatment from postnatal day 0 (when their pups were born) and a hyperthyroid group, which was given L-thyroxine (T4, Sigma) dissolved in their drinking water (0.3 mM). These drinking solutions and regular rat chow were available *ad libitum*. All treatments were continued until sacrifice on postnatal day 24 (P24). PTU was supposed to be delivered to the pups through the milk and so was T4 in the case of the hyperthyroid group till the pups suckled and later, in addition, they got the drugs directly through their drinking water, too. The colony was kept on a 16/8 hr dark/light cycle and to ensure regular water intake, the temperature was controlled and kept between 21 and 24 °C.

Tissue processing

On postnatal day 24 pups were anaesthetised with ether and blood samples were collected from the aorta for determining serum T4 levels by radioimmunassay (RIA) (T4 Kit, Pharmacia). The animals were perfused intracardially with buffered saline followed by 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The olfactory bulbs were removed, sliced with a razor blade and postfixed in phosphate buffered 1% OSO_4, then dehydrated and regularly oriented pieces were flat embedded in Araldite.

Morphometry

1-µm-thick frontal sections were cut using a pyramitome (LKB), stained with methylene blue and examined in a Zeiss light microscope at a magnification of 250x. The thickness of the EPL was measured using an ocular micrometer at 10 different positions per section which were 5 µm apart from each other. Taken together sections from 7 control, 8 prenatally PTU treated, 6 postnatally PTU treated and 7 T4 treated OBs were analysed. Data obtained were normally distributed, so we used the Student's paired t-test in order to compare the means of the treated groups with that of the control one. For electron microscopy sections were cut on a Reichert ultramicrotome, stained with lead citrate and uranyl acetate and examined with a Zeiss Opton electron microscope at a magnification of 1:2000x. Morphometric analysis of the EPL was carried out using an IBAS image analysing system within a stripe of approximately 165 µm x 3 µm per section which was parallel with the mitral cell layer. The area of mitral and granule cell dendrites and that of glial

processes were measured in the described territory. Sections from 7 control, 5 prenatally PTU treated, 4 postnatally PTU treated and 4 T4 treated OBs were examined. Since some of the obtained data were not normally distributed and the sample sizes were rather different, in the statistical analysis the Kruskal-Wallis test was used, followed by the Dunn's range test (Sokal and Rohlf, 1969).

RESULTS

Radioimmunoassay

RIA measurements indicated that both PTU and T4 treatments were effective, since the serum T4 levels of the PTU treated groups were less than 20% of the control (less than the lower limit of the detection system), whereas those of the T4 treated group were about one order of magnitude higher than the control values (Table 1).

Table 1: Serum T4 Levels of 24-day-old Rats (nmol/l)

	PTU prenat. (n = 4)	PTU postnat. (n = 3)	CONTROL (n = 4)	T4 (n = 4)
	63.5	<10	<10	589.0
	35.8	<10	<10	520.2
	36.9	<10	<10	453.6
	63.6		<10	586.8
Mean ± S.D.	49.95 ± 13.6	<10	<10	537.4 ± 55.72

Serum T4 levels were measured by RIA. PTU treatment reduced serum T4 levels, thus inducing hypothyroidism, while T4 treatment caused hyperthyroidism. S.D. = standard deviation

Light microscopy

No striking difference could be observed between the general architecture of the OBs and particularly in the appearance of the EPL in the various groups (Fig. 1).

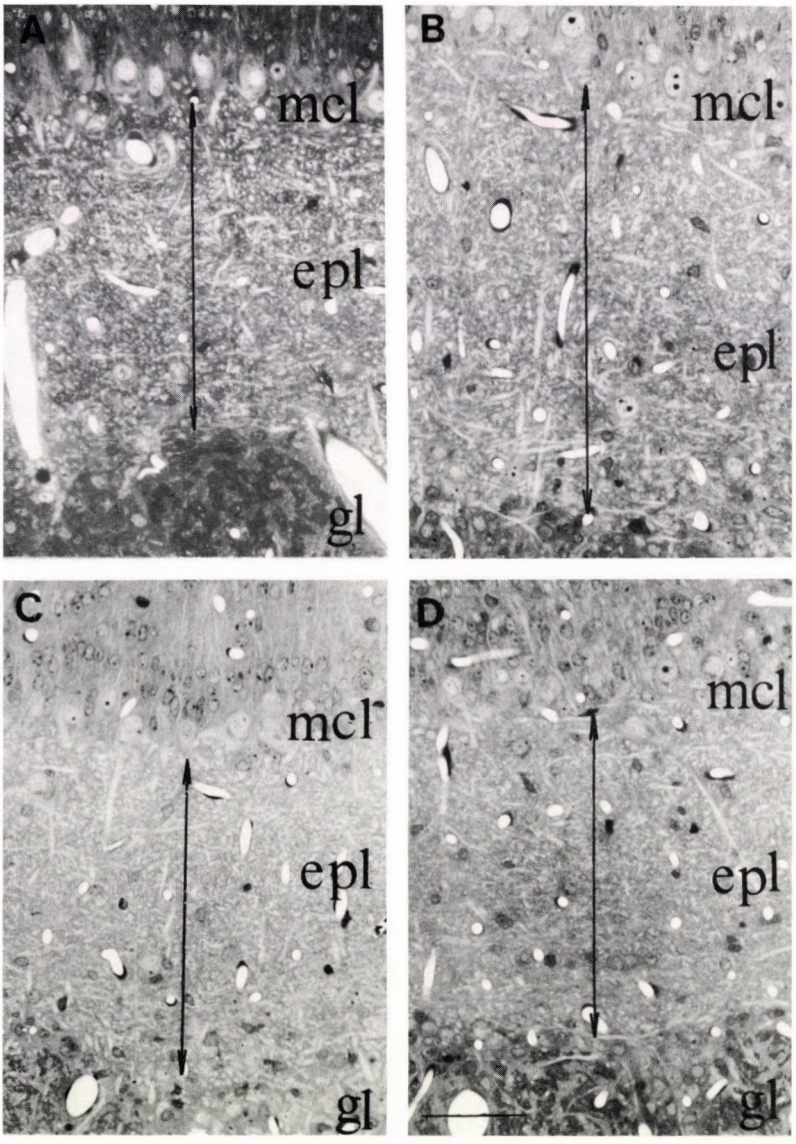


Fig. 1: Survey picture of the glomerular (gl), external plexiform (epl) and mitral cell (mcl) layer in coronal plane. No striking difference can be observed comparing the OBs of the (A) control, (B) postnatally T4 treated, (C) prenatally PTU treated and (D) postnatally PTU treated rats. Bar for (A) through (D): 50 µm

Table 2: Average Width of EPL (μm)

	CONTROL (n = 70)	PRENAT.PTU (n = 80)	POSTNAT.PTU (n = 60)	T4 (n = 70)
Mean ± S.D.	184.41 ± 3.90	159.48 ± 18.27*	173.25 ± 13.05	200.01 ± 7.44*

The width of the EPL of the various experimental groups demonstrates that hypothyroidism resulted in narrower EPL while hyperthyroidism made the EPL wider, comparing to controls. S.D. = standard deviation *: significant difference compared to control (Student's paired t-test; $p < 0.05$)

Comparison of the width of the EPL using the Student's paired t-test revealed a significant decrease (13.5% on average) in the hypothyroid bulbs; treated with PTU from gestational day 18 and a significant increase (8.5% on average) in the hyperthyroid group. No significant difference was found in the EPL of the postnatally PTU treated OBs (Table 2).

Electron microscopy

In qualitative studies – similarly to the semi-thin sections – there was no conspicuous difference between the EPLs of different groups at the ultrastructural level. Morphometric analyses were carried out on three major components of the deep part of the EPL, the mitral (tufted) cell dendrites, granule cell dendrites and glial processes (which were identified in most cases to be astrocytic, based on their electron-lucent appearance and sparse filaments). The dendrites of mitral (tufted) and granule cells were recognised mostly on the basis of their size and characteristic electron density. In addition, synapses with symmetrical or asymmetrical membrane thickenings and their opposite directions also helped to identify these two types of fibres. All aspects of cut fibre segments (should their course be parallel, oblique or perpendicular to the plane of the section) were comprised in the statistics (Fig. 2).

Statistical analysis of the mean areas occupied by these three types of processes revealed several notable differences (Table 3). When compared to controls,

A) the size of mitral cell dendrites was reduced by 17% on average in the prenatally PTU treated group,

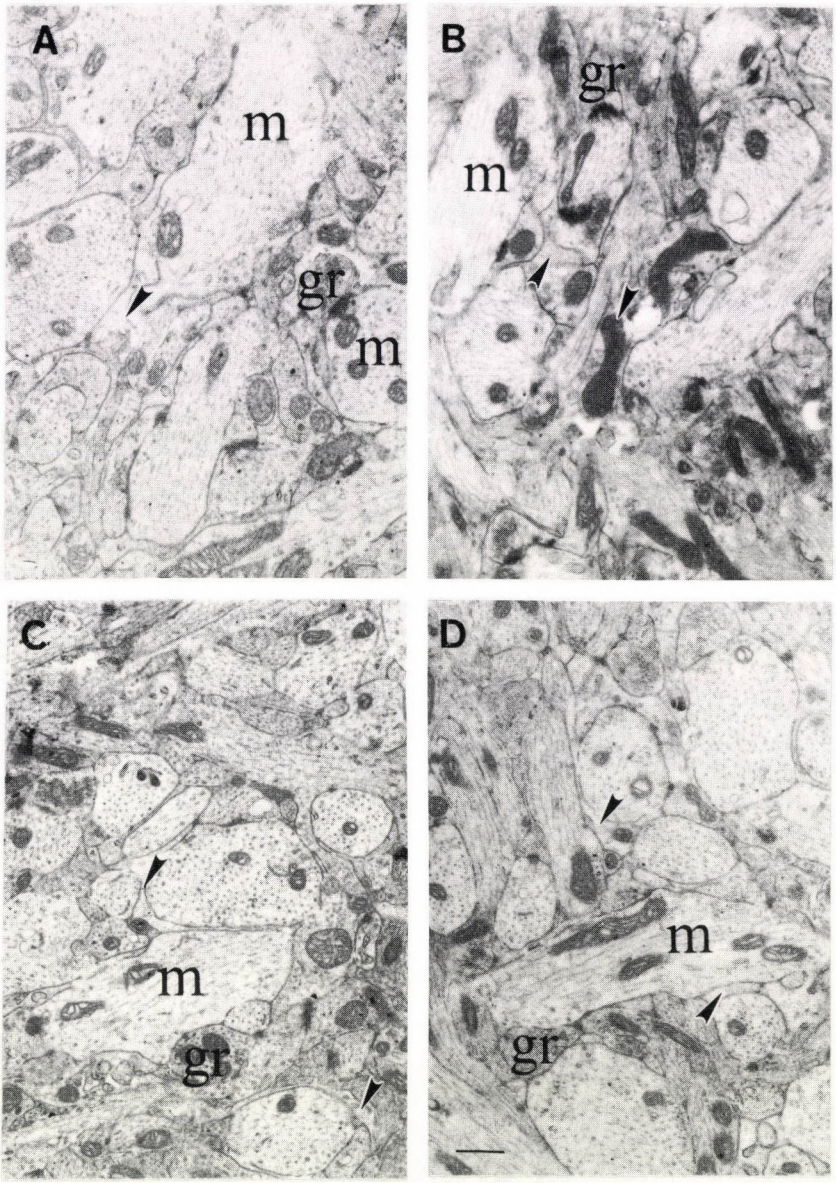


Fig. 2: Electron microscopical details of the external plexiform layer of the (A) control, (B) postnatally T4 treated, (C) prenatally PTU treated and (D) postnatally PTU treated rat olfactory bulb. m: mitral cell dendrite; gr: granule cell dendrite. Some glial processes are labelled by arrowheads . Bar for (A) through (D): 1 μm

B) the area of granule cell dendrite cross-sections showed a decrease of 27% on average in the postnatally PTU treated group, and
C) both the granule cell dendrites and those of glial processes were reduced in size by 20% and 24%, respectively, in the hyperthyroid group.

Although all kinds of treatment resulted in a decrease in the cross-sectioned area of all three tissue components we examined in this experiment, no further significant difference was found.

Table 3: Average Cross-Sectional Area of Processes (μm^2)

	EXPERIMENTAL GROUPS			
Tissue components	CONTROL	PRENAT. PTU	POSTNAT. PTU	T4
Mitral cell dendrites	1.40 ± 1.13 (n = 374)	1.16 ± 0.87* (n = 306)	1.21 ± 0.90 (n = 283)	1.16 ± 0.84 (n = 272)
Granule cell dendrites	0.44 ± 0.34 (n = 934)	0.42 ± 0.37 (n = 745)	0.32 ± 0.29* (n = 670)	0.35 ± 0.28* (n = 711)
Glial processes	0.45 ± 0.48 (n = 168)	0.40 ± 0.39 (n = 151)	0 41 ± 0.40 (n = 98)	0.34 ± 0.44* (n = 100)

All treatments resulted in a reduction of the cross-sectional area of all three types of processes, examined in this experiment when compared to controls.
Data shown are mean values ± standard deviation.
*: significant difference compared to control (Kruskal-Wallis test and Dunn's range test; $p < 0.05$)

DISCUSSION

Either elevated or depressed thyroid hormone levels can hinder the development or growth of a mammalian. Excess of thyroid hormones causes precocious maturation of the brain – it increases cell proliferation and maturation during the early postnatal period (i.e. the first two weeks) but results in a decreased body size and reduced volume of various brain areas (Balázs et al., 1971; Patel et al., 1979). The retardation caused by elevated thyroid hormone levels by the third week (in the rat brain) was explained by supposing them "to hasten the decline of cell proliferation" (Patel et al., 1979).

Thyroid hormone deficiency results in developmental disturbances involving the olfactory system too. Reduced T4 level affects seriously the sense of smell (Beard and Mackay-Sim, 1987) and also the apparent maturity of the peripheral olfactory apparatus (Mackay-Sim and Beard, 1987). According to the results we presented above, the first relay station for the nasal chemoreceptory signals, the olfactory bulb, also shows some morphological changes which accompany either reduced or elevated serum T4 levels. These changes, however, are not dramatic, qualitative alterations. Even the seemingly most vulnerable, developing granule cells are surprisingly resistant to the elevated T4 level. Former studies from our laboratory documented, however, a slight decrease in granule cell density per unit area of the layer. Simultaneously, thickness of the granule cell layer decreased, while the total volume of the bulbar cortex (as detected on weight measurements; Hoyk and Halász, unpublished) remained constant in T4 groups. Brunjes and colleagues (1982) using a Golgi-technique, detected no change in the density and dendritic arborization of the deep granule cell population ("dark" granule cells in methacrylate, Struble and Walters, 1982). Moreover, they observed a significant increase in the low-caliber category of dendritic processes which reached its maximum on P21 but persisted even at P60. Less is known on the also late-developing superficial local interneurons, the periglomerular cell population and the glomerular organisation which may also be prone to hormonal factors. More centrally, the anterior commissure (relaying olfactory signals to contralateral sides of the brain) was observed to be reduced in thickness (by 39%) under hypothyroid status but conveyed a similar number of fibers as in normal rats (Guadano-Ferraz et al., 1994).

Our findings partly support earlier data demonstrating that hypothyroidism is associated with the hypoplasticity of the neuropil (Meisami, 1979; Legrand, 1982, 1983; Timiras and Nzekwe, 1989). In 25-day-old hypothyroid bulbs, the NGF level was reduced, while the low-affinity NGF receptor expression was markedly elevated when compared to controls (Sendera and Meisami, 1995). The morphometry presented here indicates that at least some of the targets of thyroid hormone action are mechanisms influencing the neuronal architecture of the OB. These mechanisms may affect the proliferation and maturation of OB granule cells similarly to those of the cerebellum. In the hypothyroid cerebellum besides granule cells Purkinje cells were also seriously damaged (Lauder, 1977; Legrand, 1982-1983; Benjamin et al., 1988). Hajós and co-workers (1973) revealed further qualitative changes in the thyroid-deficient (induced by subcutaneous injection of

methimazole) rat cerebella: somatic spines persisted longer on Purkinje cells, maturation of granule cell dendrites was delayed and the glomeruli remained smaller and contained "large glial spaces". In our material, in contrast, all cross-sectional areas - including those of glial processes – were smaller in the treated groups when compared to control, but only some of these reductions were significant.

Our preliminary light microscopic experiments on determining the number of mature granule cells in the OB (Hoyk et al., 1992) suggest that they are reduced in number compared to controls due to both kinds of treatment (data not shown). Besides it is proven that thyroid hormones interfere with the development of the cytoskeleton by altering the expression of TAU and tubulin (Nunez, 1984; Ginzburg, 1991) and in the hypothyroid rat cerebellum the detyrosination of a-tubulin is delayed (Poddar and Sarkar, 1993). The possible changes in the structure of microtubules and in the maturation of granule cells as far as it was shown in our studies – might contribute to the observed morphological phenomena. It might also at least in part reconcile the apparent paradox that there is a decrease in process cross-sectional area in both hypo- and hyperthyroid states, but the width of the external plexiform layer is decreased only in hypothyroidism, and in hyperthyroidism even an increase is seen in the width of this layer. If the structure of microtubules is disturbed, it might result in thinner and longer processes in hyperthyroid rats but in thinner and shorter processes in hypothyroidism. More light should be shed on the regulation by thyroid hormones of cytoskeletal proteins.

Although the manipulated thyroid hormone level does not result in a restructuring of the basic circuitry of the olfactory bulb (for review, see Halász, 1990) at this age and developmental stage, apparent changes in behaviour (slowing down, being generally depressed) may have an olfactory component, too. Experiments to study these olfactory-related behavioural consequences and those of targeting the better understanding of the neurochemical changes in animals with elevated or reduced thyroid hormone levels are in progress in our laboratory.

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REFERENCES

Balázs, R., Kovács, S., Cocks, W. A., Johnson, A. L. and Eayrs, J. T. (1971) Effect of thyroid hormone on the biochemical maturation of rat brain: postnatal cell formation. Brain Res. 25, 555-570.

Beard, M. D. and Mackay-Sim, A. (1987) Loss of sense of smell in adult, hypothyroid mice. Dev. Brain Res. 36, 181-189.

Benjamin, S., Cambray-Deakin, M. A. and Burgoyne, R. D. (1988) Effect of hypothyroidism on the expression of three microtubule-associated proteins (1A, 1B and 2) in developing rat cerebellum. Neurosci. 27, 931-939.

Brunjes, P. C., Schwark, H. D. and Greenough, W. T. (1982) Olfactory granule cell development in normal and hyperthyroid rats. Dev. Brain Res. 5, 149-159.

Ginzburg, I. (1991) Neuronal polarity: targeting of microtubule components into axons and dendrites. Trends Biochem. 16, 257-261.

Guadano-Ferraz, A., Escobar del Rey, F., Morreale de Escobar, G., Innocenti, G. M. and Berbel, P. (1994) The development of the anterior commissure in normal and hypothyroid rats. Dev. Brain Res. 81, 293-308.

Hajós, F., Patel, A. J. and Balázs, R. (1973) Effect of thyroid deficiency on the synaptic organization of the rat cerebellar cortex. Brain Res. 50, 387-401.

Halász, N.: The Vertebrate Olfactory System. Chemical Neuroanatomy, Function and Development (1990) Akadémiai Kiadó, Budapest.

Hoyk, Zs., Vecsernyés, M. and Halász, N.: Effect of thyroid hormones on the development of the rat olfactory bulb: a morphometric study. ECRO X, Munich, Aug. 23-28, 1992, Abs. 59.

Izumo, S. and Mahdavi, V. (1988) Thyroid hormone receptor a isoforms generated by alternative splicing differentially activate myosin HC gene transcription. Nature 334, 539-542.

Lauder, J. M. (1977) Effects of thyroid state on development of rat cerebellar cortex. In: Thyroid Hormones and Brain Development (ed. Grave G.D.), pp. 235-252. Raven Press, New York.

Legrand, J. (1982-1983) Hormones thyroldiennes et maturation du systeme nerveux. J. Physiol. (Paris) 78, 603-652.

Mackay-Sim, A. and Beard, M. D. (1987) Hypothyroidism disrupts neural development in the olfactory epithelium of adult mice. Dev. Brain Res. 36, 190-198.

Meisami, E. (1979) The developing rat olfactory bulb: prospects of a new model system in developmental neurobiology. In: Neuronal Growth and Differentiation (eds. Meisami, E. and Brazier, M. A.), pp. 183-206. Raven Press, New York.

Nakai, A., Sakurai, A., Macchia, E., Fang, V. and Degroot, L. J. (1990) The roles of three forms of human thyroid hormone receptor in gene regulation. Mol. Cell. Endocrinol. 72, 143-148.

Nunez, J. (1984) Effects of thyroid hormones during brain differentiation. Mol. Cell. Endocrinol. 37, 125-132.

Nunez, J. (1988) Mechanism of action of thyroid hormone. In: Hormones and Their Actions. Part 1 (eds. Cooke B. A., King R. J. B. and Van Der Molen H. J.), pp. 61-80. Elsevier Science Publishers BV.

Nunez, J., Couchie, D., Aniello, F. and Bridoux, A. M. (1992) Thyroid hormone effects on neuronal differentiation during brain development. Acta Med. Austriaca, 19, Suppl. 1, 36-39.

Patel, A. J., Lewis, P. D., Balázs, R., Balley, P. and Lai, M. (1979) Effects on postnatal cell acquisition in the rat brain. Brain Res. 172, 57-72.

Paternostro, M. and Meisami, E.(1989) Selective effects of thyroid hormonal deprivation on growth and development of olfactory receptor sheet during the early postnatal period: a morphometric and cell count study in the rat. Int. J. Devl. Neurosci. 7, 243-255.

Paternostro, M. A. and Meisami, E. (1991) Lack of thyroid hormones but not their excess affects the maturation of olfactory receptor neurons: a quantitative morphologic study in the postnatal rat. Int. J. Devl. Neurosci. 9, 439-452.

Poddar, R. and Sarkar, P. K. (1993) Delayed detyrosination of Alpha-tubulin from parallel fibre axons and its correlation with impaired synaptogenesis in the hypothyroid rat cerebellum. Brain Res. 614, 233-240.

Samuels, H. H., Forman, B. M., Horowitz, Z. D. and Ye, Z.-S. (1988) Regulation of gene expression by thyroid hormone. J. Clin. Invest. 81, 957-967.

Schwartz, H. L. (1983) Effect of thyroid hormone on growth and development. In: Molecular Basis of Thyroid Hormone Action, pp. 413-444. Academic Press.

Sendera, T. and Meisami, E. (1995) Altered levels of NGF and NGF-receptor in olfactory bulb of developing hypothyroid and recovering rats. 17th Ann. Meeting of AChemS, Sarasota, Fla.

Silva, J. E. and Rudas, P. (1990) Effects of congenital hypothyroidism on microtubule-associated protein-2 expression in the cerebellum of the rat. Endocrinology 126, 1276-1282.

Sokal, R. R. and Rohlf, F. J. (1969) Biometry. W. H. Freeman and Company, San Francisco.

Struble, R. G. and Walters, C. P. (1982) Light microscopic differentiation of two populations of rat olfactory bulb granule cells. Brain Res. 236, 237-251.

Thompson, C. C., Weinberger, C., Lebo, R. and Evans, M. R. (1987) Identification of a novel thyroid hormone receptor expressed in the mammalian central nervous system. Science 237, 1610-1613.

Timiras, P. S. and Nzekwe, E. U. (1989) Thyroid hormones and nervous system development. Biol. Neonate 55, 376-385.

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Research report

PROTECTION AGAINST NON-NMDA RECEPTOR-MEDIATED EXCITOTOXICITY BY GYKI 52466 IN MATURE TELENCEPHALIC CULTURES OF THE RAT

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Summary: The neuroprotective effect of 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI 52466), a recently developed non-competitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate antagonist was demonstrated against quisqualate (10 or 15 μM) and AMPA (20 μM) excitotoxicity in mature (17-20 days in vitro) cultures of rat embryonic telencephalic cells. GYKI 52466 attenuated quisqualate (15 μM) or AMPA (20 μM) neurotoxicity in a dose-dependent manner with IC50 of 16 μM and 10 μM, respectively.
Keywords: quisqualate, AMPA, AMPA/kainate receptor, GYKI 52466, neuroprotection, NMDA receptor

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INTRODUCTION

Glutamate and related acidic amino acids account for most of the excitatory synaptic activity in the mammalian brain. Excessive or persistent activation of glutamate-gated ion channels may cause neuronal degeneration and this process is implicated in the neuropathology of several adult neurodegenerative disorders, as well as in stroke and seizures (Ames et al., 1993; Bondy and Lebel, 1993; Coyle and Puttfarcken, 1993). Ionotropic glutamate receptors are categorised pharmacologically and genetically into 3 subclasses: N-methyl-D-aspartate (NMDA) receptors, AMPA receptors and kainate receptors. Quisqualate acts as an agonist on the two latter receptors and also activates metabotropic glutamate receptors coupled to polyphosphoinositide hydrolysis (Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995).

Primary culture of neurons from the central nervous system of rat or mice is widely used to study the glutamate receptor-mediated neuronal destruction - the so-called excitotoxicity – at cellular level (Koh et al., 1990; Murphy et al., 1990; Priestley et al., 1990; Keilhoff and Erdõ 1991; Zinkand et al., 1992; May and Robison, 1993; Weller et al., 1993; Akaike et al., 1994; Cazevieille et al., 1994; Cheng and Mattson, 1994; Okamoto et al., 1994; DeCoster et al., 1995; Hajimohammadreza et al., 1995; Lakics et al., 1995; Lynch et al., 1995; May et al., 1995; Prehn et al., 1995). In the present work the neuroprotective effect of GYKI 52466, a recently developed non-competitive AMPA/kainate receptor antagonist (Tarnawa et al., 1990; Arvin et al., 1992; LePeillet et al., 1992; May and Robison, 1993; Parsons et al., 1994; Xue et al., 1994) was demonstrated against quisqualate and AM:PA toxicity in mature (17-20 days in vitro) cultures of rat telencephalic neurons.

MATERIALS AND METHODS

Cell culture

Primary culture of rat embryonic telencephalic cells was prepared from Sprague-Dawley rat embryos on the 17th to 19th day of gestation according to the method described by Madarász et al. (1984), with minor modifications. Briefly, telencephalons were dissected and after removal of meninges, mechanically dissociated in culture medium by trituration with Pasteur pipette

above a nylon mesh with pore diameter of 42 μm. $5x10^5$ cells filtered through the mesh were plated onto 24-well plates (Costar) coated with 2 μg/ml poly-L-lysine (MW>300000, Sigma) for 1 h. For dissociation, plating and maintenance HEPES-buffered Eagle's Minimal Essential Medium (MEM, Sigma) supplemented with 10% heat-inactivated fetal calf serum (FCS, Sigma), 4 mM glutamine (Sigma), 20 mM KCl, gentamicin sulfate (50 mg/l; Chinoin, Budapest, Hungary) and amphotericin-B (2.5 mgA; Sigma) was used (maintenance medium, MM). Cultures were incubated at 37 °C in a humidified atmosphere containing 2% CO_2. Medium changes were carried out 24 h and 3-4 days after plating. On the 5th-6th day in vitro the proliferation of non-neuronal cells was arrested by addition of 10 μM cytosine arabinofuranoside (CAR, Sigma) for 24 h after that the cells were shifted into a modified maintenance medium (MMM), containing 10% heat-inactivated horse serum (HS, Sigma) instead of FCS, and 12 mM glucose. MMM did not contain KCl. The last change of medium was carried out on the 12th to 14th day after plating.

Analysis of quisqualate neurotoxicity

Experiments were performed on 17 to 20-day-old cultures. Cultures were exposed for 21-23 h to quisqualate (10 or 15 μM; Tocris Neuramin) or AMPA (20 μM; Tocris Neuramin) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 50 μM; Tocris Neuramin) or GYKI 52466 (15 or 45 μM; synthesised in the Institute for Drug Research, Budapest, Hungary) by adding small volumes (<20 μl) of concentrated stock solutions, with or without changing the medium.

In experiments comparing the neurotoxicity indices MMM containing only 5% HS and 100 μM D-amino-5-phosphonopentanoic acid (D-AP5, Tocris Neuramin) was used for medium change. In the case of the dose-response curve determination, the GYKI 52466 dilutions and treatments were made in MMM containing only 5% HS and 10^5 U/l penicillin instead of gentamicin and amphotericin-B. CNQX or GYKI 52466 was added to the cultures 30 minutes before addition of quisqualate or AMPA.

The neuronal cell death was visually assessed by phase contrast microscopy and quantified by cell counting and also by measuring the activity of LDH released into the culture medium. One hour before the quisqualate treatment 150-300 neurons were counted for each culture well in a representative field. The number of surviving neurons was determined in the

same field after 21 h of quisqualate exposure and the percentage of neuronal cell death was calculated. (To identify the fields, a screen put under the plate was used.) Neurons were identified morphologically as phase bright cells bearing extensive processes (Koh and Choi, 1987; Rosenberg, 1991). (This morphological identification had been verified by immunocytochemistry using a monoclonal antibody against neurofilament 200.)

The following procedure was used for comparing the neurotoxicity indices. After 22 h of treatment with quisqualate the culture supernatants (supernatants A) were removed and replaced with MMM containing 5% HS and 500 µM glutamate to destroy all the survived neurons. After 24 h of incubation the culture supernatants (supernatants B) were removed and the remaining pure glial cultures were lysed by freeze-thaw (samples C) in ten times diluted Dulbecco's phosphate buffered saline, and the lysates were collected (glia samples). LDH activities of supernatants A and B (LDHA and LDHg) and the glia samples (LDHglia) were measured following centrifugation (5 min., 10 000 g, 4 °C).

LDH activity was determined at 37 °C, in 96-well microtiter plates in duplicate from 100 µl aliquots of culture supernatants and 40 µl of glia samples, respectively. The decrease of NADH absorbance at 340 nm was measured by a kinetic ELISA reader (Biotek). The LDH activity of 100 µl MMM containing 5% HS was subtracted as background in the case of culture supernatants. Three different neurotoxicitv indices (NIs), including a novel one were calculated.

NI_1. LDH activities of supernatants A (LDHA) were scaled to the LDH released due to complete neuronal cell death in selected culture wells (in 4 wells of a 24-well plate). In our culture system medium change was used to achieve complete neuronal cell death: LDHA,MC. N1 is one of the most widely used indices described by Koh and Choi (1987).

NI_2. 100x[LDHA/(LDHA+LDHg+LDHglia)]. The net result is the other conventional neurotoxicity index, which includes LDH measurement from the excitotoxically indifferent glial cells as well (e.g. Murphy et al., 1990; Keilhoff and Erdõ, 1991; May et al., 1995).

NI_3. 100x[LDHA/(LDHA+LDHg)] – the newly developed index, that gives the extent of neuronal cell death as percentage of LDH activity characteristic for the total neuron number in the same culture well. LDHA+LDHB corresponds to LDHneurons.

The dose-response curves for GYKI 52466 were determined using the NI_3 neurotoxicity index.

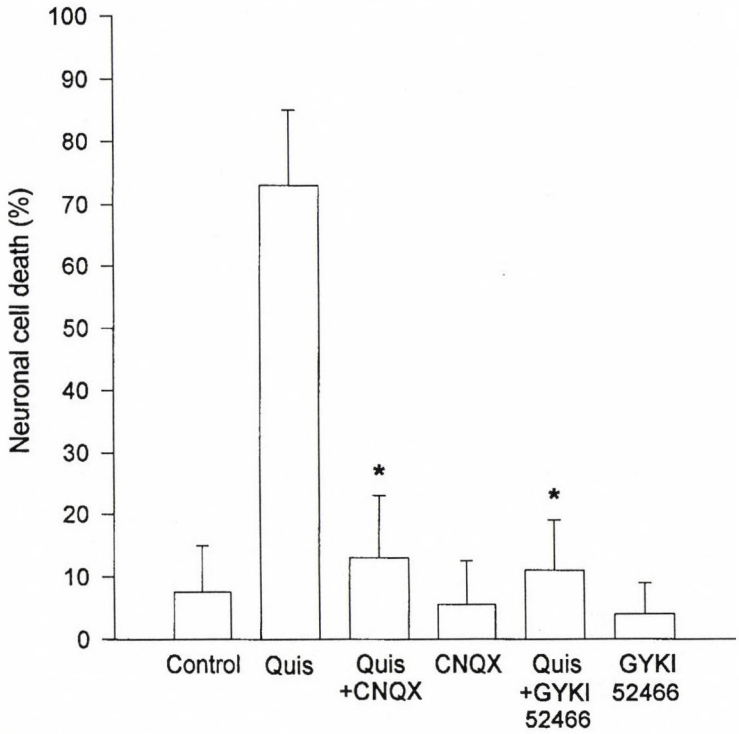


Fig. 1: Quantification by cell counting of quisqualate neurotoxicity and neuroprotection of AMPA/kainate receptor antagonists in mature cultures of rat telencephalic cells. Cultures of 18-20 days in vitro were exposed for 21 h to 10 μM quisqualate and/or to its antagonists (50 μM CNQX or 45 μM GYKI 52466). Treatments were performed without changing the medium by adding small volumes (<20 μl) of concentrated stock solutions. Columns and bars represent mean ± SD of three independent experiments, each in quadruplicate. Asterisks indicate significant difference as compared to quisqualate treatment alone by Bonferroni t-test following one-way ANOVA (*P<0.001)

RESULTS

Neurons in tissue culture from rat embryonic telencephalon become very sensitive tc glutamate receptor-mediated toxicity after two weeks in vitro, and thereafter medium change completely destroys them in our conditions. Rosenberg (1991) and Driscoll et al. (1991, 1993) reported that medium change causes neuronal cell death in mature cultures of cortical and mesencephalic neurons, respectively. According to their explanation the

primary cause of this phenomenon was the glutamate contamination of the medium supplement glutamin. However, in our culture system glutamin proved not to be toxic at all (data not shown).

To avoid the unidentified neurotoxic effect of fresh medium, cultures were exposed to quisqualate and to its antagonists (GYKI 52466 and CNQX) without medium change. In this case the cell counting was the only usable method for the quantification of neuronal cell loss (Fig. 1). GYKI 52466 (45 μM) as well as CNQX (50 μM) fully protected cultured neurons against quisqualate toxicity. We were not able to quantify excitotoxicity by LDH determination because of the high and variable amount of LDH, accumulated in the culture supernatants since the last medium change. When LDH content was measured in a small sample of each well supernatants before excitotoxic injury and subtracted from the final total value of LDH, the results were contradictory (data not shown). Experiments were performed to prevent the neuronal cell death caused by medium change.

Hundred μM D-AP5 (a competitive NMDA antagonist) proved to be fully protective against the neurotoxic effect of fresh medium (see Fig. 2B). 50 μM dizocilpine (MK-801; an NMDA channel blocker) was also effective, but neither 50 μM CNQX nor 60 μM GYKI 52466 (both are AMPA/kainate antagonists) inhibited this type of neuronal destruction (data not shown). Identification of the neurotoxic component(s) of the medium is in progress, and prelirninary results clearly show that the amphotericin-B and gentamicin preparations are responsible for the neurotoxic effect of medium change.

The neurotoxicity of quisqualate (10 μM) and the neuroprotective effect of GYKI 52466 (15 and 45 μM) were determined in the presence of 100 μM D-AP5. Treatments were carried out following medium change. Fig. 2 demonstrates that the presence of 100 μM D-AP5 in the culture medium does not influence the neurotoxic potency of quisqualate and the neuroprotection afforded by GYKI 52466. Fig. 3 shows the summary of results of three independent quisqualate toxicity experiments. For evaluation of neurotoxicity, cell counting as well as three different neurotoxicity indices (NIs), based on LDH measurements, were use (see Materials and Methods).

The neuroprotective efficacy of GYKI 52466 was examined against quisqualate (15 μM) and AMPA (20 μM) in penicillin-containing medium (Fig. 4.).

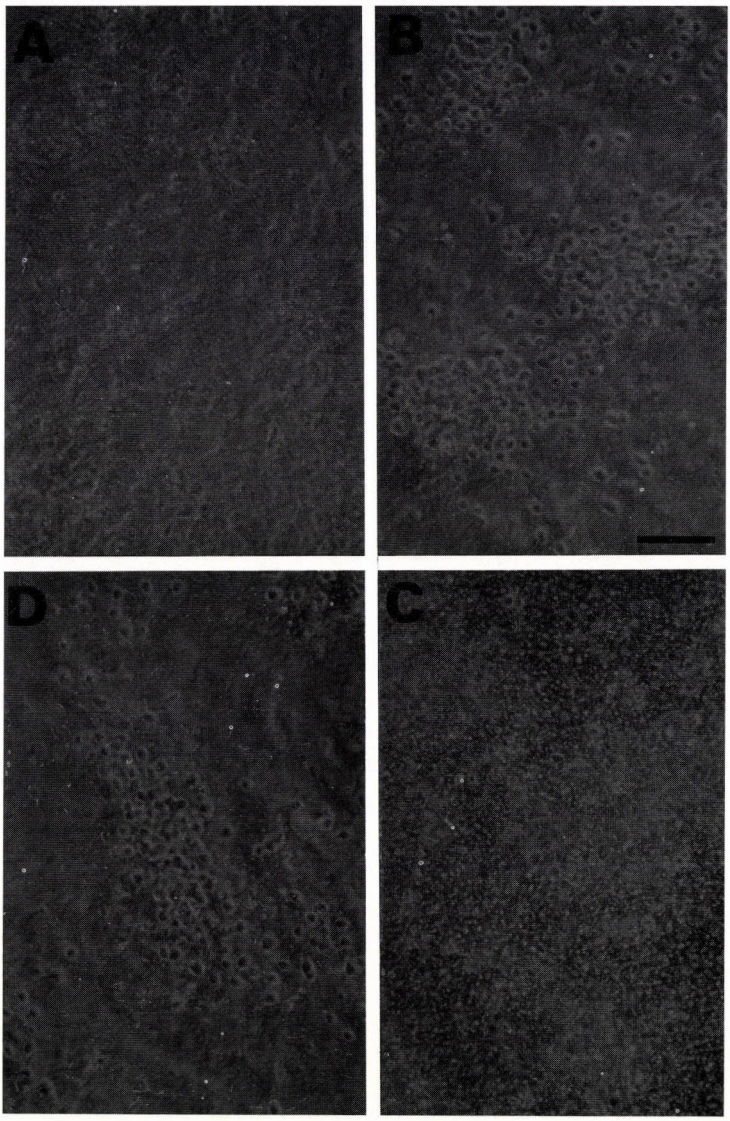


Fig. 2: Neuroprotection against quisqualate excitotoxicity by GYKI 52466. Phase-contrast photomicrographs of telencephalic cultures (19 days in vitro) after 23 h of treatments. A: Medium change (MC). B: MC+100 µM D-AP5. C: MC+100 µM D-AP5+10 µM quisqualate. D: MC+100 µM D-AP5+10 IlM quisqualate+45 µM GYKI 52466. Scale bar, 100 µm

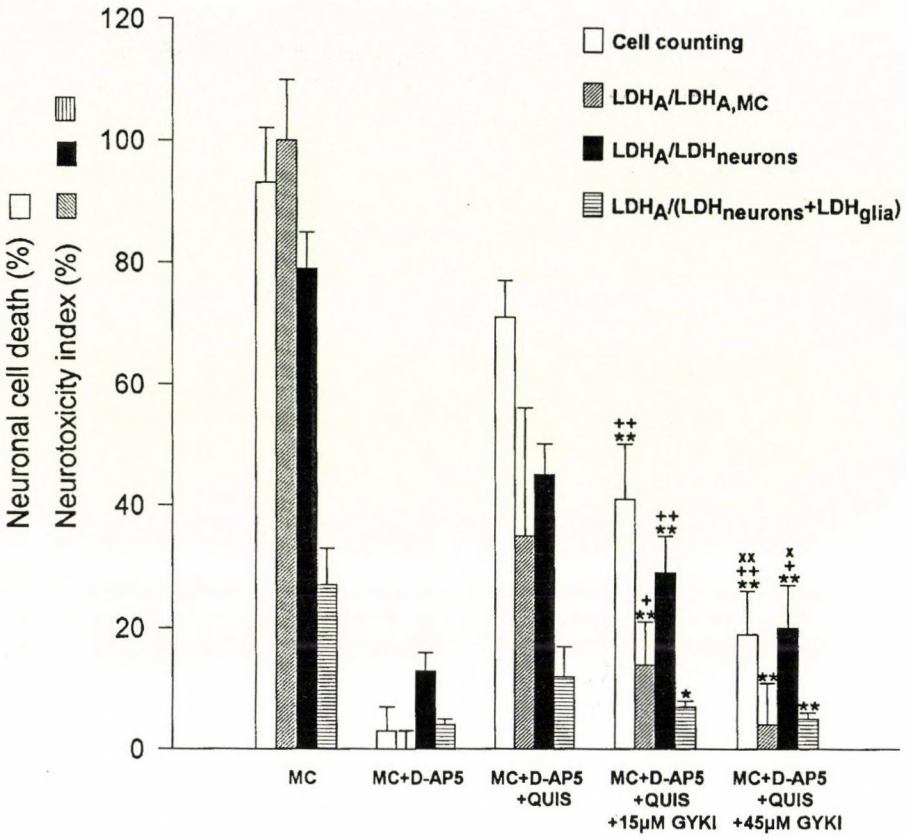


Fig. 3: Quisqualate neurotoxicity as measured by cell counting and characterized by three different neurotoxicity indices as well. Cultures of 17-19 days in vitro were exposed for 22 h to 10 μM quisqualate (Quis) and 15 or 45 μM GYKI 52466 in the presence of 100 μM D-AP5. Treatments were carried out with medium change (MC). The neurotoxicity indices were calculated from LDH activities determined in the culture supernatants after quisqualate exposure and in the surviving neurons and glial cells, as detailed in Materials and Methods. Columns and bars represent mean ± SD of three independent experiments, each in quadruplicate. LDH_A: LDH activity of the culture supernatant after quisqualate treatment for 22 h; $LDH_{A,MC}$: LDH activity of the culture supernatant in the 22nd h following a medium change (100% neuronal cell death). Significance of differences between the treatments was calculated by Bonferroni t-test following one-way ANOVA. Comparison to quisqualate exposure alone: *PC0.05; **$P<0.001$. Comparison to MC+D-AP5: $^{+}P<0.05$; $^{++}P<0.001$. Comparison to (MC+D-AP5+Quis+15 IlM GYKI 52466): $^{x}P<0.01$; $^{xx}P<0.001$

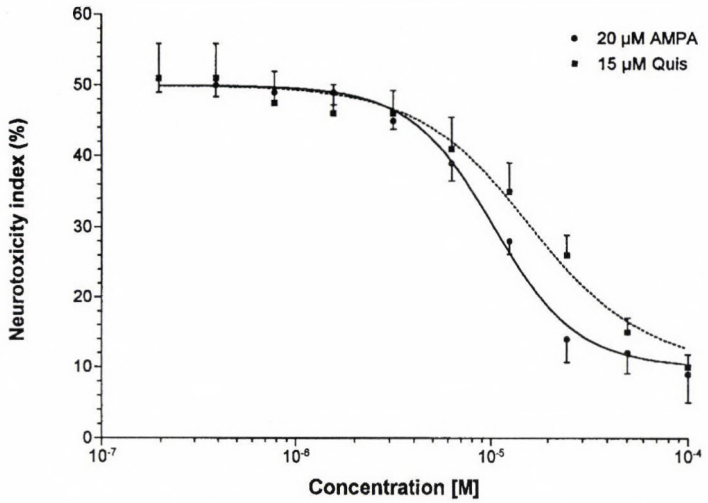


Fig. 4: Concentration-dependent neuroprotection by GYKI 52466 against quisqualate and AMPA excitotoxicity. Cultures of 18-19 days in vitro were treated for 23 h with 15 µM quisqualate (Quis) or 20 µM AMPA and various concentration of GYKI 52466. The medium used for treatments contained penicillin as antibiotic instead of gentamicin and amphotericin-B. The NI3 neurotoxicity index was calculated as described in Materials and Methods. Points are mean ±SD of three independent experiments, each in duplicate. IC_{50} values were determined by nonlinear curve fitting (GraphPad Prism): 16 µM against quisqualate and 10 µM against AMPA

The medium used for treatments contained penicillin as antibiotic instead of gentamicin and amphotericin-B. Medium change with this type of medium did not cause significant neuronal cell death (data not shown). IC_{50} values for neuroprotection by GYKI 52466 were 16 µM against quisqualate and 10 µIM against AMPA, as determined by nonlinear curve fitting (Graphpad Prism).

DISCUSSION

There are several studies demonstrating GYKI 52466 to be an effective neuroprotectant in vivo (Arvin et al., 1992; LePeillet et al., 1992; Xue et al., 1994). Only May and Robison (1993) examined, however, the neuroprotective effect of GYKI 52466 in primary cultures of neurons. They used hippocampal cultures and kainate as neurotoxin. Here we demonstrated GYKI 52466 to be

an effective neuroprotective compound against quisqualate excitotoxicity in mature cultures from rat telencephalon. The sensitivity of the two culture systems to excitotoxins is significantly different despite the similar in vitro age (14-20 and 17-20 days in the case of hippocampal and telencephalic cultures, respectively). In the hippocampal cultures, used by May and Robison (1993), 500 μM kainate was needed to achieve the near maximal neuronal cell death. In our culture system 10 μM quisqualate (Fig. 1), 20 μM AMPA (Fig. 4) or 40μM kainate (data not shown) was sufficient for maximal neuronal degeneration.

This may partly be a consequence of co-culturing the cortical, striatal and hippocampal neurons in our telencephalic cultures. Galarraga et al. (1990) demonstrated that quinolinate and kainate neurotoxicity in neostriatal cultures is potentiated by co-culturing with neocortical neurons Another cause of the increased sensitivity may be the 25 mM KCl present in the medium during the first week after plating. The dramatic effect of K^+ concentration on the expression of AMPA receptor subunits was reported recently (Condorelli et al., 1993).

Quisqualate activates not only AMPA/kainate receptors but also the polyphosphoinositide hydrolysis coupled metabotropic glutamate receptors (Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995). Activation of this type of metabotropic glutamate receptor results mobilization of calcium from intracellular stores and this might contribute to the neurotoxic effect of quisqualate, because the highly increased level of intracellular calcium plays a crucial role in the excitotoxic cell death (Bondy and Lebel, 1993; Coyle and Puttfarcken, 1993). CNQX and GYKI 52466, however, both are AM:PA/kainate receptor antagonists, give complete neuroprotection against quisqualate in our primary cultures (see Fig. 1), indicating that solely activation of quisqualate sensitive metabotropic glutamate receptor does not cause neurotoxicity, at least in our culture conditions. This presumption was supported by experiments in which the dose-response curves for GYKI 52466 neuroprotection were determined against quisqualate and AM:PA excitotoxicity (Fig. 4). The two curves did not differ significantly. Recently interest has turned to the research of new AMPA/kainate receptor antagonists as therapeutic agents for treatment of several types of brain insults, including hypoxia, ischemia and hypoglycemia, and age related neurodegenerative disorders, such as Parkinson's disease and Huntington's disease (Gill, 1994). The AMPA/kainate antagonist GYKI 52466 can serve as starting point for molecular design and synthesis of new 2,3-benzodiazepines with improved

neuroprotective properties. The mature telencephalic cultures described in this report are suitable for selecting the effective compounds.

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REFERENCES

Akaike, A., Kaneko, S., Tamura, Y., Nakata, N., Shiomi, H., Ushikubi, F. and Narumiya, S. (1994) Prostaglandin E2 protects cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. Brain Res. 663, 237-243.

Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA 90, 7915-7922.

Arvin, B., Moncada, C., LePeillet, E., Chapman, A. and Meldrum, B.S. (1992) GYKI 52466 blocks the increase in extracellular glutamate induced by ischemia. Neuroreport 3, 235238.

Bondy, S.C. and Lebel, C.P. (1993) The relationship between excitotoxicity and oxidative stress in the central nervous system. Free Radic. Biol. Med. 14, 633-642.

Cazevieille, C., Muller, A., Meynier, F., Dutrait, N. and Bonne, C. (1994) Protection by prostaglandins from glutamate toxicity in cortical neurons. Neurochem. Int. 24, 395-398.

Cheng, B. and Mattson, M.P. (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. Brain Res. 640, 56-67.

Condorelli, D.F., Dell'Albani, P., Aronica, E., Genazzani, A.A., Casabona, G., Corsaro, M., Balázs, R. and Nicoletti, F. (1993) Growth conditions differentially regulate the expression of α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor subunits in cultured neurons. J. Neurochem. 61, 2133-2139.

Coyle, J.T. and Puttfarcken, P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262, 689-695.

DeCoster, M.A., Klette, K.L., Knight, E.S. and Tortella, F.C. (1995) α–receptor-mediated neuroprotection against glutamate toxicity in primary rat neuronal cultures. Brain Res. 671, 45-53.

Driscoll, B.F., Law, M.J. and Crane, A.M. (1991) Cell damage associated with changing the medium of mesencephalic cultures in serum-free medium is mediated via N-methyl-D-aspartate receptors. J. Neurochem. 56, 1201-1206.

Driscoll, B.F., Deibler, G.E., Law, M.J. and Crane, A.M. (1993) Damage to neurons in culture following medium change: role of glutamine and extracellular generation of glutamate. J. Neurochem. 61, 1795-1800.

Galarraga, E., Surmeier, D.J. and Kitai, S.T. (1990) Quinolinate and kainate neurotoxicity in neostriatal cultures is potentiated by co-culturing with neocortical neurons. Brain Res. 512, 269-276.

Gill, R. (1994) The pharmacology of α–amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate antagonists and their role in cerebral ischemia. Cerebrovasc. Brain Metab. Rev. 6, 225-256.

Hajimohammadreza, I., Probert, A.W., Coughenour, L.L., Borosky, S.A., Marcoux, F.W., Boxer, P.A. and Wang, K.K.W. (1995) A specific inhibitor of calcium/calmodulin-dependent protein kinase-II provides neuroprotection against NMDA- and hypoxia/hypoglycemia-induced cell death. J. Neurosci. 15, 4093-4101.

Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31-108.

Keilhoff, G. and Erdõ, S.L. (1991) Parallel development of excitotoxic vulnerability to N-methyl-D-aspartate and kainate in dispersed cultures of the rat cerebral cortex. Neuroscience 43, 35-40.

Koh, J.-Y. and Choi, D.W. (1987) Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. J. Neurosci. Methods 20, 83-90.

Koh, J.-Y., Goldberg, M.P., Hartley, D.M. and Choi, D.W. (1990) Non-NMDA receptor mediated neurotoxicity in cortical culture. J. Neurosci. 10, 693-705.

Lakics, V., Sebestyén, M.G. and Erdõ, S.L. (1995) Vinpocetine is a highly potent neuroprotectant against veratridine-induced cell death in primary cultures of rat cerebral cortex. Neurosci. Lett. 185, 127-130.

LePeillet, E., Arvin, B., Moncada, C. and Meldrum, B.S. (1992) The non-NMDA antagonists, N13QX and GYKI 52466, protect against cortical and striatal cell loss following transient global ischemia in the rat. Brain Res. 571, 115-120.

Lynch, J.J., Yu, S.P., Canzoniero, L.M.T., Sensi, S.L. and Choi, D.W. (1995) Sodium channel blockers reduce oxygen-glucose deprivation-induced

cortical neuronal injury when combined with glutamate receptor antagonist. J. Pharmacol. Exp. Ther. 273, 554-560.

Madarász, E., Kiss, J. and Bartók, I. (1984) Cell production and morphological pattern formation in primary brain cell cultures. I. Pattern formation within the basal layer(s). Brain. Res. 304, 339-349.

May, G.R., Rowand, W.S., McCormick, J.G. and Sheridan, R.D. (1995) Neuroprotective profile of lifarazine (RS-87476) in rat cerebrocortical neurons in culture. Br. J. Pharmacol. 114, 1365- 1370.

May, P.C. and Robison, P.M. (1993) GYKI 52466 protects against non-NMDA receptor-mediated excitotoxicity in primary rat hippocampal cultures. Neurosci. Lett. 152, 169-172.

Murphy, T.H., Schnaar, R.L. and Coyle, J.T. (1990) Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystin uptake. FASEB J. 4, 1624-1633.

Okamoto, M., Mori, S. and Endo, H. (1994) A protective action of chondroitin sulfate proteoglycans against neuronal cell death induced by glutamate. Brain Res. 637, 57-67.

Parsons, C.G., Gruner, R. and Rozental, J. (1994) Comparative patch clamp studies on the kinetics and selectivity of glutamate receptor antagonism by 2,3-dihydroxy-6-nitro-7sulfamoyl-benzo(F)quinoxaline (NBQX) and 1-(4-amino-phenyl)-4-methyl-7,8methylendioxyl-5H-2,3-benzodiazepine (GYKI 52466). Neuropharmacology 33, 589-604.

Pin, J.-P. and Duvoisin, R. (1995) Review. Neurotransmitter receptors I. The metabotropic glutamate receptors: structure and function. Neuropharmacology 34, 1-26.

Prehn, J.H.M., Lippert, K. and Krieglstein, J. (1995) Are NMDA or AMPA/kainate receptor antagonists more efficacious in delayed treatment of excitotoxic neuronal injury? Eur. J. Pharmacol. 292, 179-189.

Priestley, T., Horne, A.L., McKernan, R.M. and Kemp, J.A. (1990) The effect of NMDA receptor glycine site antagonists on hypoxia-induced neurodegeneration of rat cortical cell cultures. Brain Res. 531, 183-188.

Rosenberg, P.A. (1991) Accumulation of extracellular glutamate and neuronal death in astrocyte-poor cortical cultures exposed to glutamine. Glia 4, 91-100.

Tarnawa, I., Engberg, I. and Flatman, J.A. (1990) GYKI 52466, an inhibitor of spinal reflexes is a potent quisqualate antagonist. In: Amino acids:

Chemistry, Biology and Medicine (eds. Lubec, G. and Rosenthal, G.A.) pp. 538-546. Escom, Leiden.

Weller, M., Finiels-Marlier, F. and Paul, S.M. (1993) NMDA receptor-mediated glutamate toxicity of cultured cerebellar, cortical and mesencephalic neurons: neuroprotective properties of amantadine and memantine. Brain Res. 613, 143-148.

Xue, D., Huang, Z.G., Barnes, K., Lesiuk, H.J., Smith, K.E. and Buchan, A.M. (1994) Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. J. Cereb. Blood Flow Metab. 14, 251-261.

Zinkand, W.C., DeFeo, P.A., Thompson, C., Hargrove, H., Salama, A.I. and Patel, J. (1992) Quisqualate neurotoxicity in rat cortical cultures: pharmacology and mechanisms. Eur. J. Pharmacol. 212, 129-136.

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Research report

IMMUNOHISTOCHEMICAL LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE IN THE TERETE NUCLEUS OF THE RAT HYPOTHALAMUS

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Summary: The topographical distribution of calcitonin gene-related peptide (CGRP)containing neuronal elements in the posterior hypothalamus has been re-examined by means of indirect labeled immunohistochemistry in the rat. In contrast to previous findings, no CGRP-like immunoreactive neuronal perikarya were present in the premamillary nuclei, but they form a small group of cells, which is identical to the recently described terete nucleus. In coronal sections, this small and rounded cluster of intermingled CGRP-immunopositive perikarya and fibers is situated in the lateral hypothalamus at the premamillary level, ventrolateral to the fornix. Perikarya, dendrites, presynaptic terminals, as well as nonmyelinated and some myelinated axons were labeled by CGRP-like immunoreactive material within and in the immediate vicinity of the terete nucleus. On some of the CGRP-positive neurons, neuropeptide Y-immunoreactive axon terminals establish synaptic contacts.

Keywords: Terete nucleus; CGRP; Immunohistochemistry; Posterior hypothalamus

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INTRODUCTION

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide encoded and generated by alternative RNA processing of the calcitonin gene (Amara et al., 1982; Rosenfeld et al., 1983). Previous studies have provided evidence that CGRP may act as a neurotransmitter and/or neuromodulator in the central and peripherial nervous system (Dennis et al., 1990; Wackym et al., 1991; Drumheller et al., 1992), and exerts profound effect on central cardiovascular control (Nguyen et al., 1986) CGRP-like immunoreactivity has been shown to be widely distributed in the brain (Rosenfeld et al., 1983; Skofitsch and Jacobowitz, 1985; Kruger et al., 1988a,b). In the hypothalamus CGRP has been found in neuronal perikarya of the medial preoptic, periventricular, anterior hypothalamic and ventral premamillary nuclei, as well as the perifornical and lateral hypothalamic areas (Kawai et al., 1985; Skofitsch and Jacobowitz, 1985; Kruger et al., 1988a). The terete nucleus is a "smooth, cylindrical" group of small neurons in the premamillary region of the posterior hypothalamus. It is less discernible in Nissl preparations, but its high content of zinc makes it easily identifiable on sections stained with Timm's sulfide-silver method (Timm, 1958). This infrequent trait helped Paxinos and Danscher to recognize and describe it as a separate hypothalamic nucleus in 1986 (Paxinos and Watson, 1986). No comparative neuroanatomical study or data has been published about this tiny nucleus so far. However, most probably it may correspond to one of the lateral tuberal nuclei of the primate hypothalamus (Haymaker et al., 1969). In the rat, the terete nucleus extends rostrocaudally from 3,200 μm to 4,200 μm behind the plane of the bregma. In coronal sections, the terete nucleus is visible as a rounded neuronal aggregation with an average diameter of 200-250 μm. Its rostral half is bordered medially by the medial tuberal nucleus (Bleier and Byne, 1985; Paxinos and Watson, 1986) and, more caudally, by the ventral premamillary nucleus. Dorsally it is contiguous with the lateral hypothalamic area (wherein it is actually "embedded"), while ventrally cells of the terete nucleus almost reach the basal surface of the hypothalamus. At the caudalmost part, the terete nucleus becomes neighbouring with the lateral subdivision of the tuberomamillary nucleus. The chemical character of the neuronal structures of the terete nucleus is barely known, although a few authors described CGRP-immunoreactive (ir) perikarya within the same hypothalamic area (Kawai et

al., 1985; Skofitsch and Jacobowitz, 1985; Kruger et al., 1988a) without recognizing the entity of this small cell group.

MATERIALS AND METHODS

Eight male Wistar-Kyoto rats (weight 300±10 g) were injected with colchicine (150 g in 15 µl saline; Sigma) intracerebroventricularly under deep anesthesia (80 mg/kg b.w. ketamin and 16 mg/kg b.w. xylazin i.m.). Then, 48 hours later the animals were perfused transcardially with 50 ml saline, followed by 500 ml Zamboni's fixative (4% paraformaldehyde, 0.19% picric acid in 0.1 M phosphate buffer (PB, pH 7.35). For electron microscopy, 0.1% glutaraldehyde was added to the fixative. The brains were removed from the skull, and postfixed overnight in glutaraldehyde-free fixative solution.

Light microscopic immunohistochemistry.

Blocks containing the hypothalamus were immersed in 15% sucrose for cryoprotection until they sank. Coronal sections, 50 µm thick, were cut on a freezing microtome, washed with 0.1 M PB and processed for immunohistochemistry. The free-floating sections were permeabilized with 0.5% Triton X-100 containing PB overnight, at 4°C. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PB for 10 minutes. The nonspecific binding sites were blocked with 10% normal goat serum for 1 hour. The sections were incubated in the primary antiserum against CGRP (working dilution 1:20,000) for 48 hours at 4°C with continuous agitation. For immunostaining the avidin-biotin-peroxidase complex (ABC) technique (Hsu et al., 1981) was employed. Briefly, the sections were incubated with secondary antibody (biotinylated antirabbit IgG; Vector Lab.) diluted at 1:500, overnight at 4°C, then with ABC, diluted at 1:250, for 2 hours at room temperature. The primary and secondary antibodies were diluted with PB containing 1% normal goat serum and 0.1% sodium azide. The ABC was diluted only with PB. Between incubation steps, sections were washed 3 times 15 minutes, each in PB. The tissue-bound peroxidase activity was visualized with nickel-intensified diaminobenzidine (DAB) chromogen reaction (0.025% 3,3'-diamino-benzidine, 0.3% nickel-ammoniumsulphate, 0.0015% H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.5) for 5 to 25 minutes, at room temperature.

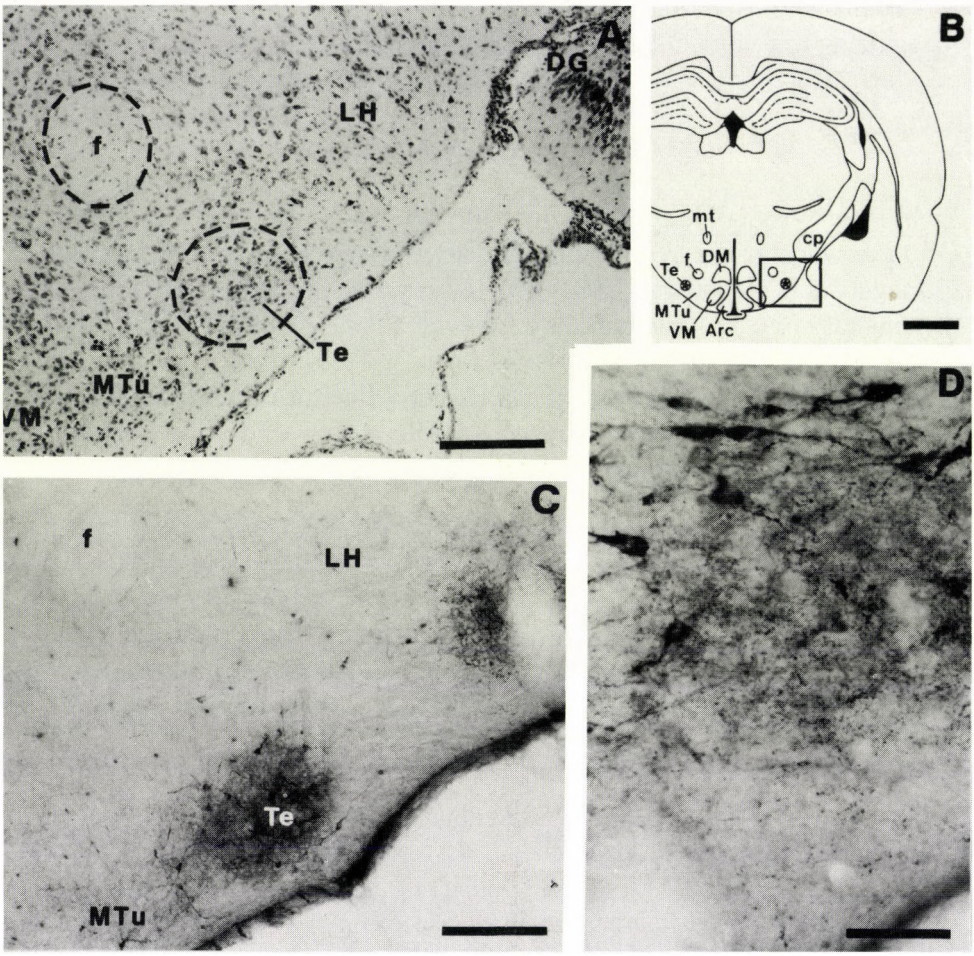


Fig. 1. A: Nissl-stained coronal section of the rat brain at 3,600 μm caudal to the bregma level showing the ventrolateral part of the posterior hypothalamus (framed area on B). B: Schematic drawing illustrates the regional topography. Modified after Paxinos and Watson (1986). C: Light microscopic immunolabeling for CGRP in the area corresponding to A. The CGRP-ir perikarya and nerve fibers are conspicuously clustered in the terete nucleus (Te) practically delineating its round shape. Scattered CGRP-positive cell bodies and fibers are visible in the medial tuberal nucleus (MTu) as well. D: CGRP-ir neuronal elements in the terete nucleus (high magnification). Abbreviations: Arc, arcuate hypothalamic nucleus; cp, cerebral peduncle; DG, dentate gyrus; DM, dorsomedial hypothalamic nucleus; f, fornix; LH, lateral hypothalamic area; mt, mamillothalamic tract; VM, ventromedial hypothalamic nucleus; Scale bars: A,C = 200 μm,B = 2 mm,D = 50 μm.

Sections were mounted on gelatinized slides, air dried, cleared with toluene and coverslipped with Cytoseal mounting medium (Stephens Scientific).

Electron microscopic immunocytochemistry: double labeling

Fifty μm thick coronal sections were cut on a vibratome. They were washed in PB, transferred to 10, 20 and 30% sucrose containing saline (30 minutes in each solution), and freeze-thawed to enhance the penetration of the antibodies. Then, the sections were washed 3 times 15 minutes in PB and immunostained with the first antiserum (CGRP, working dilution 1:20,000) similarly as described above. Tissue bound peroxidase was visualized with DAB chromogen reaction (without nickel-ammoniumsulphate). Sections were washed 3 times 15 minutes in PB, and treated with 8% thioglicolic acid for 3 hours at room temperature. Then, the silver-gold intensificatlon was carried out, as reported previously (Görcs et al., 1986b; Van den Pol and Görcs, 1986). In the second labeling step, the silver-gold-intensified CGRP-ir sections were selected and immunostained for NPY (with NPY-antiserum, working dilution 1:32,000) using the same protocol desribed above, and the end product (DAB) was not intensified. The double labeled sections were postosmicated (1% OsO4 in PB forl hour), dehydrated through graded ethanol (70% ethanol contained 1% uranyl acetate, 30 minutes), and finally flat-embedded in Durcupan. Ultrathin sections were cut on an ultramicrotom (Ultracut E, Reichert), mounted onto single slot grids, contrasted with lead citrate and examined using a JEOL 1200 EX electron microscope.

Antisera specificity and controls

The CGRP- and NPY-antisera were generated against the representative synthetic peptides coupled to bovine thyroglobulin (Sigma Type I) with glutaraldehyde as a cross-linking reagent in mixed breed rabbits. Characterization of these antisera wlth enzyme-linked immunosorbent assay and immunohistochemistry was performed as published previously (Görcs et al., 1986a; Görcs et al., 1988; Csiffáry et al., 1990; Baffi et al., 1992). With ELISA the CGRP antibody did not crossreact with CCK or the other peptides tested (Baffi et al., 1992). Preabsorption with 8 μg CGRP resulted in complete disappearance of immunolabeling, while similar preabsorption with other peptides (50 μg each) failed to have any such effect. The NPY antibody, with ELISA gave 90% cross-reactivity with avian pancreatic polypeptide.

Preabsorption with 10 μg NPY, or 15 μg APP resulted in complete disappearance of immunolabeling. Similar preabsorption with other peptides (50 μg each) had no effect on the intensity of immunolabeling. In control experiments the primary antisera were omitted, replaced with normal rabbit serum, or preadsorbed of their working dilutions with synthetic CGRP or NPY, respectively (see above). All the three tests resulted in complete disappearance of immunoreactivity.

RESULTS

Light microscopy

The distribution of CGRP-ir neuronal structures was examined at six equidistant (approx. 150 μm) rostro-caudal levels. A moderate number of CGRP immunolabeled perikarya was found in the posterior hypothalamus. Most of these cells apparently grouped within a circumscribed area in the lateral hypothalamus, ventrolaterally to the fornix known as *terete nucleus* (Fig. 1A,C). They were small to medium in size, fusiform or polymorphic, and possessed two to five well-stained dendrites (Fig. 1D). The density of CGRP-immunopositive cell bodies (and fibers) was consistent throughout the entire nucleus. In addition to these, some CGRP-ir neurons were scattered around the terete nucleus in the lateral hypothalamic area, especially in the medial tuberal nucleus (Fig. 1C) and in the caudal portion of the arcuate nucleus, which extends into the premamillary area.

The CGRP-ir neuronal fibers formed a dense cluster in the terete nucleus (Fig. 1C), which virtually adumbrated its shape and borders. They appeared mainly as small puncta or pieces of varicous axons intermingled with large dendritic fragments (Fig. 1D). CGRP-immunoreactive fibers and terminals were considerably less dense around the nucleus; only a few of them were disseminated in the medial tuberal, tuberomamillary, and ventral premamillary nuclei, and in the lateral hypothalamic area (Fig. 1C).

NPY-ir fibers, but no perikarya were scattered in the territory of the terete nucleus. The density of NPY-ir fibers was far less than that in the structures medial to the terete nucleus: in the arcuate nucleus and the postinfundibular median eminence (Lantos et al., 1995).

Electron microscopy

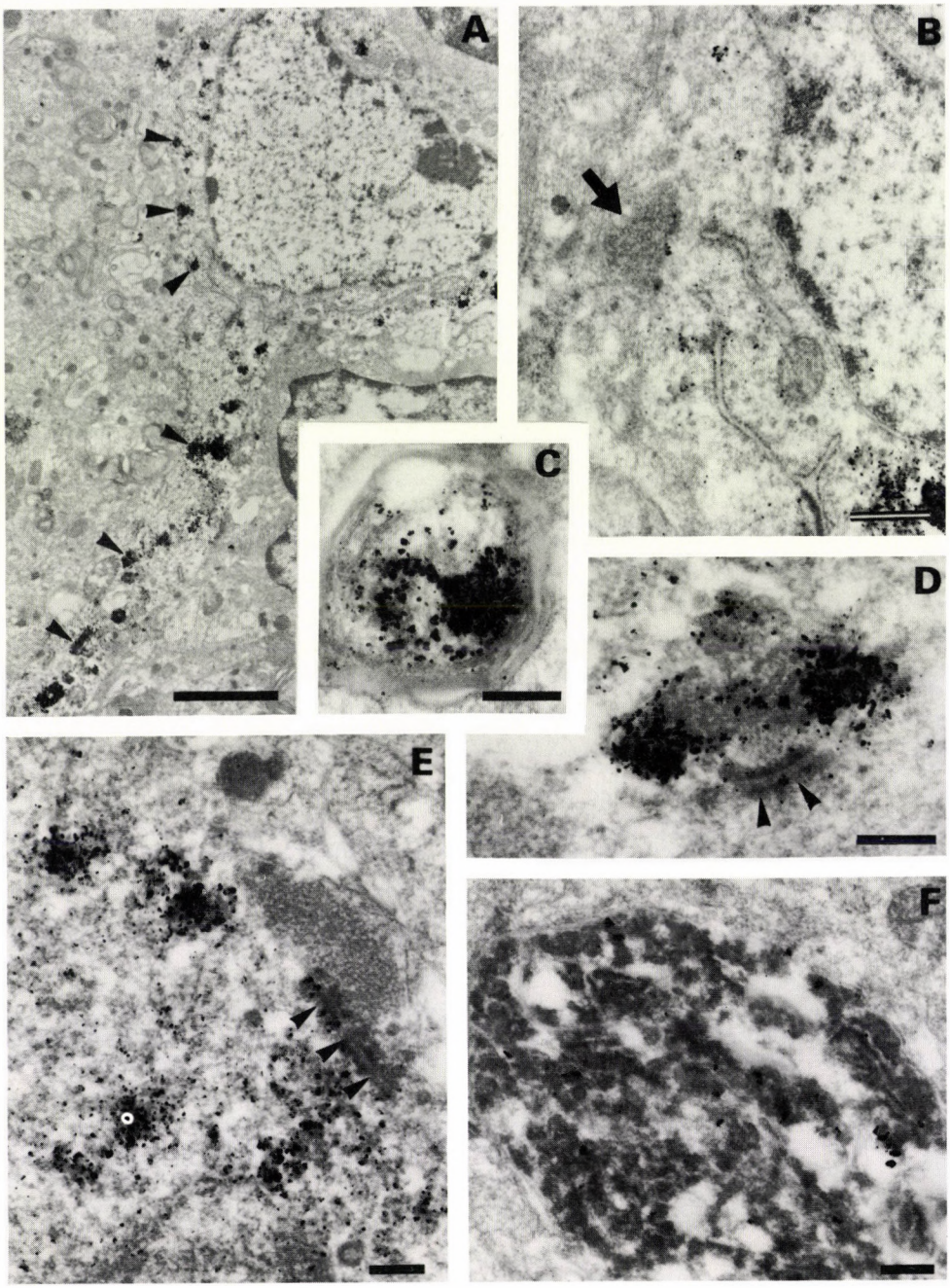
Silver-gold-labeled CGRP-ir particles were found in neuronal elements of the terete nucleus. Immunopositivity was localized in the cytoplasm of perikarya, dendrites (Fig. 2A,B,E), and nerve terminals (Fig. 2D). Immunolabeled nonmyelinated axons occurred in a fairly high number in the nucleus while some immunolabeled myelinated axons were visible in the immediate vicinity of the terete nucleus (Fig. 2C). NPY-immunopositivity was observable only in nerve terminals (Fig. 2E), some of them in synaptic contact with CGRP-immunopositive perikarya (Fig. 2E).

DISCUSSION

The present results are seemingly in disagreement with previous observations which localized CGRP-ir perikarya in the posterior hypothalamus. Kawai and his colleagues described CGRP-ir perikarya "at the most basal region" of the lateral hypothalamus (Kawai et al., 1985), while others in the ventral premamillary (Skofitsch and Jacobowitz, 1985) or in the "medial premammillary nucleus" (Kruger et al., 1988a). It is most probably, that these authors had no knowledge of the existence of the terete nucleus, which was described as a separate cell growp only in 1986 (Paxinos and Watson, 1986). According to the given topographical coordinates of CGRP-ir neurons in the premamillary region, the above mentioned authors described but not recognized the CGRP-ir terete nucleus. In the human hypothalamus, the only concerning report (Takahashi et al., 1989) failed to detect CGRP-ir perikarya

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Fig. 2. Ultrastructural characteristics of CGRP-ir neuronal elements in the terete nucleus from double immunolabeled material. A: Low-power electron micrograph showing a CGRP-positive (silver-gold-intensified DAB) neuron. Silver-gold particles are distributed throughout the cell body and dendrite (arrowheads). B: Unlabeled axon terminal (arrow) in close association with a CGRP-positive perikaryon. C: CGRP-positive myelinated axon. D: CGRP-positive axon termlinal form symmetrical synaptic contact (arrowheads) with an unlabeled neuronal element. E: Symmetrical synaptic contact (arrowheads) between NPY-positive (non-intensified DAB) axon terminal and CGRP-positive perikaryon. F: CGRP-immunopositive axonal profile. Note the scattered silver-gold particles over the DAB labeling. Scale bars: A = 2 μm, B = 500 nm, C,D,E,F= 200 nm


A
B
C
D
E
F

in the corresponding area (the existence of the terete nucleus in the human brain has not been documented, yet), but some labeled perikarya and neuronal fibers were found in this part of the posterior hypothalamus (Lantos et al. unpublished observation). CGRP-like immunoreactivity was seen in both pre- and postsynaptic sites. The synaptic boutons may represent the existence of intranuclear CGRP-positive connections, but considering the CGRP-ir neurons scattered throughout the posterior hypothalamus, the extranuclear origin of CGRP-ir terminals cannot be excluded in the terete nucleus. A fraction of immunolabeled axon terminals was NPY-positive. The NPY-ir nerve fibers could be of either hypothalamic – arcuate nucleus, (Bai et al., 1985; Lantos et al., 1995), or extrahypothalamic – brainstem, but not local origin. Inside the terete nucleus no NPY-ir perikarya were detected (Lantos et al., 1995).

The possible functional significance of the presence of CGRP in the terete nucleus awaits further investigations. Neither the projections of these cells, nor their neuronal inputs are known. Neurons in the premamillary region in general, have no direct projection to the median eminence or the posterior pituitary (Lechan et al., 1982; Canteras et al., 1992), thus the participation of the terete nucleus in neuroendocrine regulation is very unlikely. The close association of this nucleus with the lateral hypothalamus tempts to hypothesize a possible relay function for long ascending or descending signals along the forebrain-brainstem pathways.

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REFERENCES

Amara, S.G., Jonas, V., Rosenfeld, M.G., Ong, E.S. and Evans, R.M. (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 298, 240-244.

Baffi, J., Görcs, T., Slovik, F., Horváth, M., Lekka, N., Pásztor, E. and Palkovits, M. (1992) Neuropeptides in the human superior cervical ganglion. Brain Res. 570, 272-278.

Bai, F.L., Yamano, M., Shiotani, Y., Emson, P.C., Smith, A.D., Powell, J.F. and Tohyama, M. (1985) An arcuato-paraventricular and dorsomedial

hypothalamic neuropeptide Y-containing system which lacks norepinephrine in the rat. Brain Res. 331, 172-175.

Bleier, R. and Byne, W. (1985) Septum and hypothalamus. In The rat nervous system (ed. Paxinos, G.) Vol. 1, Academic Press, Sydney.

Canteras N. S., Simerly, R. B. and Swanson, L. W. (1992) Projections of the ventral premammillary nucleus, J. Comp. Neurol., 324, 195-212.

Csiffáry, A., Görcs, T.J. and Palkovits, M. (1990) Neuropeptide Y innervation of ACTH immunoreactive neurons in the arcuate nucleus of rats: a correlated light and electron microscopic double immunolabeling study. Brain Res. 506, 215-222.

Dennis, T., Fournier, A., Cadieux, A., Pomerleau, F., Jolicoeur, F.B., St. Pierre, S. and Quirion, R. (1990) hCGRPg-27, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related peptide receptor heterogeneity in brain and periphery. J. Pharmacol. Exp. Ther. 254, 123-128.

Drumheller, A., Menard, D., Fournier, A. and Jolicoeur, F.B. (1992) Neurochemical effects of CGRP, Ann. N. Y. Acad. Sci. 657, 546-548.

Görcs, T.J., Gottschall, P. Cov. D. and Arimura, A. (1986a) Possible recognition of the GnRH receptor by an antiserum against peptide encoded by RNA sequences complementary to mRNA of a GnRH precursor peptide. Peptides 7, 1137-1145.

Görcs, T.J., Leránth, Cs. and MacLusky, N.J. (1986b) The use of gold-substituted silver-intensified diaminobenzidine (DAB) and non-intensified DAB for simultaneous electron microscopic immunoperoxidase labeling of thyrosine hydroxilase and glutamic acid decarboxilase immunoreactivity in the rat medial preoptic area. J. Histochem., Cytochem. 34, 1439-1447.

Görcs, T.J., Gottschall, P.E., Arimura, A. and Coy, D.H. (1988) Possible prediction of receptor like peptides from peptide hormone DNA sequences? In Peptides Chemistry, Biology, Interactions with Proteins (ed. Penke B. and Török A.) de Gruyter, Berlin. pp. 149-156.

Haymaker, W., Anderson, E. and Nauta, W.J.H. (1969) The Hypothalamus. Charles C. Thomas Publisher, Springfield, Illinois, U.S.A.

Hsu, S-M., Raine, L. and Fager, H. (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Kawai, Y., Takami, K., Shiosaka, S., Emson, P.C., Hillyard, C.J., Girgis, S., MacIntyre, I. and Tohyama, M. (1985) Topographic localization of calcitonin gene-related peptide in the rat brain: an immunohistochemical analysis. Neuroscience 15, 747-763.

Kruger, L., Mantyh, P.W., Sternini, C., Brecha, N.C. and Mantyh, C.R. (1988a) Calcitonin gene-related peptide (CGRP) in the rat central nervous system: pattern of immunoreactivity and receptor binding sites. Brain Res. 463, 223-244.

Kruger, L., Sternini, C., Brecha, N.C. and Mantyh, P.W. (1988b) Distribution of calcitonin gene-related peptide immunoreactivity in relation to the rat central somatosensory projections. J. Comp. Neurol. 273, 147-162.

Lantos, T.A., Görcs, T.J. and Palkovits, M. (1995) Immunohistochemical mapping of neuropeptides in the premamillary region of the hypothalamus in rats. Brain Res. Rev. 20, 209-249.

Lechan, R.M., Nestler, J.L. and Jacobson, S. (1982) The tuberoinfundibular system of the rat as demonstrated by immunohistochemical localization of retrogradely transported wheat germ agglutinin (WGA) from the median eminence. Brain Res. 245, 1-15.

Nguyen, K.Q., Sills, M.A. and Jacobowitz, D.M. (1986) Cardiovascular effects produced by microinjection of calcitonin gene-related peptide into the rat central amygdaloid nucleus. Peptides 7, 337-339.

Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotaxic Coordinates, 2nd edition. Academic Press, New York.

Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W. and Evans, R.M. (1983) Production of novel neuropeptide encoded by the calcitonin gene via tissue specific RNA processing. Nature 304, 59-72., 129-135.

Skofitsch, G. and Jacobowitz, D.M. (1985) Calcitonin gene-related peptide: detailed immunohistochemical distribution in the central nervous system. Peptides 6, 721-745.

Takahashi, K., Mouri, T., Sone, M., Murakami, O., Itoi, K., Imai, Y., Ohneda, M., Yoshinaga, K. and Sasano, N. (1989) Calcitonin gene-related peptide in the human hypothalamus. Endocrinol. Japon. 36, 409-415.

Timm, F. (1958) Zur Histochemie des Ammonshornsgebietes. Z. Zellforsch. 48, 548-555.

Van den Pol, A.N. and Görcs, T.J. (1986) Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptide, vasoactive intestinal polypeptide, and glutamate decarboxilase immunoreactivity in

the suprachiasmatic nucleus: dual ultrastructural immunocytochemistry with gold-substituted silver peroxidase. J. Comp. Neurol. 252, 507-521.

Wackym, P.A., Popper, P., Ward, P.H. and Micevych, P.E. (1991) Cell and molecular anatomy of nicotinic acetylcholine receptor subunits and calcitonin gene-related peptide in the rat verstibular system. Otolaryngol. Head neck Surg. 105, 493-510.

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Research report

C-FOS EXPRESSION IN THE BRAINSTEM AFTER VOLUNTARY INGESTION OF SUCROSE IN THE RAT

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Summary: Neurons showing activity after sweet taste stimulation, were detected with *c-fos* immunocytochemistry, to identify brainstem nuclei involved in processing taste information. The distribution of the evoked expression of *c-fos* was visualized in the rat brainstem after voluntary ingestion of sucrose.

Sucrose-taste-specific *c-fos* immunoreactive neurons were found within the medial part of the nucleus of the solitary tract, the dorsal motor nucleus of the vagus at caudal levels, the paratrigeminal nucleus and the lateral and medial part of the parabrachial nucleus. These nuclei are known to be involved in the processing of information related to ingestive behavior such as information about taste quality. The present study has shown that *c-fos* is a useful anatomical marker for activated neurons in the rat brainstem, and that the above-mentioned brainstem areas are involved in the processing of sweet taste information.

Keywords: sweet taste-stimulation, *c-fos* expression, brainstem, NTS, rat

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INTRODUCTION

Taste information may alert the body for the arrival of food. Various organs show an anticipatory response, such as an endocrine or exocrine secretion. An example is the vagally mediated preabsorptive insulin response (PIR) by the endocrine pancreas (Strubbe and Steffens, 1975; Steffens, 1976; Grill et al., 1984; Flynn et al., 1986; Berthoud and Powley, 1990). To study the role of taste information eliciting the PIR, we first tried to trace pathways that convey taste information to the endocrine pancreas. Descending vagal projections from the rostral NTS (rNTS) to the endocrine pancreas were traced, using a retrograde transneuronal viral tracing technique (Streefland et al., 1994). Results suggested that the intermediate and/or caudal NTS or the medullary reticular formation form part of the pathway from the rNTS to the medial dorsal motor nucleus of the vagus (DMnX), which in turn projects to the pancreas. A direct projection from the rNTS to the DMnX was not substantiated by experimental findings.

In this study, we use *c-fos* immunocytochemistry to detect sweet taste-sensitive neurons in the brainstem. The proto-oncogene *c-fos* belongs to a large class of genes, referred to as cellular immediate early genes (IEG's) (Sheng and Greenburg, 1990; Morgan and Curran, 1989, 1991), which are induced rapidly and transiently by extracellular stimuli. Its protein product Fos is induced in transsynaptically activated neurons (Sagar et al., 1988). Several studies have demonstrated a coincidence between Fos-like immunoreactivity and (^{14}C)-2-deoxyglucose uptake (Sagar et al., 1988; Sharp et al., 1989), thus suggesting a tight correlation between neuronal activity and the expression of *c-fos*. The effects of several types of stimulation on *c-fos* expression have been studied, including electrical (Sandner et al., 1992; Krukoff et al., 1992; Sheng et al., 1993; Yousfi-Malki and Puizillout, 1994), pharmacological (Messina et al., 1992; Nakagawa et al., 1992; Olson et al., 1992) and sensory stimulation (Hunt et al., 1987; Menetrey et al., 1989; Sagar and Sharp, 1990; Onoda, 1992). Taste-induced *c-fos* expression was observed in several brain areas of the rat by Yamamoto et al. (1991,1993). Furthermore, Fos-like immunoreactivity was correlated with conditioned taste aversion (Houpt et al., 1994) and altered food intake (Olson et al., 1993). We investigated *c-fos* expression in brainstem nuclei after voluntary ingestion of a sucrose solution in unanesthetized rats, to elucidate efferent projections conveying gustatory information.

MATERIALS AND METHODS

All procedures used in this study were approved by the Committee on Animal Bioethics of the University of Groningen.

Experimental design

Twenty male Wistar rats of our own breeding (± 250 g) were divided into five groups (Al, A2, B, C and D, four animals per group) and housed in pairs on a reversed 12 h light/dark cycle. Lights were on between 21.00 h and 9.00 h. During one week, the animals received ad libitum water and were food-deprived, except for the first two hours of the dark period. In these hours the rats in groups A1, A2, B and C were trained to drink a 1 M sucrose solution and subsequently to eat standard rat chow (5 g per animal, Hope Farms, NL) as a supplement to the sucrose diet. Animals in group D received standard rat chow during these two hours. Every day, the weights of the rats and the amount of liquid they drank were determined.

After one week training the final feeding session took place during the first 90 minutes of the dark period. Rats in group A received a 1 M sucrose solution, rats in group B water, rats in groups C and D received neither water nor sucrose.

Immunocytochemical procedure

After the 90 min test period the rats were deeply anaesthetized with sodium pentobarbital (i.p., 270 mg/kg) and rats in groups Al, B, C and D were perfused transcardially with 400 ml of fixative (3.8% Borax, 4% paraformaldehyde in distilled water, pH 9.5), preceded by a short prerinse with heparinized saline. Rats in group A2 were perfused with 400 ml of a 4% paraformaldehyde fixative (in phosphate buffer (PB), pH 7.4). Two fixatives were used to allow different immunocytochemical stainings afterwards brains were removed and cryoprotected by overnight storage at 4°C in 30% sucrose in 0.1 M PB. Transverse sections were cut at 40 µm thickness on a cryostat microtome. The sections were collected in 0.1 M PB.

c-fos was visualized with an imrnunocytochemical avidin-biotin staining technique. Prior to the first antibody incubation, sections were immersed for 10 min in 0.3% H_2O_2 in 0.0l M phosphate buffered saline (PBS) to exhaust

endogenous peroxidase activity. The sections were then incubated for 1 h at room temperature (RT) in 5% normal rabbit serum (NRS) to suppress non-specific antibody binding. Thereafter the sections were incubated for 72 h at 4°C in a sheep-anti-Fos primary antibody solution (1:2000; Cambridge Research Biochemicals, UK). Subsequently, the sections were incubated in 3% NRS for 1 h: at RT, in a rabbit-anti-sheep IgG biotinylated secondary antibody solution (1:800; Zymed, San Francisco, USA) for 90 min at RT and in the ABC complex (Vectastain Elite kit, NL) for 90 min at RT. The secondary antibody solution and the ABC solution were used again to repeat the latter two steps for 30 min each. The primary and secondary antibodies were diluted in PBS, containing 1% NRS and 0.3% triton-X100. Between all steps, the sections were rinsed thoroughly with 0.0l M PBS. Finally the sections were rinsed in 0.l M Na-Acetate (pH 6.0) and processed by the diaminobenzidine (DAB)-H_2O_2 reaction (30 mg DAB, 0.03% nickel ammoniumsulphate and 0.01 % H_2O_2/100 ml 0.lM NaAcetate, pH 6.0).

After the immunocytochemical staining the sections were mounted, air dried, dehydrated, cleared in xylene and coverslipped with DPX mountant (BDH-Pool, UK).

Analysis

The atlas of the rat brain, according to Paxinos and Watson (1986) was used to localize neurons showing *c-fos* expression. *c-fos* expression within brainstem nuclei was semi-quantified and presented in Table 1. Fos-immunoreactivity within the medial NTS (at caudal, intermediate and rostral levels) of group A animals was quantified by counting positively stained nuclei and presented in Fig. 2.

RESULTS

Low level *c-fos* expression was observed in all groups within several brainstem nuclei (Table 1), although hardly any Fos-immunoreactivity (Fos-

Table 1. *c-fos* expression within the brainstem, in group A, B, C, and D, after a 90 min feeding session with 1 M sucrose solution (A), water (B) and neither sucrose nor water (C and D)

Group	A	B	C	D
Medulla Oblongata				
dorsal motor nucleus of the vagus	++	—	—	—
nucleus of the solitary tract	++	—	—	—
area postrema	+/—	++	++	++
subpostremal region	—	++	++	++
paratrigeminal nucleus	+	—	—	—
parvocellular reticular formation	+	+	+	+
raphe nuclei (obscurus, pallidus, magnus)	+	+	+	+
lateral reticular nucleus, S5	+	+	+	+
gigantocellular reticular nucleus, ventral	+	+	+	+
medullary reticular nucleus, ventrolateral	+	+	+	+
A5 noradrenergic region	+	+	+	+
caudal periolivary nucleus	+	+	+	+
supragenual nucleus	+	+	+	+
spinal trigeminal nucleus, caudal/oral	+	+/—	+/—	+/—
dorsal cochlear nucleus	+	+	+	+
Pons				
parabrachial nucleus, ventrolateral	++	—	—	—
parabrachial nucleus, external lateral	++	—	—	—
parabrachial nucleus, dorsolateral	++	++	—	++
parabrachial nucleus 'waist area'	++	—	—	—
Kölliker-Fuse nucleus	+	+	—	+
locus coeruleus	+	+	+	+

++ = moderate *c-fos* expression, + = low level, basal *c-fos* expression,
- = no *c-fos* expression, +/- = *c-fos* expression in at least 50% of the rats in the group.

IR) was present at rostral medullary and pontine level. Very few differences in Fos labeling were observed between the different groups (Table 1), except for the *c-fos* expression within the dorsal motor nucleus of the vagus (DMnX), nucleus of the solitary tract (NTS), the paratrigeminal nucleus (Pa5) and the parabrachial nucleus (PBN).

c-fos expression in the Medulla Oblongata

In group A, Fos-IR was detected in the medial part of the DMnX at caudal medullary levels (Fig. lA,B,C), this DMnX labeling diminished in rostral direction, and was not found at levels rostral to the area postrema (AP).

Furthermore, group A animals showed clear *c-fos* expression in the medial part of the NTS (mNTS) at caudal, intermediate and rostral levels (Figs. 1,2,3).

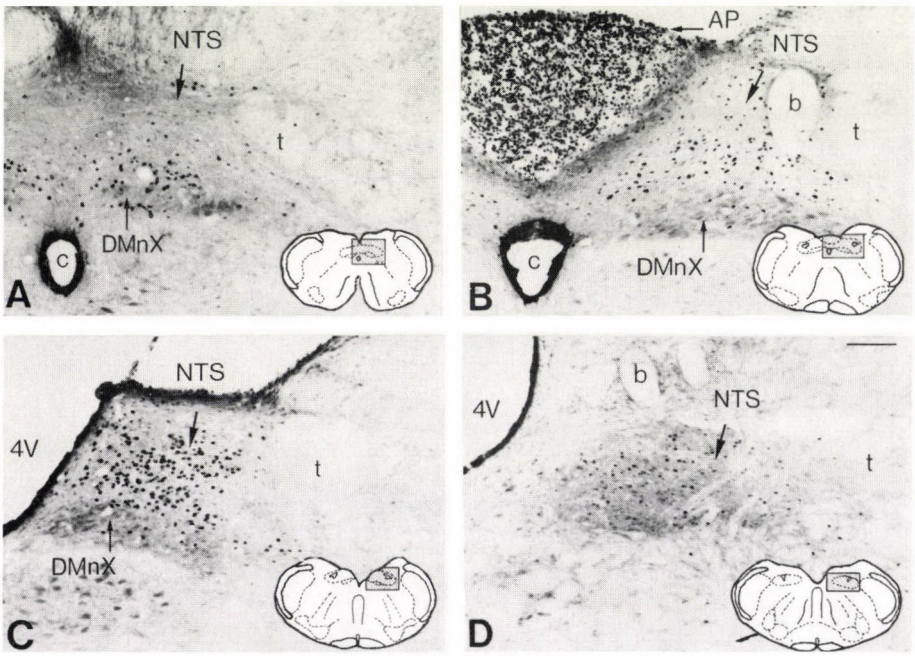


Fig. 1. *c-fos* expression wilthin the brainstem, in DMnX AP and throughout the caudal (A), intermediate (B,C) and rostral (D) NTS after voluntary intake of sucrose (Group A). NTS, nucleus of the solitary tract; DMnX, dorsal motor nucleus of the vagus; AP, area postrema; b, blood vessel; c, central canal; t, solitary tract; 4V, fourth ventricle. Scale bar = 100 μm

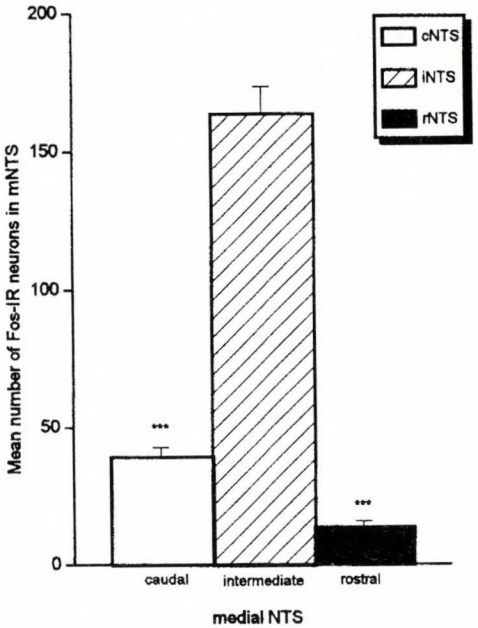


Fig. 2. Quantification of *c-fos* expression within the NTS, after sucrose ingestion by group A animals. Positively stained nuclei were counted within the medial NTS bilaterally, at caudal, intermediate and rostral levels. Cell counts were averaged across animals (n=8) by NTS subregion. Among the group A animals, only rats Al-4 and A2-1,2,3 showed *c-fos* expression within the extreme rostral NTS (+ Bregma -12.3), and contributed to the mean number of Fos-labeled neurons in this subnucleus *** rostral vs intermediate NTS and caudal vs intermediate NTS, $p < 0.001$ (ANOVA).

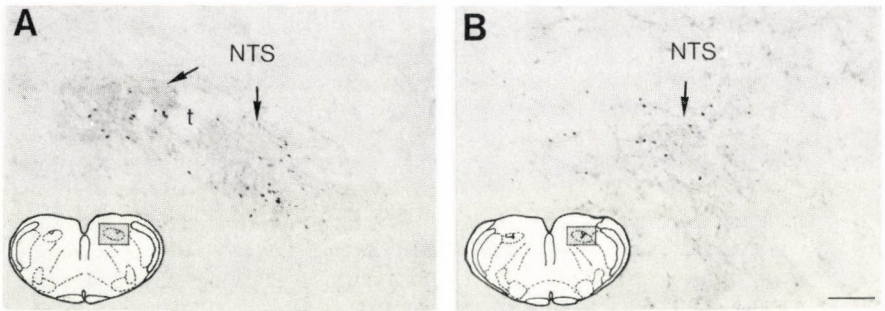


Fig. 3. *c-fos* expression within the rostral gustatory part of the NTS after voluntary intake of sucrose (group Al-4, group A2-1,2,3) at Bregma -12.3 (A) and Bregma -11.8 (B). NTS, nucleus of the solitary tract; t, solitary tract. Scale bar = 100 μm

The highest number of positively stained nuclei was found at the intermediate NTS level, diminishing in both density and strength in rostral and caudal direction (Fig. 2). In four of the eight animals, the mNTS labeling was found up to the level of the intermediate NTS (Bregma -12.5) (Fig. lD). The remaining four rats (Al-4 and A2-1,2,3) showed additional, scattered Fos-IR in rostral direction, within the mNTS up to the level where the NTS contacts the spinal trigeminal nucleus (Fig. 3A,B), sometimes extending laterally into the lateral NTS (INTS) (Fig. 3B) or medullary reticular formation.

c-fos expression was found in groups B, C and D in the AP and in the subpostremal region (Fig. 4A) but not in NTS or DMnX (Fig. 4B). The density of Fos labeled cells in the AP of six of the eight animals of group A (Fig. lB) was higher than in the control groups (Fig. 4A); however, animals A2-2 and 4 did not show any *c-fos* expression in the AP. No Fos-positive neurons were observed in the subpostremal region of group A animals. Furthermore, group A animals showed a few scattered Fos positive neurons in the paratrigeminal nucleus, in contrast to the animals in groups B, C and D, showing no Fos-IR in this nucleus.

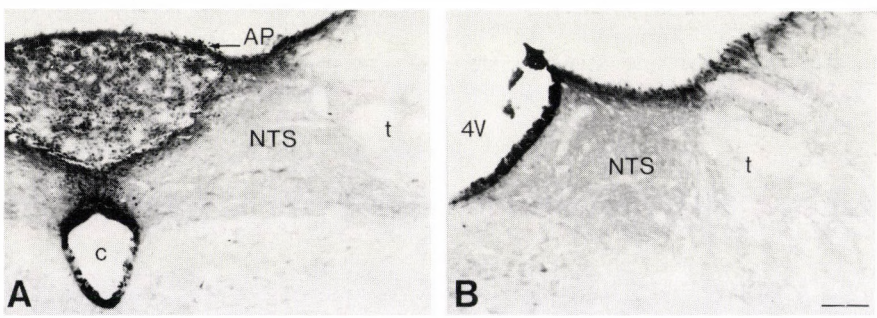


Fig. 4. Fos-immunoreactivity in the brainstem after water intake (control group B). *c-fos* expression was found solely in the AP and in the subpostremal area (A), no *c-fos* expression was detected within the NTS and DMnX (B). A similar distribution of Fos-positive neurons was observed in groups C and D. NTS, nucleus of the solitary tract; DMnX, dorsal motor nucleus of the vagus; AP, area postrema; c, central canal; t, solitary tract; 4V, fourth ventricle. Scale bar = 100 μm

c-fos expression in the Pons

At pontine levels, groups A, B and D animals showed moderate density Fos-IR in the dorsolateral parabrachial nucleus (PBN) at caudal levels (Fig. 5). In the same groups, scattered Fos labeled cells were detected in the Kölliker-Fuse nucleus. Within group A, additional Fos labeling was observed in a zone that includes the ventrolateral and external lateral subnuclei and the 'vaist' area of the caudal PBN (Bregma -9.7 to -9.2) (Fig. 5A,B).

DISCUSSION

In this study, we investigated *c-fos* expression in brainstem nuclei after voluntary ingestion of a sucrose solution in unanaesthetized rats, to elucidate

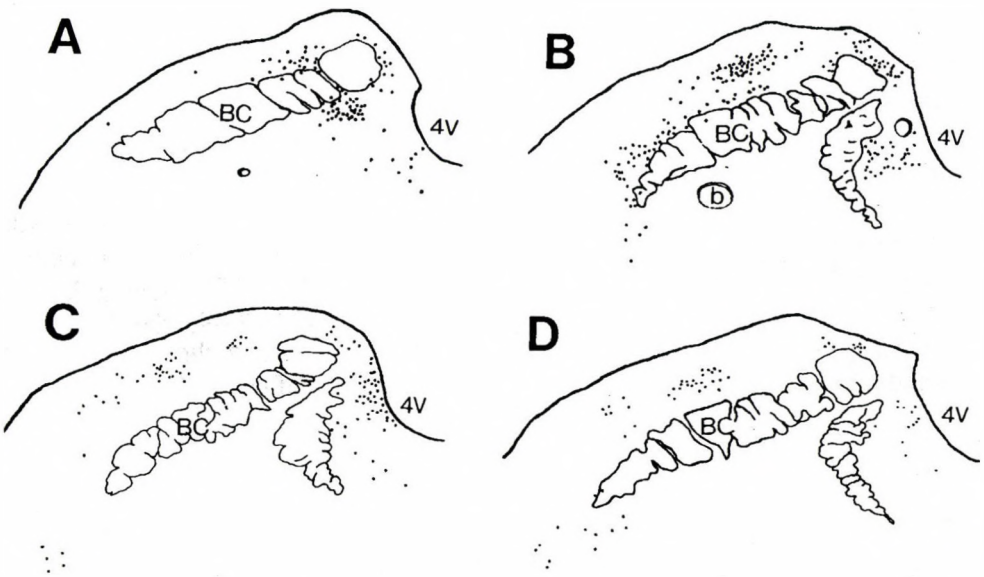


Fig. 5. Camera lucida drawings of *c-fos* expression within the PBN after voluntary intake of sucrose (group A) (A,B), after water intake (group B) (C) and without drinking or eating anything (group D) (D). Group C did not show Fos-IR within the PBN. BC, brachium conjunctivum; b, blood vessel; 4V, fourth ventricle

efferent projections conveying gustatory information. Fos-immunoreactive (Fos-IR) neurons were found mainly within the medial part of the nucleus of the solitary tract (mNTS), the dorsal motor nucleus of the vagus (DMnX) at caudal medullary levels, the paratrigeminal nucleus (PaS) and the lateral and medial part of the parabrachial nucleus (PBN).

Electrophysiological and neuroanatomical studies have shown that afferent nerves conveying taste information from the rat oral cavity have their first synapse in the rostral pole of the NTS (rNTS) (Norgren, 1981, 1984, 1985; Hamilton and Norgren, 1984; Halsell et al., 1993; Nakamura and Norgren, 1991, 1993). Viral tracing of descending vagal projections from the rNTS to the endocrine pancreas (Streefland et al., 1994), suggested that the intermediate and/or caudal NTS or the medullary reticular formation could play a role as intermediate station(s) and excluded a direct projection from the rNTS to the DMnX. To obtain further evidence of the gustatory involvement of these nuclei, the sweet taste-induced expression of *c-fos* was used as a neuronal activity marker.

To study stimulation of sweet taste receptors in the rat oral cavity and esophagus and subsequent *c-fos* expression within the rat brainstem, we used 5 groups of rats. In the final test period (see: Materials and Methods), rats in groups Al and A2 received a sweet taste stimulation by drinking a sucrose solution. Rats in group B drank water during this period, showing the effects of sweet taste on *c-fos* expression if compared with group A. Rats in group C received neither sucrose nor water, so that comparison with group B will showed the effects of drinking behavior (mouth and tongue movements, swallowing, etc.) on *c-fos* expression. Group D rats were allowed to eat standard rat chow during the test week and nothing during the final test period. Comparison with group C will show the effects of a chronically raised blood glucose or insulin level in group C animals, after the high intake of sucrose during the previous week, on *c-fos* expression.

The brainstem structures of the animals of groups B, C and D exhibited minimal *c-fos* expression, except for the AP and the PBN, which showed moderate Fos-labeling. This basal *c-fos* expression may reflect the basal activity level of these neurons, or may be due to unintentional stimulation, such as light, noise and touch. Apart from the Fos labeling in the PBN, no clear differences were observed between the control groups. In groups B and D, Fos-IR was found in the dorsolateral and Kölliker-Fuse (KF) subnuclei of the PBN, in contrast to group C. These differences are difficult to explain.

However, motivational changes, stress or frustration, caused by the different food presented, might induce *c-fos* expression in the PBN-KF complex, which is an important site in the neuronal circuit for descending sympathetic projections involved in stress-related cardiovascular responses (Korte et al., 1992).

c-fos expression induced by sweet taste stimulation, should have been present in group A animals only, and, indeed, was observed in the DMnX medial NTS, several parabrachial subnuclei and in the Pa5.

At caudal medullary levels, scattered Fos labeled cells were detected in the medial part of the DMnX up to the level of the area postrema. This part of the DMnX is known to project to the pancreas and mediate insulin release through its gastric branches (Berthoud and Powley, 1990). Four of the eight animals in group A showed moderate Fos-IR within the medial part of the rostral gustatory pole of the NTS (rNTS), the remaining four did not. In a comparable study, Yamamoto et al. (1991), did not find taste-induced *c-fos* expression in the rNTS either. We found this surprising, since electrophysiological studies yielded clear-cut single-unit responses to sucrose in the rNTS (Maes and Erickson, 1984; Norgren, 1984; Nakamura and Norgren, 1993; Halsell et al., 1993). A possible explanation for this finding is that sweet taste stimulation activates rNTS neurons without activation of the appropriate tools for induction of *c-fos* expression. This idea is supported by the observation that the ventrolateral and external lateral subnuclei of the PBN and the so-called waist area of the PBN (all part of the pontine taste area (PTA)) (Norgren and Leonard, 1972), receive taste input from the rostral gustatory NTS (Norgren and Pfaffmann, 1975; Perrotto and Scott, 1976; Nishijo and Norgren, 1990; Herbert et al., 1990), do show *c-fos* expression (Fig. 4A). At present it is not clear which factors cause the intraindividual differences with respect to *c-fos* expression in the rNTS.

Part of the *c-fos* expression in the caudal NTS of the animals in group A might be due to the occurrence of postingestive changes such as gastric distension (Fraser and Davison, 1994) and elevated blood-glucose and insulin levels (Giza and Scott, 1983; Giza et al., 1992). Direct activation of NTS neurons could take place through glucoreceptors and insulin receptors, that might be sensitive to endogenous glucose and insulin levels (Unger and Livingston, 1993). Indirect activation of NTS neurons could be effected through visceral afferents, such as hepatic afferents conveying information about glycemia (Timo-Iara et al., 1993). Incoming gustatory and visceral

information can be transmitted in caudal direction, from the NTS through the vagal and autonomic nerves, as integrated homeostatic response patterns.

Lower brainstem pathways conveying taste information to the pancreas

The circuit conveying taste information from the tongue to the pancreas to initiate the PIR, should involve the rNTS and the medial columns of the DMnX (mDMnX), since the rNTS is considered the first taste relay and, at the other hand, the PIR is mediated via the gastric branches of the vagus nerve. Transneuronal viral tracing from the rNTS to the pancreas showed that a direct connection between the rNTS and the mDMnX is very unlikely and suggested the presence of the following intermediate station(s); a) the medullary reticular formation (MdRF); b) the parabrachial nucleus (PBN); c) the intermediate or caudal NTS. d) The first relay for the taste information inducing the PIR, may not be at the rNTS, but may reside in more caudal NTS regions (Streefland et al., 1994). Based on the present Fos-data we can reconsider these possibilities or add new ones.

a) Although anterograde and retrograde tracing studies confirm rNTS projections to the reticular formation (Norgren, 1978; Hermann et al., 1983; Herbert et al., 1990; Travers, 1988; Becker and Travers, 1990; Beckman and Whitehead, 1991), no *c-fos* expression was found within the medullary reticular formation in this study. However, since the absence of *c-fos* expression is not strictly correlated with the absence of neuronal activity, we cannot definitely exclude the MdRF as a possible intermediate station.

b) Projections from the gustatory NTS to various subdivisions of the PBN have been found (Ricardo and Koh, 1978; Travers, 1988; Herbert et al. 1990; Beckman and Whitehead, 1991). However, since PBN neurons, according to our present knowledge, can reach the DMnX solely throughout the MdRF (Saper and Loewy, 1980; Herbert et al., 1990; Krukoff et al., 1993), which does not show Fos-IR, the PBN may be excluded as a link between taste receptors and the pancreas, despite the Fos labeling observed in this nucleus.

c) rNTS neurons might project to the pancreas via the intermediate or caudal NTS by means of intra-NTS interneurons (Beckman and Whitehead, 1991), since Fos labeling was detected in the mNTS at caudal and intermediate levels.

d) Another possibility is that the first relay for taste fibers conveying information to initiate the PIR is not situated in the rNTS, but in more caudal regions of the NTS. This would fit in with the Fos labeling we found at cNTS and iNTS levels. Furthermore, it implicates that ascending and descending pathways conveying gustatory information, diverge already before the first relay station. From the intermediate and caudal NTS, direct projections to the DMnX have already been reported (Ricardo and Koh, 1978; Fox and Powley, 1985; Beckman and Whitehead, 1991). It is known that taste fibers terminate mainly in the rostrolateral NTS (Arango et al., 1988), but also, at diminishing density, at intermediate and caudal NTS levels (Contreras et al., 1982; Hamilton and Norgren, 1984). Several authors found indications that posterior receptive fields in the oropharyngeal cavity are important in the rat's sensitivity to sucrose (Travers et al., 1986; Spector and Grill, 1992). For example, the superior laryngeal branch (SL) of the vagal (Xth) nerve, which innervates taste receptors in the region of the larynx, terminates at intermediate NTS levels (Hamilton and Norgren, 1984). In hamster, the SL does not seem remarkably responsive to sucrose (Dickman and Smith, 1988). In dog, however, stlimulation of the back of the throat with sucrose, led to pancreatic exocrine and endocrine secretion (Powers et al., 1990), which lead to the conclusion that the SL branch of the vagus nerve is the primary mediator of the PIR

e) A final possible intermediate station in the descending pathway conveying gustatory information, is the paratrigeminal nucleus (Pa5), where low level Fos-IR was found. The Pa5 is located in the trigeminal complex, which receives information from the oral cavity. However, since no NTS-Pa5 projections are reported and the Pa5 is not involved in projections to the pancreas, according to our viral tracing experiments (Streefland et al., 1994), we think that the Pa5 is not involved in processing sweet taste information to the pancreas. Future electrophysiological experiments will be necessary to probe the remaining possible projections from the tongue to the pancreas.

REFERENCES

Arango, V., Ruggiero, D.A., Callaway, J.L., Anwar, M., Mann, J.J. and Reis, D.J. (1988) Catecholaminergic neurons in the ventrolateral medulla and nucleus of the solitary tract in human. J.Comp.Neurol. 273, 224-240.

Becker, D. and Travers, S. (1990) Projections of electrophysiologically identified loci in the orally-responsive solitary nucleus. Soc.Neurosci.Abstr. 16, 404.

Beckman, M.E. and Whitehead, M.C. (1991) Intramedullary connections of the rostral nucleus of the solitary tract in the hamster. Brain Res. 557, 265-279.

Berthoud, H.R and Powley, T.L. (1990) Identification of vagal preganglionics that mediate cephalic phase insulin response. Am.J.Physiol. 258, R523-R530.

Contreras, R.J., Beckstead, R.M. and Norgren, R. (1982) The central projections of the trigeminal, facial, glossopharyngeal and vagus nerves: An autoradiographic study. J.Auton.Nerv.Syst. 6, 303-322.

Dickman, J.D. and Smith, D.V. (1988) Response properties of fibers in the hamster superior laryngeal nerve. Brain Res. 450, 25-38.

Flynn, F.W., Berridge, K.C. and Grill, H.J. (1986) Pre- and postabsorptive insulin secretion in chronic decerebrate rats. Am.J.Physiol. 250, R539-R548.

Fox, E.A. and Powley, T.L. (1985) Longitudinal organization within the dorsal motor nucleus represents separate branches of the abdominal vagus. Brain Res. 341, 269-282.

Fraser, K.A. and Davison, J.S. (1994) Gastric distension induces *c-fos* immunoreactivity in the rat brainstem. Cholecystokinin 713, 164-166.

Giza, B.K. and Scott, T.R. (1983) Blood glucose selectively affects taste-evoked activity in rat nucleus tractus solitarius. Physiol.Behav 31, 643-650.

Giza, B.K., Scott, T.R. and Vanderweele, D.A. (1992) Administration of satiety factors and gustatory responsiveness in the nucleus tractus solitarius of the rat. Brain Res.Bull. 28, 637-639.

Grill, H.J., Berridge, K.C. and Ganster, D.J. (1984) Oral glucose is the prime elicitor of preabsorptive insulin secretion. Am.J.Physiol. 246, R88-R95.

Halsell, C.B., Travers, J.B. and Travers, S.P. (1993) Gustatory and tactile stimulation of the posterior tongue activate overlapping but distinctive regions within the nucleus of the solitary tract. Brain Res. 632, 161-173.

Hamilton, R .and Norgren, R. (1984) Central projections of gustatory nerves in the rat. J.Comp.Neurol. 222, 560-577.

Herbert, H., Moga, M.M. and Saper, C.B. (1990) Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. J.Comp.Neurol. 293, 540-580.

Hermann, G.E., Kohlerman, N.J. and Rogers, R.C. (1983) Hepatic-vagal and gustatory afferent interactions in the brainstem of the rat. J.Auton.Nerv.Syst. 9, 477-495 .

Houpt, T.A., Philopena, J.M., Wessel, T.C., Joh, T.H. and Smith, G.P. (1994) Increased *c-fos* expression in the nucleus of the solitary tract correlated with conditioned taste aversion to sucrose in rats. Neurosci.Lett. 172, 1-5.

Hunt, S.P., Pini, A. and Evan, G. (1987) Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature 328, 632-633.

Korte, S.M., Jaarsma, D., Luiten, P.G.M. and Bohus B. (1992) Mesencephalic cuneiform nucleus and its ascending and descending projections serve stress-related cardiovascular responses in the rat. J.Auton.Nerv.Syst. 41, 157-176.

Krukoff, T.L., Harris, K.H. and Jhamandas, J.H. (1993) Efferent projections from the parabrachial nucleus demonstrated with the anterograde tracer phaseolus vulgaris leucoagglutinin. Brain Res.Bull. 30, 163-172.

Krukoff,T.L., Morton, T.L., Harris, K.H. and Jhamandas, J.H. (1992) Expression of c-fos protein in rat brain elicited by electrical stimulation of the pontine parabrachial nucleus. J.Neurosci. 12, 3582-3590.

Maes, F.W. and Erickson, R.P. (1984) Gustatory intensity discrimination in rat NTS: a tool for evaluation of neural coding theories. J.Comp.Physiol.A 155, 271 -282.

Menetrey, D., Gannon, A., Levine, J.D. and Basbaum, A.I. (1989) Expression of *c-fos* protein in interneurons and projection neurons of the rat spinal cord in response to noxious somatic, articular, and visceral stimulation. J.Comp.Neurol. 285, 177-195.

Messina, J.L., Standaert, M.L., Ishizuka, T., Weinstock, R.S. and Farese, R.V. (1992) Role of protein kinase C in insulin's regulation of *c-fos* transcription. J.Biol.Chem. 267, 9223-9228.

Morgan, J.I. and Curran, T. (1989) Stimulus-transcription coupling in neurons: Role of cellular immediate-early genes. TINS 11, 459-462.

Morgan, J.I. and Curran, T. (1991) Stimulus-transcription coupling in the nervous system: Involvement of the inducible proto-oncogenes Fos and Jun. Ann.Rev.Neurosci. 14, 421-451.

Nakagawa, Y., Rivera, V. and Larner, A.C. (1992) A role for the Na/K-ATPase in the control of human *c-fos* and *c-jun* transcription. J.Biol.Chem. 267, 8785-8788.

Nakamura, K. and Norgren, R. (1991) Gustatory responses of neurons in the nucleus of the solitary tract of behaving rats. J.Neurophysiol. 66, 1232-1248.

Nakamura, K. and Norgren, R. (1993) Taste responses of neurons in the nucleus of the solitary tract of awake rats: An extended stimulus array. J.Neurophysiol. 70, 879-891.

Nishijo, H. and Norgren, R. (1990) Responses from parabrachial gustatory neurons in behaving rats. J.Neurophysiol. 63, 707-724.

Norgren, R. (1978) Projections from the nucleus of the solitary tract in the rat. Neurosci. 3, 207-218.

Norgren, R. (1981) The central organization of the gustatory and visceral afferent systems in the nucleus of the solitary tract. In: Brain mechanisms of sensation (eds. Katsuki, Y., Norgren, R. and Sato, M.) pp. 143-160.

Norgren, R. (1984) Central neural mechanisms of taste. In: Handbook of physiology – The nervous system III, Section II: Sensory processes (ed. I. Darien Smith,) pp. 1087-1128. Amer.Physiol.Soc., Bethesda.

Norgren, R. (1985) Taste and the autonomic nervous system. Chem.Senses 10, 143-165.

Norgren, R. and Leonard, C.M. (1972) Ascending central gustatory pathways. J.Comp.Neurol. 150, 217-238.

Norgren, R. and Pfaffmann, C. (1975) The pontine taste area in the rat. Brain Res. 91, 99-117.

Olson, B.R. Freilino, M., Hoffman, G.E., Stricker, E.M., Sved, A.F. and Verbalis, J.G. (1993) *C-fos* expression in rat brain and brainstem nuclei in response to treatments at alter food intake and gastric motility. Mol.Cell.Neurosci. 4, 93-106.

Olson, B.R. Hoffman, G.E., Sved, A.F., Stricker, E.M. and Verbalis, J.G. (1992) Cholecystokinin induces *c-fos* expression in hypothalamic oxytocinergic neurons projecting to the dorsal vagal complex. Brain Res. 569, 238-248.

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Onoda, N. (1992) Odor-induced fos-like immunoreactivity in the rat olfactory bulb. Neurosci.Lett. 137, 157-160.

Paxinos, G. and Watson, C. (1986) The rat brain in stereotaxic coordinates. Academic Press, New York.

Perrotto, R.S. and Scott, T.R. (1976) Gustatory neural coding in the pons. Brain Res. 110, 283-300.

Powers, M.A., Schiffman, S.S., Lawson, D.C., Pappas, T.N. and Taylor, I.L. (1990) The effect of taste on gastric and pancreatic responses in dogs. Physiol.Behav. 47, 1295-1297.

Ricardo, J.A. and Koh, E.T. (1978) Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain stuctures in the rat. Brain Res. 153, 1-26.

Sagar, S.M. and Sharp, F.R. (1990) Light induces a Fos-like nuclear antigen in retinal neurons.Molec. Brain Res.7, 17-21

Sagar, S.M., Sharp, F.R. and Curran, T. (1988) Expression of *c-fos* protein in brain: Metabolic mapping at the cellular level. Science 240, 1328-1331.

Sandner, G., Scala, Rocha, B. and Angst, M.J. (1992) *C-fos* immunoreactivity in the brain following unilateral electrical stimulation of the dorsal periaqueductal gray in freely moving rats. Brain Res. 573, 276-283.

Saper, C.B. and Loewy, A.D. (1980) Efferent connections of the parabrachial nucleus in the rat. Brain Res. 197, 291-317.

Sharp, F.R. Gonzalez, M.F., Sharp, J.W. and Sagar, S.M. (1989) *C-fos* expression and (^{14}C)-2-deoxyglucose uptake in the caudal cerebellum of the rat during motor/sensory cortex stimulation. J.Comp.Neurol. 284, 621-636.

Sheng, H.Z., Fields, R.D. and Nelson, P.G. (1993) Specific regulation of immediate early genes by patterned neuronal activity. J.Neurosci.Res. 35, 459-467.

Sheng, M. and Greenburg, M.E. (1990) The regulation and function of *c-fos* and other immediate early genes in the nervous system. Neuron 4, 477-485.

Spector, A.C. and Grill, H.J. (1992) Salt taste discrimination after bilateral section of the chorda tympani or glossopharyngeal nerves. Am.J.Physiol. 263, R169-R176.

Steffens, A.B. (1976) Influence of the oral cavity on insulin release in the rat. Am.J.Physiol. 230,1411-1415.

Streefland, C., Ter Horst, G.J. and Maes, F.W. (1994) Brainstem projections to the pancreas: A retrograde transneuronal viral tracing study in the rat (submitted).

Strubbe, J.H. and Steffens, A.B. (1975) Rapid insulin release after ingestion of a meal in the unanesthesized rat. Am.J.Physiol. 229, 1019-1022.

Ter Horst, G.J., Luiten, P.G.M. and Kuipers, F. (1984) Descending pathways from hypothalamus to dorsal motor vagus and ambigous nuclei in the rat. J.Auton.Nerv.Syst. 11, 59-75.

Timo-Iara, C., Fraga da Silva, E.C. and Hell, N.S. (1993) Nuclei of the solitary tract and regulation of glycemia. In: Nucleus of the solitary tract (ed. Barraco, I.R.A.) Ch. 31, pp. 385-398. CRC Press, Boca Raton.

Travers, J.B. (1988) Efferent projections from the anterior nucleus of the solitary tract of the hamster. Brain Res. 457, 1-11.

Travers, S.P., Pfaffmann, C. and Norgren, R. (1986) Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract. Brain Res. 365, 305-320.

Unger, J. and Livingston, J.N. (1993) Insulin receptors in the nucleus of the solitary tract and related neuronal circuits. In: Nucleus of the solitary tract (ed. Barraco, I.R.A.) Ch. 31, pp. 377-384. CRC Press, Boca Raton.

Yamamoto, T., Shimura, T., Sako, N., Sakai, N., Tanimizu, T. and Wakisaka, S. (1993) *C-fos* expression in the parabrachial nucleus after ingestion of sodium chloride in the rat. Neuroreport 4, 1223-1226.

Yamamoto, T., Shimura, T., Azuma, S., Bai, W.-Zh., Mochizuki, R. and Wakisaka, S. (1991) Distribution of c-fos-like immunoreactivity in the rat brain following taste stimulation. JASTS XXV, 17, 93.

Yousfi-Malki, M. and Puizillout, J.J. (1994) Induction of Fos-like protein in neurons of the medulla oblongata after electrical stimulation of the vagus nerve in anesthetized rabbit. Brain Res. 635, 317-322.

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Preliminary note

NITRIC OXIDE SYNTHASE INHIBITOR FACILITATES AMINOPYRIDINE INDUCED NEOCORTICAL SEIZURE

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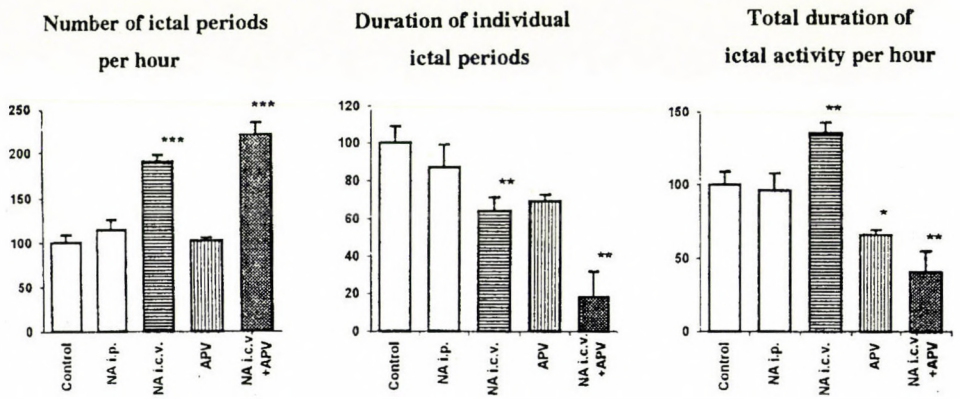
The effects of the diffusible messenger nitric oxide (NO) in the pathophysiology of epilepsy are still controversial. Most of the studies were carried out in different *in vitro* models of epileptiform activity. In the present study we have examined, how the inhibition of NO synthase (NOS) by L-nitro-l-arginine (NA) affects 3-aminopyridine (Ap) induced focal primary and secondary electrographic ictal seizures in anaesthetised rats.

Epileptiform activity was induced by the application of Ap to the surface of the somatosensory cortex. In the homologous area of the contralateral hemisphere secondary seizure activity developed (mirror focus: Mf). Electroencephalograms were monitored by four silver-ball electrodes for one hour after induction of Pf. In one group of animals NA was injected intraperitoneally (i.p.) twice daily, for four days. In another group NA was injected into the lateral cerebral ventricle (i.c.v.) on the side of Pf, 30 minutes before the application of Ap. In other experiments D(-)2-amino-5-phosphonovaleric acid (APV), a specific blocker of NMDA receptors, was applied to the surface of the cortex, 10 minutes before the induction of Pf in non-treated and NA i.c.v. treated animals.

NA, injected both i.p. and i.c.v. markedly facilitated propagation of seizure activity (up to 80% in contrary to 20% in non-treated animals). In addition,

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intracerebral NA increased the number of ictal events per hour, while the duration of individual periods decreased. As a result, the total duration of ictal episodes was significantly increased. APV by itself did not change the number of ictal episodes but decreased their duration, resulting in a decrease of the total duration of ictal activity. When animals were pre-treated with APV and by NA injected i.c.v., the number of ictal periods increased and their duration synergically diminished. The sum of these effects was an increase in the duration of paroxysmal activity during the one hour inspection time. Results in % of control are shown in Pf in Fig. 1. Epileptiform activity in the Mf did not show any specific changes compared to the activity of Pf, as a consequence of drug treatments.



We describe here a paradoxical effect of NA on the Ap-induced ictal epileptiform activity in the neocortex *in vivo*. The lack of NO strongly increased the number of ictal periods per hour, while significantly decreased their duration. The propagation of epileptiform events to the entire cortex was markedly facilitated. These effects of NOS inhibition were not prevented by APV, therefore we suggest that they have an origin probably different from NMDA receptor. In conclusion NO seems to be anticonvulsive in seizure induction and propagation, while it seems to be proconvulsive in seizure maintenance.

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Preliminary note

EFFECTS OF GALANINS pGAL1-29 AND hGAL1-30 AND THEIR FRAGMENTS ON CHOLINERGIC NEURONS PRESENT IN THE NUCLEUS OF THE VERTICAL DIAGONAL BAND

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Galanin (GAL) is a 29/30-amino acid neuropeptide. It was isolated first from the porcine upper intestine (pGAL1-29) (6) and later from human tissues (hGAL1-30). It has been shown that first 16 N-terminal amino acids of the peptide are both necessary and sufficient for receptor recognition and receptor activation (1). By immunohistochemical means, GAL has been localized in the different areas of the brain in various species of animals. In humans, GAL-immunoreactive neurons appear to be present in the basal part of the brain and GAL is colocalized with acetylcholine (ACh). In the nucleus basalis of Meynert (nbM), the choline acetyltransferase (ChAT)- immunostained neurons are surrounded by GAL-positive nerve fibers. In Alzheimer's disease (AD), where the number of cholinergic neurons in the nbM is reduced, the GAL-containing axons hyperinnervate the remaining cholinergic neurons (2). Neuropharmacological experiments have demonstrated the inhibitory function of GAL on the evoked release of ACh. The results indicate that GAL may play a role in modulating the cholinergic function and could be of importance in human AD (3). We have already presented evidence that the effects of pGAL1-29 and hGAL1-30 are different in the various parts of the rat brain (dorsal and ventral hippocampus, striatum and olfactory bulb) (4) .

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The aim of the present investigation was to compare the effects of pGAL1-29 and hGAL1-30, hGAL1-19, the fragments hGAL21-30 and hGAL26-30 and different combinations of these substances with either K^+ or electrical (4 Hz, 2 ms, 720 pulses) stimulation [applied at the 9th (S1) and 21st (S2) min of superfusion] on the basal (spontaneous) release and the K^+-evoked release of ACh in the nucleus of the vertical diagonal band (VDBN) of rat.

The release of ACh from the VDBN was studied as described for other areas of the rat brain (5).

Fig. 1

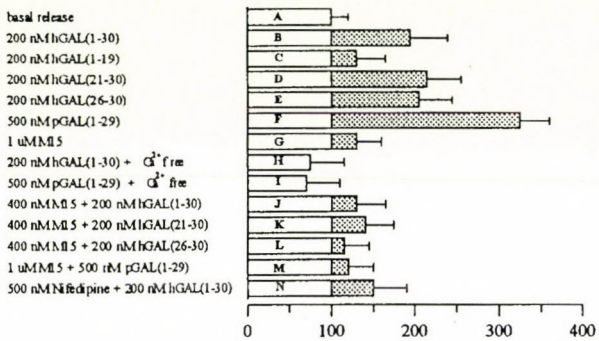
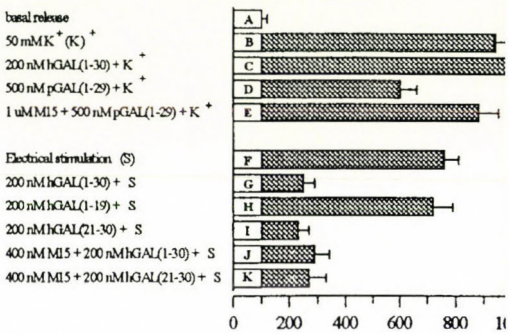


Fig. 2



(100% = 1,12 fmol ACh/mg protein/6 min)

Fig. 1: Effects of hGAL1-30, hGALl-l9 and the fragments hGAL21-30 and hGAL26-30 and pGALl-29, alone or in combination with GAL receptor antagonist (M15) on ACh release in rat VDBN. The ACh release in B, D, E and F was significantly ($P<0.0l$) different from that in A. M15 significantly ($P<0.01$) reduced the stimulatory effects of hGALl-30, hGAL21-30, hGAL26-30 and pGAL1-29 on ACh release (compare B with J, D with K, E with L and F with M)

Fig. 2: *In vitro* release of ACh in the VDBN of rat following application of different combinations of K^+ or electrical stimulation and M15 with the different GALs and fragments

Conclusions

1. hGAL1-30 (but not hGALl-l9) and the fragments hGAL21-30, hGAL26-30 and pGAL1-29 induce ACh release from the VDBN of rat; this can be antagonized by the use of M15.

2. The carboxy terminal of hGAL is more effective on ACh release than the amino terminal of the neuropeptide.
3. M15 can be used as an effective antagonist of GAL-induced ACh release.
4. K^+-induced ACh release can be reduced by pGAL1-29, but not by hGAL1-30.
5. The ACh release evoked by electrical stimulation can be reduced significantly ($P<0.01$) by hGAL1-30 and hGAL21-30, but not by hGAL1-19.

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References

1. Bedecs, K. et al., Int. J. Biochem. Cell Biol. 27, 337-349 (1995)
2. Chan-Palay, V.L., J. Comp. Neurol., 273, 543-557 (1988)
3. Hökfelt, T. et al., Experientia 43, 768-780 (1987)
4. Kása, P. et al., 3rd Int. Springfield Symp. Abstr. p 109 (1994)
5. Kása, P. et al., Neuroscience (in press, 1996)
6. Tatemoto, K. et al., FEBS Lett. 164, 124-128 (1983)

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Preliminary note

FEEDING AND ACETHYLCHOLINE IN THE AMYGDALA: MICRODIALYSIS IN FREELY MOVING RATS

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Several researchers have suggested that acetylcholine (ACh) facilitates feeding behavior as demonstrated by application of cholinergic agonists in different brain regions. Cholinergic activation of the hypothalamus and the amygdala (AMY) has also been shown to induce drinking.However, the effect of feeding on endogenous ACh is not yet known. Therefore, the following questions may arise: 1) whether the activation of limbic ACh system changes during feeding, and 2) which peripheral component of ingestion (or feeding in general) exerts a feedback control on central cholinergic mechanisms involved in the regulation of feeding. In order to elucidate the effect of feeding on limbic ACh, we measured extracellular ACh levels in the central nucleus of the AMY by using microdialysis in 20 min intervals before, during, and after 1 hr food access in 20 hr food-deprived freely moving rats. In addition to determine whether the observed effects on ACh release were consequent to hyperglycemia and/or hyperinsulinaemia associated with ingestion of food, effects of peripheral injection of glucose and insulin on AMY ACh were also examined in non-deprived animals. The results showed an increase in extracellular ACh in the AMY following free-feeding in 20 hr deprived rats (Fig. 1). Similar increase in ACh levels was found after systemic injection of glucose in non-deprived animals. Furthermore, two different doses of insulin also increased the extracellular concentration of ACh in the AMY (Fig. 2).

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The "low" and "high" doses of insulin had similar effects on ACh release even though they had different hypoglycemic potency revealed in separate tests.

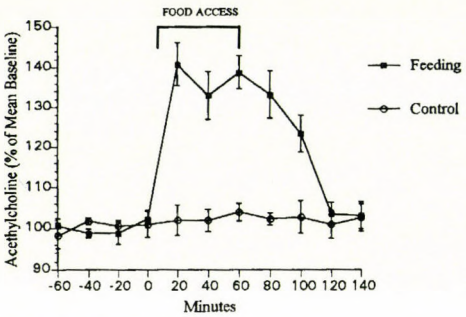


Fig. 1

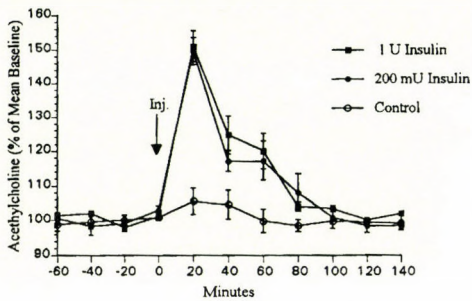


Fig. 2

These findings demonstrate that ACh release in the AMY can be potentiated by ingestive behavior. Moreover, on the basis of the present results one may suppose that insulin can act partly independently from its hypoglycemic effect. On the other hand, it can also be hypothesized that the observed effects of feeding or glucose injections on the AMY ACh caused by the insulin mobilizing effect of absorbing glucose rather than the direct caloric effect of meal.

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Preliminary note

THE DIFFERENTIAL INVOLVEMENT OF ADRENOCEPTORS IN CHALLENGES OF DIFFERENT INTENSITIES

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While presynaptic alpha-2 adrenoceptors inhibit the negative feedback of norepinephrine release, the postsynaptic alpha-2 adrenoceptors partake in the regulation of many central and peripheral processes. As a consequence, alpha-2 adrenoceptor agonists activate postsynaptic receptors but decrease the liberation of norepinephrine, by this decreasing beta or alpha-1 adrenoceptor-mediated effects. Alpha-2 adrenoceptor blockers in contrast increase the liberation of norepinephrine and induce an increase in beta and alpha-1 adrenoceptor mediated processes while postsynaptic alpha-2 adrenoceptors are blocked (Fig. 1). All these might mean that the effects of alpha-2 adrenoceptor blockade have two origins. They may reflect the consequences of postsynaptic adrenoceptor antagonism, but they also may be consequences of the postsynaptic beta or alpha-1 activation. The involvement of the two mechanisms in behavioral changes elicited by alpha-2 adrenoceptor blockers is largely unknown.

To search an answer to the above-outlined problem we have submitted male Wistar rats to electric shocks in a Shuttle-box apparatus (intense behavioral challenge). Active responses (gate crossings) were reduced dramatically after the alpha-2 adrenoceptor antagonists idazoxan and CH38083. Beta and alpha-1 antagonism (propranolol and prazosin, respectively) was not able to change this response, i.e. in this behavioral test the postsynaptic alpha-2 adrenoceptors appear to be involved (Fig. 2).

When the rats were exposed to a novel cage (mild behavioral challenge) the alpha-2 adrenoceptor idazoxan stimulated the active response (exploration) at 1 mg/kg while at 5 mg/kg it caused an inhibition (first and third experiment) or did not affect behavior (second experiment). The beta

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adrenoceptor blocker propranolol was able to abolish the increase in exploration induced by idazoxan (Fig. 3). The alpha-1 adrenoceptor blocker

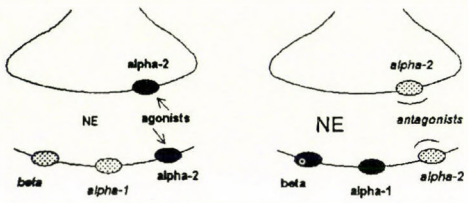


Fig. 1. The complex effects of alpha-2 adrenoceptor agonists and antagonists on adrenoceptor function

Fig. 2. Idazoxan (Idaz) inhibits active response (gate crossing) in the shuttle box. Propranolol (Pro) or prazosin (Pra) do not affect this change

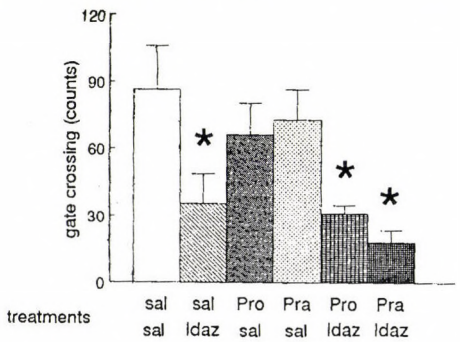
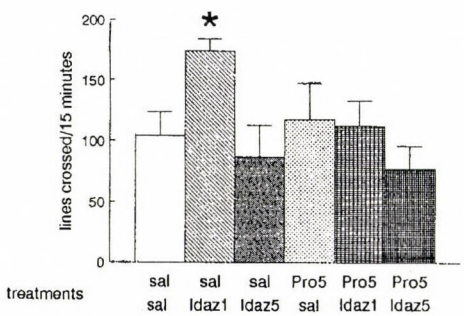


Fig. 3. Idazoxan (Idaz) inhibits actiYe response (gate crossing) in the shuttle box. Propranolol (Pro) or prazosin (Pra) do not affect this change (Idaz1)



prazosin caused a very significant reduction in exploration which was unaffected by the alpha-2 adrenoceptor blockade. In conclusion, the behavioral response in the novel cage was largely dependent on beta and especially on alpha-1 adrenoceptors, and the effects induced by alpha-2 adrenoceptor blockers can be attributed to a presynaptic mechanism.

It is concluded that the mechanisms by wich alpha-2 adrenoceptor blockers affect behavior are context dependent. The presynaptic mechanism appears to work during a mild environmental challenge (novel cage), while during intense challenges the postsynaptic mechanism prevails. It also appears that challenges of different types involve different types of adrenoceptors.

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Preliminary note

ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECT OF DIFFERENT SOMATOSTATIN-ANALOGS

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A variety of effects of the neuropeptide somatostatin has already been examined, including modulation of hormone- and neurotransmitter release, cognitive and behavioural processes, the endocrine system, the gastrointestinal tract and the cardiovascular system and also has tumor growth inhibiting effects. These effects of the molecule are due to five different somatostatin-receptor subtypes. Many new somatostatin-analogs have recently been synthetized in order to study the relative importance of specific substitutions in relation to selectivity between endocrine and antitumor effects. We demonstrated earlier that local neurogenic inflammation has systemic anti-inflammatory effect. Participation of somatostatin in the observed anti-inflammatory responses has been assumed since this peptide is released from the activated capsaicin-sensitive afferent nerve endings. As the efferent function of the C-fibers is neurogenic inflammation and the afferent is nociception, somatostatin is possible to have an effect on nociception as well. The aim of the present study was to examine the effect of somatostatin, octreotide (Sandostatin), and three stable, cyclic analogs, TT-250, TT-248, and TT-232, synthetized in the Central Research Institute for Chemistry of the Hungarian Academy of Sciences on neurogenic and non-neurogenic inflammation and chemonociception.

For assesment of neurogenic inflammation plasma extravasation was measured by the Evans Blue tracer method in the paw-skin of the rat. The

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inflammation was evoked by topical application of mustard oil in 1% concentration after acute, bilateral denervation of the hindleg (sciatic nerves and saphenous nerves). Non-neurogenic inflammation was induced by giving subplantar injection of dextran (0.1 ml 0.5%) 5 days after bilateral denervation of the hindleg. Oedema was measured by plethysmometer. Somatostatin (10 μg/kg i.p.) significantly inhibited both neurogenic and non-neurogenic inflammation. Furthermore the stable, cyclic, synthetic analogs (TT-250, TT-248, and TT-232) were more effective. TT-232 had the greatest inhibiting effect, which – according to previous reports – does not have any endocrine effect, but this is the best in inhibiting tumor-cell proliferation (Figs 1, 2).

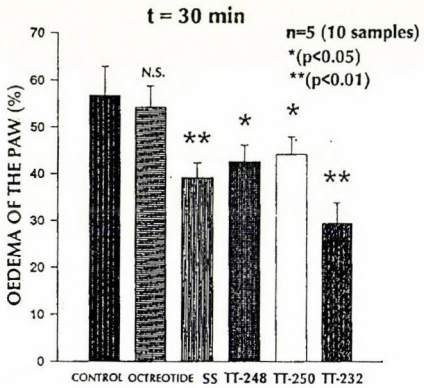


Fig. 1. Effect of somatostatin-analogs on neurogenic inflammation evoked by mustard oil (1%)

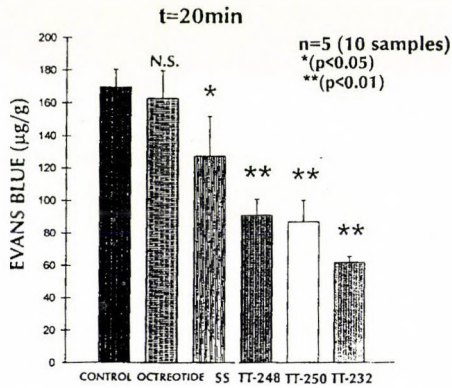


Fig. 2. Effect of somatostatin-analogs on non-neurogenic inflammation evoked by dextran (0.5%, s.p.)

For examining antinociceptive effects different vegetative reflexes (blood-pressure, heart-rate, respiration) to nociceptive stimulations were recorded in rats anaesthetized with urethan. These nociceptive stimuli were topical application of mustard oil on the hindpaw and capsaicin solution into the eyes in 100μg/ml concentration. The responses were evaluated with the help of a special computer-program. In control animals nociceptive stimuli elicited a significant enhancement in blood-pressure and heart-rate elevation and an increase in respiration. These different efferent responses were

inhibited by pretreatement with somatostatin, TT-250, TT248, and TT-232 (10μg/kg i.p.).

Octreotide had no influence on either neurogenic, or non-neurogenic inflammation, and did not induce an antinociceptive effect.

These data suggest, that the receptor-family (R2, R3, R5) being responsible for the endocrine effect of somatostatin is not the one which is responsible for the anti-inflammatory and anti-nociceptive effects, because octreotide, a potent analog acting on them was ineffective in this respect. TT-232 seemed to be the best and most promising anti-inflammatory and antinociceptive compound, as this is the only analog which has no endocrine effect.

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Preliminary note

EFFECT OF HYPOCHOLINERGIC ACTIVITY ON THE IMMUNOREACTIVITY OF β-AMYLOID PRECURSOR PROTEIN IN THE CENTRAL NERVOUS SYSTEM

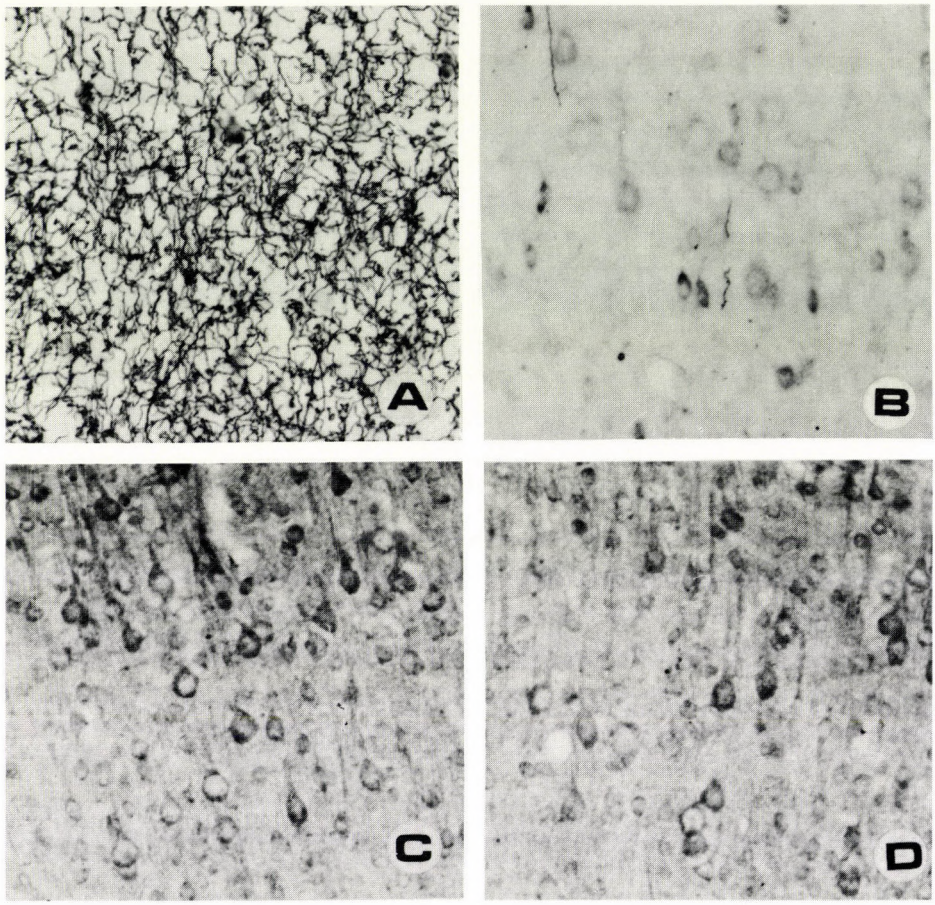
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Alzheimer's disease (AD) is a chronic, neurodegenerative disorder in which amyloid β-protein (Aβ) containing "neuritic" or senile plaques (SP) accumulate in the brain, a great number of neurofibrillary tangles (NFT) appear and there is dramatic reduction in the elements of the cholinergic system (choline acetyltransferase: ChAT; acetylcholine; acetylcholinesterase: AChE; muscarinic acetylcholine receptors; nicotinic acetylcholine receptors). The ChAT deficit correlates with the histopathologic changes and memory dysfunction. Quantitative evaluation of the number of SP in the cerebral cortex revealed a strong correlation with the loss of cholinergic neurons in the nucleus basalis of Meynert (nbM) (1, 4). This finding indicated that changes in the cortical cholinergic innervation which arise from the nbM are an important feature in the pathogenesis of SP. It has been suggested that an altered processing of the amyloid precursor protein (APP) is a central event in the formation of Aβ deposits in the brain of individuals with AD. It was recently demonstrated that subcortical neurotransmitter system lesions alter the expression of secreted APP (5) the precursor of Aβ. Stimulation of m_1 and m_3

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AChR subtypes with carbachol increased the basal release of APP derivatives (3), whereas activation of protein kinase C enhanced the secretory cleavage



Figs A-D. Demonstration of AChE positivity in the frontal cortex of untreated rat (Fig. A) and in a rat treated with 192-IgG-saporin for 42 days (Fig. B). APP immunohistochemical staining in the control frontal cortex (Fig. C) and after treatment with 192-IgG-saporin for 42 days (Fig. D). Note the difference in the AChE staining (Fig. A and Fig. B), whereas the APP immunoreactivity is identical in the control (Fig. C) and in the treated sample (Fig. D)

within the Aβ domain, resulting in an increased release of soluble APP and inhibited the cellular production and the release of Aβ (2).

The main objective of the present investigation was to determine whether the cholinergic hypoactivity in the rat can induce any changes in the production of APP.

Rats were treated intraventricularly with either 2 or 4 μg 192-IgG-saporin. After different time intervals (6, 10, 14, 20, 28, 31 or 42 days), the reduced AChE staining was observed within both the cortical layers and the hippocampal layers. Consecutive sections of formalin-fixed tissue sections were processed for the demonstration of AChE activity and for the immunohistochemical visualization of APP (Boehringer).

The results showed that, after the different time intervals, 192-IgG-saporin resulted in a dramatic reduction of AChE activity in the frontal and parietal cortices (Fig. B) and in the hippocampus in the cholinotoxin-injected animals, while there were no changes in the same areas of the control samples (Fig. A). The immunohistochemical staining for APP revealed no changes in the staining intensity in the pyramidal cells in the cholinotoxin-treated samples (Fig. D) as compared with the controls (Fig. C).

This experiment therefore, demonstrated that the cholinergic hypoactivity induced by the specific cholinotoxin 192-IgG-SAP on the presynaptic side does not alter the APP immunostaining within the postsynaptic neurons. It is suggested that following specific lesion of cholinergic axons (neurons), which does not lead to pathologic changes in the postsynaptic structure, the abnormal processing of the APP will not appear.

Acknowledgement: This work was supported by the OTKA (2723), ETT (T-123 and T04,602/93) and MKM (279).

References

1. Arendt, Y. et al., Neurosci. Lett. 13, 81-85 (1984)
2. Hung, A.Y. et al., J. Biol. Chem., 268, 22959-22962 (1993)
3. Nitsch, M.R. et al., Science 258, 304-307 (1992)
4. Perry, E.K. et al., Br. Med. J. 2, 1457-1459 (1979)
5. Wallace, W.C. et al., J. Neurosci. 15, 4896-4905 (1995)

Neurobiology; 4 (1996) 123-126

Preliminary note

NEUROPATHOLOGIC CHANGES IN THE OLFACTORY BULB IN ALZHEIMER'S DISEASE

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The principal features of Alzheimer's disease (AD) are the presence of neurofibrillary tangles (NFTs) and senile plaques (SPs) and the loss of cholinergic axons. In AD, the brain structures related to the olfactory system (the olfactory bulb (OB), olfactory tracts, olfactory tubercles, pyriform cortex, medial group of amygdaloid nuclei and ventrolateral entorhinal area) are severely damaged. Most of these structures contain the elements of the cholinergic system.

Previous histochemical (4) and immunohistochemical (3) observations in our laboratory have demonstrated the fine structural organization of the elements of the cholinergic system (choline-acetyltransferase; acetylcholinesterase: AChE) in the rat OB, which has attracted special interest because it is thought that the chemical organization may be the same in identical morphological structures. However, to our knowledge there are no data in the literature on the morphological localization of cholinergic structures in the human OB. Furthermore, increased numbers of SPs and NFTs in the human OB in the anterior olfactory nucleus (AON), have been revealed in AD brain samples. It is suggested that there is a close correlation between the impairment of olfactory processes and the course of AD. Since it has been postulated that AD may begin in the nose (6), the purpose of the present study was to determine whether the elements of the cholinergic system in human are structurally identical to those can be found in rodents, or whether differences

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may be revealed and whether the numbers of SPs and NFTs can be correlated with the alterations of in cholinergic system in the human OB.

Materials

OB of fifteen AD patients were investigated selected from samples according to neuropathologic criteria (5). The control material consisted of five OBs from corresponding age-matched cases who had neurologic disorders. The post-mortem delay ranged from 3 to 24 h.

Methods

For the demonstration of AChE activity in the different structures a histochemical technique (7) was applied. To study the neuropathologic alterations in the different layers, silver impregnation (1, 9) and the thioflavine-S fluorescent technique was used. The aluminum positivity in the neurons, SPs and NFTs was studied by using a modified solochrome azurine technique (2).

Results

This study describes the distribution pattern of AChE in the human OB, and neuropathologic changes not demonstrated before.

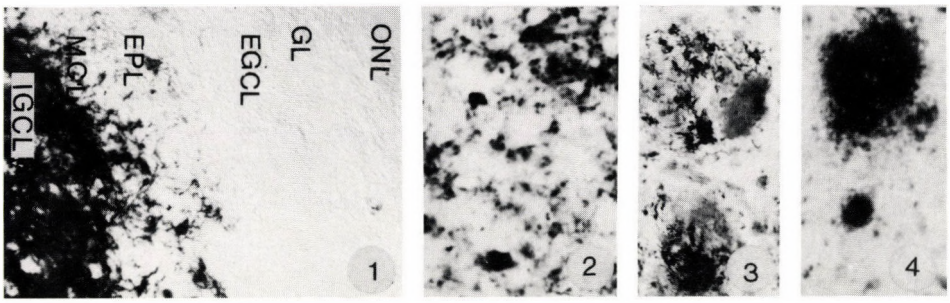
Localization of AChE: A specific and sensitive histochemical technique for study of the distribution of AChE-positive structures demonstrated the absence of cholinergic structures from the olfactory nerve layer (ONL), the glomerular layer (GL) and the external granule cell layer (EGCL), and the presence of the enzyme in the external plexiform layer (EPL) the internal plexiform layer (IPL), the internal granule cell layer (IGCL), the white matter (WM) and the AON (Fig. 1). Neurons in the mitral cell layer (MCL), short-axon neurons in the IGCL and different types of cells (small and large, bipolar and multipolar) were enzyme-positive within the AON (Fig. 2).

Neuropathology: Although cholinergic (AChE-positive) nerve fibers were evidently absent from the GL, diffuse amyloid staining could be detected inside the glomeruli (Fig. 3). This staining is thought to be localized in or near the olfactory sensory axons, where amyloid precursor-like protein (APLP2) (8) is present. In the EPL, MCL, IGCL and WM, very few if any diffuse

plaques were present. Primary and diffuse plaques were most numerous in the AON (Fig. 4). In the control samples, the number of SPs was much less than in the AD samples. Both in the control group and in the AD samples, very few NFTs were revealed in the MCL. In contrast, a number of NFTs were present within the AON in the AD samples.

Summary

1. For the first time we have described the structural localization of AChE in the different layers of the human OB. In contrast with the rat, no cholinergic structures were detected in the GL and the outer part of the EPL in the human OB.
2. No correlation was found between the AChE (and perhaps the cholinergic axon) content and the neuropathologic changes.
3. Diffuse amyloid β-protein staining was present in or near the olfactory sensory axons within the GL in the AD samples.
4. Rats may not serve as an accurate model for AD as the cholinergic fibers are more diffusely distributed in the different layers than in the human OB..



Acknowledgement: This work was supported by the ETT (T-123, T04,602/93), OTKA (2723) and MKM (279).

References

1. Gallyas, F., Acta Morphol. Acad. Sci. Hung. 19, 1-8 (1971)
2. Kása, P. et al., Acta Neuropathol. 90, 526-531 (1995)

3. Kása, P. et al., Neuroscience 67, 667-677 (1995)
4. Kása, P. et al., Neuroscience (in press, 1996)
5. Khachaturian, Z.S., Arch. Neurol. 42, 1097-1105 (1985)
6. Roberts, E., Neurobiol. Aging 7, 561-567 (1986)
7. Tago, H. et al., J. Histochem. Cytochem. 34, 1431-1438 (1986)
8. Thinakaran, G. et al., J. Neurosci., 15, 6314-6326 (1995).
9. Yamaguchi, H. et al., Acta Neuropathol. 79. 569-572 (1990)

ABSTRACTS

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Organizer:

J. Szolcsányi

ACTH$_{4-10}$ ANALOGS WITH NOOTROPIC PROPERTIES

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The representatives of several peptide families exert the properties of nootropic drugs (memory stimulation, enhancing of brain blood circulation and antihypoxic activity): olygopeptide fragments of vasopressin, corticotropin$_{4-10}$ analogs and particularly peptide MEHFPGP - semax (V.Nesavibathko et.al.). Semax demonstrates long-term (20-24 h) enhancement of mental work efficiency after intranasal administration in dose about 2-4 mg per human. It also exerts antihypoxic effects in white rats (hypobaric and circulatory hypoxia) and in humans (testing of EEG during hyperventillation). Semax activity is similar to typical nootrop pyracetam. In the experiments with genetically stroke-prone rats (KM) semax demonstrates a protective effect against the development of haemorragic stroke. Putative nootrops are also some fragments of substance P. In future it is reasonable to investigate the combine nootropic properties of peptides mentioned above.

STIMULATED RELEASE OF CALCITONIN GENE - RELATED PEPTIDE, SUBSTANCE P AND PROSTAGLANDIN E_2 FROM RAT SKIN, IN VITRO

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Institut für Physiologie I, Universitätsstraße 17, D-91054 Erlangen

Inflammatory mediators acting directly on nociceptors induce neuropeptide release and prostaglandin secretion from the surrounding tissue matrix. The following method allows to determine basal and stimulated release of CGRP, SP and PGE_2 from rat skin in order to investigate nociceptor excitation as well as neurogenic and non-neurogenic mechanisms of release.

Hairy skin from the back of rats (bw < 100 g) was subcutaneously excised, mounted on acrylic rods with the corium side exposed and washed for 30 min in "synthetic interstitial fluid" (SIF) at 32°C. The skin flap was then placed for 5 min in each of 6 consecutive glass tubes mounted in a shaking bath (32°C). All tubes were filled with 3 ml of carbogen gassed SIF except for the third tube containing the inflammatory mediators BK 10^{-5}M, "IS" (BK, HIS, 5-HT, all 10^{-5}M) or phosphate buffered SIF at pH 5.2. CGRP, SP and PGE_2 contents of the eluates were measured using enzyme immuno assays (SPIbio, France; Cayman, USA; Brune et al., FEBS 186, 46-50, 1985).

BK and 3 times more effectively the combination "IS" augmented the parallel release of CGRP, SP and PGE_2 from the skin, significantly and reversibly, whereas low pH, equipotent with BK, only increased the release of the two neuropeptides. PGE_2 secretion, on the contrary, was significantly decreased. The findings support electrophysiological evidence for a synergism of inflammatory mediators in exciting nociceptors which involves secondary PGE_2 in certain innervation territories. Pain from tissue acidosis, however, should not depend on PGE_2 synthesis which is actually suppressed. This work is supported by DFG, SFB 353 - Z2.

SOLID-PHASE SYNTHESIS OF GALANINS, THEIR SEGMENTS AND ANALOGS, STRUCTURE-ACTIVITY RELATIONSHIPS

Lajos Baláspiri, Tamás Janáky, Gábor Blazsó*, Tamás Takács**, Péter Kása***, and Marianna Mák****
Department of Medical Chemistry, *Department of Pharmacodynamics, **Firs Department of Medicine, ***Alzheimer Research Group, Albert Szent-Györgyi Medical University, Szeged; ****Central Research Institute of HASc, Budapest, Hungary

Since the discovery of the first galanin (porcine galanin) in 1983, the sequences of a further twelve, among them human and chicken galanins (Gal), have been determined. All galanins are linear, multifunctional peptide hormones and neuropeptides. We focused on the two endogenous human galanins and chicken galanin. Human Gal 1-19-OH (GWTLNSAGYLLGPHAVGNH), human Gal 1-30-OH (GWTLNSAGYLLGPHAVGNHRSFSDKNGLTS) and chicken Gal 1-29-NH_2 (GWTLNSAGYLLGPHAVDNHRSFNDKHGFT), and as controls the well-studied porcine Gal 1-29-NH_2, rat Gal 1-29-NH_2 and Galantid (M-15) antagonist were synthesized. For study of the N-terminal and C-terminal parts of human and chicken galanins, we also prepared the N-terminal Gal 1-15-OH segment (the same in all galanins), its amide analog Gal 1-15-NH_2, human and chicken Gal 1-16-OH segments and the amide analog, Gal 1-16-NH_2, human Gal 17-30-OH and chicken Gal 17-29-NH_2 segments.

All syntheses were conducted by using the methods of solid-phase peptide synthesis, Boc chemistry, with 1% Merrifield and MBHA resins. The crude products were purified by reverse-phase HPLC (RP-HPLC) in several steps. The pure products were characterized by TLC, RP-HPLC, amino acid analysis, FAB-MS, ESI-MS, capillary electrophoresis and in some cases different sequence deterinations. Circular dicroism spectra were also recorded in water, trifluoroethanol (TFE) and different mixtures of water and TFE.

The physical data and some CNS and hormonal biological data were compared with data on other galanins and with the literature. Some structure-activity relationships aspects will be presented.

TRANSMISSION FROM CAPSAICIN-SENSITIVE AFFERENTS TO MYENTERIC NEURONS IN THE SMALL INTESTINE

L. Barthó and V. Halmai (Department of Pharmacology, University Medical School Pécs, H-7643 Pécs, Hungary)

Capsaicin (CAP) causes cholinergic contractions of the guinea-pig isolated ileum by stimulating sensory neurons of the gut wall which in turn activate neurons of the myenteric plexus (NS Arch Pharmac. 305: 75; 305: 83, 1978). The nature of the transmitter substance(s) that stimulate myenteric neurons is not clear. Substance P tachyphylaxis has been shown to diminish the response to CAP (J. Physiol. 332: 157, 1982) but an antagonist selective for the NK_3 receptors failed to do so (Eur. J. Pharmac. 278: 17, 1995). CCK-like peptides or CGRP are probably not involved in the effect of CAP (Br. J. Pharmac. 90: 753, 1987; Neuropeptides 25: 325, 1993).

In the present study, we have tested the possible involvement of various putative neurotransmitters that have been reported to appear in primary sensory neurons, on the neuronally-mediated contractile effect of CAP (2 μM) in the isolated ileum of the guinea-pig. The response was suppressed by half by a combination of the NK_3 receptor blocker SR 142801 (0.1 μM) and the NK_1 blocker GR 82,334 (3 μM). Either drug alone had no effect. An NK_2 receptor blocker (MEN 10,627, 3 μM) was ineffective alone or in combination with either the NK_1 or the NK_3 antagonist. Tachyphylaxis to neuropeptide K or neuropeptide gamma, bombesin, endothelin-1, alpha, beta-methylene ATP or muscimol, as well as blocking of NO synthase by N^G-nitro-L-arginine was practically without effect. Tachyphylaxis to the neurogenic contractile actions of VIP or PACAP, as well as of TRH or CRF also failed to reduce CAP-evoked contractions.

We conclude that a combined action of tachykinins on NK_1 and NK_3 receptors is involved in the contractile effect of CAP but other, yet unidentified substances also participate.

Supported by ETT and OTKA grants (Hungary) and the A. Menarini Foundation (Italy).

NGF INDUCES A LATE PHASE OEDEMA IN RAT SKIN WHICH IS NOT INHIBITED BY MAST CELL AMINES OR AN NK_1 RECEPTOR ANTAGONIST

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NGF is known to regulate neuropeptide levels in inflammation. However, intradermal (i.d.) NGF in rat skin induces a histamine-dependent inflammation (Otten *et al,* 1985).We have investigated the time dependent effect of NGF on oedema formation in the rat skin of anaesthetized rats. Oedema formation was measured by the extravas cular accumulation of ^{125}I-albumin i.v. (Brain & Williams, 1985)

Results shown in the table are mean±s.e.m. µl plasma/skin site, n=6-12 for extravasation during time period shown.* p<0.05; **p<0.01; ***p,<0.001 from Tyrode sites, ††p<0.01 from NGF site.

Test Agents i.d. at 0h	*0-30min*	*2-3h*	*3-5h*
Tyrode (control, 0.1ml/site)	12.1±1.2	12.7±1.0	11.6±0.4
NGF (8pmol)	54.4±6.4***	20.8±2.2	45.2±4.8**
NGF (8pmol) + mepyramine (2.8nmol/site) and methysergide (1.9nmol/site)	10.1±1.6††		42.1±4.5*
NGF (8pmol) + SR140333 (1nmol/site)			51.0±9.8***

Results show early NGF-induced extravasation is inhibited by the coinjected H_1 antagonist mepyramine and 5-HT antagonist methysergide, as expected. The late phase of plasma extravasation was not inhibited with either mepyramine and methysergide or the NK_1-antagonist SR140333, given at 0h (or 3h, results not shown). These results indicate that NGF produces a late (3-5h) oedema, which appears to be independent of the early oedema.

Brain,S.D. & Williams,T.J. (1985) *Br.J.Pharmacol.* **86** 855-860.
Otten,U. *et al,* (1985) *Eur.J.Pharmacol.* **372** 379-393.

EXAMINATION OF NEUROPEPTIDES IN HUMAN SURAL NERVE BIOPSIES AND CORRELATION TO SENSORY AND AUTONOMIC DEFICITS

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Taking a biopsy from the sural nerve of patients suffering from polyneuropathic diseases is a commonly used technique for diagnostic evaluation. In this study we examined unmyelinated nerve fibres with an immunocytochemical method, using antibodies against different neuropeptides. We correlated the results with neurophysiological testings of the patients before the biopsy. Patients with moderate sensory and autonomic function deficits, that underwent sural nerve biopsy for diagnostic reasons, were examined. Before performing the biopsy we tested thermal thresholds for warm and cold sensation, for heat pain and for impact induced pain. Furthermore flare reaction after histamine iontophoresis was analysed and sympathetically mediated sweat production was measured after acetylcholine-iontophoresis and during mental stress. Areas on the lateral part of the foot were compared with unaffected areas on the upper leg.

Subpopulations of unmyelinated nerve fibres were examined after staining with antibodies against CGRP and substance P (SP) for afferent fibres, tyrosin hydroxylase (TH) and neuropeptide Y (NPY) for efferent sympathetic fibres. The total areas of marked clusters of unmyelinated nerve fibres were measured. These results were compared to the total area of nerve fibres, stained with the panneuronal marker PGP9.5. Correlations between relative neuropeptide losses in the sural nerve and functional tests were found in case of sympathetic functions with TH-content of the nerve fibres, in some cases also SP content and sensory thresholds were positively correlated. It is concluded, that the neuropeptide content in sural nerve fibres, made visible in light microscopy, can be used as a marker for the peripheral function of unmyelinated nerve fibres.

REGULATION OF THE EXPRESSION OF THE ENKEPHALINE GENE IN C6 RAT GLIOMA CELLS BY ANTISENSE OLIGONUCLEOTIDES

W. Bilecki, R. Przewłocki
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Proenkephalin (PENK) gene is widely expressed in brain structures as well as in non-neuronal peripheral tissues and it is thought to play a role in phenomena of stress, pain and seizures. An understanding of the regulation of PENK biosynthesis by numerous transcription factors is important for clarifying the physiological functions of the enkephalins. One way to identify the transcription factors involved in the regulation of opioid peptide gene expression is the application of antisense oligonucleotides to cell cultures *in vitro*. PENK mRNA has been detected in various cell lines. A great abundance of PENK mRNA was found in cultured C6 rat glioma cells. In this cell line activation of β-adrenergic receptors by norepinephrine (NA) increases cAMP levels and stimulates PENK mRNA.

The abundance of PENK mRNA was determined by blot hybrydization methods and semiquantitative reverse transcription - polymerase chain reaction. Treatment of C6 cells for 7 h with 10 μM NA strongly elevated PENK mRNA. In the presence of 10μM antisense oligonucleotide (AS-ODN) against CREB mRNA the level of PENK mRNA was significantly reduced. AS-ODN against c-fos mRNA as well as controls (sense and scramble oligonucleotides) were ineffective. The application of Lipofectin Reagent™, which in some cases improved uptake of oligonucleotides did not enhace the effect of used antisenses.

These data suggest that the PENK gene may be a physiological target for CREB but not C-FOS in C6 glioma cells.

This paper is supported by European Comission grant C.I.P.A.CT 94-0226

SUMATRIPTAN REDUCES THE ELECTRICALLY-EVOKED RELEASE OF CGRP- AND SUBSTANCE P-LIKE MATERIALS FROM THE RAT SPINAL CORD

L. Arvieu, A. Mauborgne, M. Hamon, F. Cesselin, S. Bourgoin (INSERM U 288, 75634 Paris cedex 13, France)

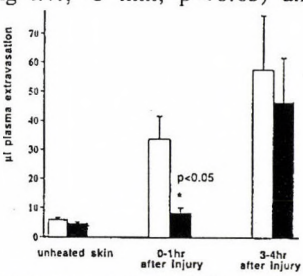
The aim of this investigation was to examine whether the anti-migraine drug sumatriptan (SU) could modulate the release of CGRP- and substance P (SP)-like materials (LM) from primary afferent fibers (PAF) entering the dorsal horn of the spinal cord. For this purpose, hemisected rat lumbosacral slices with dorsal roots were mounted in a three compartment chamber. The tissue was perfused in the central compartment with an artificial CSF (ACSF) at 0.4 ml/min. The dorsal roots were placed across bipolar electrodes and immersed in mineral oil in the lateral compartments. After a 2 h wash, 30 fractions of 2 ml were collected and their CGRPLM and SPLM content determined using specific radioimmunoassays. Two electrical stimulations (1 Hz, 2 ms, 5 mA) were applied during the collection of fractions 7-8 (s1) and 19-20 (s2). Test compounds were added to the ACSF from the 15th fraction to the end of the experiment. Under control conditions, the ratio of s2- over s1-evoked release of each peptide was remarkably stable: 0.67±0.01 for CGRPLM and 0.65±0.01 for SPLM. SU (0.01, 0.1 and 1 μM) significantly reduced the release of both peptides in a concentration-dependent manner: CGRPLM: -16%, -24% and -33%, respectively, SPLM: -23%, -35% and -45%, respectively. Both effects could be prevented by (-)tertatolol (1 μM) and methiothepin (1 μM), two mixed 5-$HT_{1A/1B}$ receptor antagonists, but not by WAY 100635 (0.1-1μM), a selective 5-HT_{1A} receptor antagonist. These data indicate that SU reduces the release of CGRPLM and SPLM from the rat dorsal horn very likely through the stimulation 5-HT_{1B} receptors on PAF, providing the first direct evidence for an inhibitory action of this anti-migraine drug on nociceptive neuronal pathways.

NK_1 AND BRADYKININ B_2 ANTAGONISTS AND INDOMETHACIN INHIBIT THE EARLY (0-1h) BUT NOT LATE (3-4h) OEDEMA INDUCED BY THERMAL INJURY.

S.D. Brain and L.Siney (Pharmacology Group, King's College, Manresa Road, LONDON SW3 6LX, UK)

Thermal injury (48°C for 5 min) of a 10mm diameter area of dorsal skin of anaesthetised rats leads to oedema formation, assessed by the extravascular accumulation of intravenous ^{125}I-albumin, which is highly significant when measured over the following 25 min. The oedema is substantially inhibited by either the CGRP antagonist $CGRP_{8-37}$ or the NK_1 receptor antagonist SR140333 (Siney and Brain *in press* and see Saria 1984). The early thermal injury is also inhibited by the bradykinin antagonist HOE140 (80 nmol/kg i.v., -5 min; $p<0.05$) and by indomethacin (10 nmol/kg i.p.-30 min; $p<0.01$).

Results indicate that even when the bradykinin antagonist and cyclo-oxygenase inhibitor are given in combination with SR140333 (120 nmol/kg i.v., -5 min), all at effective doses, a lack of inhibitory effect on the later phase of oedema formation is observed, see figure. These results suggest that although these agents contribute to the early neurogenic inflammation, they have little effect on the ongoing inflammatory response.



The response to topical thermal injury, with shaded columns showing the effect of systemic treatment with HOE140, indomethacin and SR140333 (doses as above). Mean ±s.e.m, n=5-12.

Saria, A. (1984) *Br. J. Pharmacol.*, 82, 217

Siney, L. & Brain, S.D. *Br. J. Pharmacol.*, in press.

This study is supported by the British Heart Foundation.

5-HYDROXYTRYPTAMINE IN GASTROINTESTINAL MOTILITY

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Most scientists in the field nowadays consider 5-hydroxytryptamine (serotonin; 5-HT) as a neurotransmitter of the enteric nervous system. Besides in enteric neurons, it is contained in much larger quantities in the enterochromaffine cells, which have been suggested to function as pressure transducers. 5-HT is involved in the regulation of secretion and motility of the gut. At least 14 5-HT receptor subtypes are now recognised, of which already 6 have been identified to occur in the gastrointestinal tract. Some 5-HT receptors do not fit into the currently applied classification scheme, the so-called orphan receptors. The diversity in receptor subtypes, the different transduction mechanisms, and the different possible localisations (nerves or smooth muscle) highlight the complexity of 5-HT pharmacology. As a model, the guinea-pig (especially the ileum) has been subject of extensive study. In recent years many new highly selective agonists and antagonists have become available, and results obtained with such compounds made it clear that the data obtained with guinea-pig intestine is difficult to extrapolate to the human gastrointestinal tract. Therefore, data obtained in other animal species and man is needed, and is fortunately currently appearing in literature. One of the questions that has popped up from these novel insights is the question why a prokinetic benzamide like cisapride can stimulate colonic motility in patients, whereas *in vitro* it induces relaxation and a decrease of spontaneous activity of human colonic circular muscle. Another unsolved issue is the one of the putative $5\text{-}HT_{1P}$ receptor, an orphan 5-HT receptor subtype which has so far only been identified in electrophysiological experiments. As the gastrointestinal prokinetic benzamides, like cisapride, are antagonists of these putative receptors, their identity and function needs to be clarified. These are just some of the many intriguing questions which remain to be resolved in the 5-HT field.

VISCERO-VISCERO REFLEXES IN GASTROINTESTINAL FUNCTION

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There is increasing evidence that functioning of the gastro-intestinal tract depends upon numerous long reflexes involving the central nervous system. Most of afferent messages are triggered from both splanchnic and vagal afferents mainly projecting to the NTS and DMNV, the afferent pathway activation being modulated by higher structures such as the hypothalamic nuclei.

These reflexes are also modulated by peripheral pathogens activating afferent fibers. Several neuropeptides like CCK and enkephalins play a major role in vago-vagal reflexes involved in nutrients-induced gastric and pyloric or colonic motor adaptation to the meal.

Colonic hypersecretion related to oral enterotoxin-administration is often linked to activation of primary afferents via local activation of immunocytes which trigger the CNS release of neurotransmitter such as neurokinin A or 5-HT as well as cytokines such as $IL_{1\beta}$ which is turns activate vagal efferents to induce water and ions secretion. Similarly in endotoxinic shock or allergic challenge in sensitized animal the supraspinal release of $IL_{1\beta}$ is responsible of gut motor alterations. Inhibition of proximal gut motility and activation of colonic muscles are mediated through the secondary release of prostaglandins and corticotropin-releasing factor respectively.

Rectal distension is also able to trigger a delayed colonic hypersecretion also involving activation of extrinsic neural pathway in which both neurokin receptors mainly NK_2 but also the CNS release of $IL_{1\beta}$ play a role. In all these models, local mast-cell degranulation appears as a priming events to generate such visceral hypersensitivity.

In local inflammatory processes such those initiated by an hapten like trinitrobenzene sulfonic acid, or acetic acid, the release of bradykinin acting through BK_2 receptor subtypes in essentiel to sensitize terminal endings of primary afferents generating motor alterations through activating of CGRP and SP containing C fibers afferents.

Finally, all these data support the view that alterations in viscero-visceral reflexes in response to pathogens is of paramount importance in the genesis of associated motor and secretory disturbances and subacute hyperalgesic states.

MECHANISMS BY WHICH PROSTAGLANDINS SENSITISE SENSORY NEURONES TO BRADYKININ

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Prostaglandins, including prostaglandin E_2 (PGE_2) cause sensitisation of sensory neurones to noxious stimuli, including bradykinin (Bk). The mechanism(s) underlying this sensitisation is unclear, but it has been suggested that cAMP may be involved (e.g. Hintgen et al., 1995). Bk increased $[Ca^{2+}]_i$ in single, small diameter, capsaicin-sensitive dorsal root ganglion (DRG) neurones and stimulated release of substance P (SP) from DRG cultures. PGE_2 also elevated $[Ca^{2+}]_i$ in this population of cells and caused a small increase in SP release, but, in contrast to Bk, it did not stimulate phosphoinositidase C (PIC). Pretreatment with PGE_2 enhanced Bk-evoked increases $[Ca^{2+}]_i$ in single neurones. Most strikingly, it increased the number of cells that responded to low concentrations of Bk. PGE_2 also potentiated Bk-evoked SP release, inducing a leftward-shift in the concentration-response curve. PGE_2 (1μM) had no effect on Bk-evoked [^{3}H]inositol trisphosphate formation, suggesting that its action was downstream of receptor-PIC interactions. PGE_2 stimulated adenylate cyclase activity in DRG cultures, at concentrations and times consistent with that required for both the direct and sensitising effects of PGE_2 on $[Ca^{2+}]_i$ and SP responses. The PGE_2-mediated sensitisation of these responses to Bk could be mimicked by the cAMP analogue, dbcAMP and were partially inhibited by the cAMP-dependent protein kinase (PKA) inhibitor H89, suggesting a role for PKA-mediated phosphorylation in sensitisation.

Hintgen, C.M., Waite, K. J. and Vasko, M.R. (1995) J. Neurosci. ***15****, 5411-5419.*

THE EFFECT OF THE NK-1 AND NK-3 RECEPTOR ANTAGONISTS, SR 140333 AND SR 142801, ON THE MORPHINE WITHDRAWAL RESPONSE IN THE GUINEA-PIG

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It has been proposed that substance P or other tachykinins are mediators of the opioid withdrawal response in the enteric and central nervous systems. The aim of the present study was to determine whether the morphine withdrawal response in guinea-pigs was inhibited by the NK-1 receptor antagonist, SR 140333, or the NK-3 receptor antagonist, SR 142801. Guinea-pigs were given subcutaneous (sc) injections of increasing doses of morphine sulphate twice daily for three days (total dose, 410 mg/kg). Thirty min after the final dose of morphine, the guinea-pigs were given 3 mg/kg sc of either SR 140333 or SR 142801. Control animals were given saline, vehicle (50% ethanol) or inactive enantiomer of the NK-1 receptor antagonist, SR 140603. Withdrawal was induced 30 min later with naltrexone hydrochloride, 15 mg/kg sc. Locomotor activity and behavioural responses were measured for 90 min. Animals were killed and expression of Fos, the protein product of the immediate-early gene, *c-fos*, was measured using immunohistochemical techniques. Neither the NK-1 nor the NK-3 receptor antagonist significantly reduced the locomotor response to morphine withdrawal, although the number of body shakes was reduced by SR 142801. Both SR 140333 and SR 142801 significantly reduced the number of Fos stained neurons in the piriform cortex, habenular, and substantia nigra compacta, and SR 140333 also reduced the number of Fos positive neurons in the cingulate cortex. It is concluded that NK-1 and NK-3 receptors are involved less in the motor behaviours elicited by morphine withdrawal than in other aspects of the response, possibly in the emotional or distress components.

SR 140333, SR 142801 AND SR 140603 KINDLY DONATED BY DR X. EMONDS-ALT, SANOFI RECHERCHE.

EFFECTS OF TACHININIS ON ELECTRODERMAL CONDUCTIVITY

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The influence of systemic administration of tachikinins (TCH): SP, NKA, kassinin (KSN) as well as of galanin (GAL) was investigated in rats' epileptical motor seizures induced by PTZ. KSN and GAL possessed antiepileptic action and simultaneously normalized the peripheral reflex regulation. TCH, which realized the influence through NK1 receptors, induced a decrease of electrical conductivity and asymmetry reaction of the homotop skin regions. NKA did not prevent motor seizures but decreased the electrical conductivity of the skin during and after motor seizures. GAL increased the latency of clonic-tonic seizures and prevented PTZ-induced motor epileptical fits in adult rats and rat's pups. These data confirmed the antiepileptic effects of GAL demonstrated by A.A. Shandra & A.M. Mazarati (1994). The synthetic predecessor of KSN was more effective than NKA for central epileptical fit prevention. PreKSN inhibited afterdischarges in rat's cortex and hippocampus. Dissociation of central and peripherical effectiveness of preKSN during epilepsy is caused by the TCH receptors distribution in the CNS and peripheral tissues. These data can be explained on the basis of the TCH effects on the blood flow and vascular permeability (J. Szolcsányi, 1992). TCH induce an increase in the permeability of post capillary venules leading to plasma extravasation.The research was supported by the ISF (Soros, USA), Grant NB1000.

SPINAL CORD LOCALISATION OF KININS AND THEIR RECEPTORS

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A putative role for bradykinin (BK) has been suggested in the control of nociceptive and autonomic functions at the level of the spinal cord. Studies were undertaken to assess the detailed anatomical distribution of both BK-like immunoreactivity (BK-LI) (by classical immunocytochemistry and confocal microscopy immunofluorescence) and kinin B_2 receptors (by in vitro autoradiography) in all segmental levels of the rat spinal cord. Moreover, changes of receptor binding sites were compared with those of BK-LI following various surgical lesions and pharmacological treatments. BK-LI was lightly located in all laminae of the grey matter with particular strong concentrations in ventral horn motoneuron perikaryas and proximal processes, islet and antenna-type cells in the dorsal horn and on compact cells in the nucleus dorsalis. In contrast, [^{125}I-Tyr^8]BK receptor binding sites, characterized as a B_2 subtype, were predominantly located to the substantia gelatinosa of the dorsal horn. The ventral horn showed a rather homogenous distribution of [^{125}I-Tyr^8]BK binding over the neuropil, with silver grain alignments surrounding motoneuron perikaryas and proximal processes. Marked increases of BK-LI in the dorsal horn and decreases in the ventral horn were seen following either unilateral dorsal rhizotomy or sciatic nerve section. The same lesions decreased [^{125}I-Tyr^8]BK binding sites in ipsilateral laminae I-III. Decreases of receptor binding sites were also seen in laminae I-VII after neonatal treatment with capsaicin or destruction of spinal noradrenergic fibers with intrathecal 6-hydroxydopamine. These results reveal: a) the enriched presynaptic localisation of B_2 receptors on both primary sensory fibers (Aδ and C) and spinal noradrenergic terminals, b) a non sensory origin for BK, c) an up-regulation of the content of BK-LI associated with a down-regulation of B_2 receptor in the dorsal horn. These neuroanatomical findings substantiate earlier functional implications reported for kinins in nociception. In addition, a role for kinins in motor control is suggested at the level of the spinal cord. [Supported by the Medical Research Council of Canada].

THE EFFECTS OF NEUROPEPTIDE Y AND RELATED PEPTIDES UPON GASTROINTESTINAL EPITHELIA

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Neuropeptide Y (NPY) peptide YY (PYY) and pancreatic polypeptide (PP) exert numerous effects upon gastrointestinal (GI) processes. One common response observed in many mammalian GI mucosal preparations, is a reduction in both basal and secretagogue-stimulated electrogenic anion secretion. Antisecretory effects exerted by the enteric neurotransmitter, NPY and its hormonal analogue, PYY are mediated by either Y_1- or Y_2-like receptors (or a combination of the two) present on either prejunctional terminals or postjunctional, epithelial surfaces. NPY is a prominent enteric neuropeptide which is completely co-localised with vasoactive intestinal polypeptide (VIP) specificallly in rat jejunum myenteric and submucous neurones. Double-label immunogold techniques have shown that both peptides are located in the same 80 nm diameter, dense-cored vesicles. These unrelated neuropeptides are functional antagonists, VIP exerting well characterised long-lasting secretory effects while NPY is inhibitory. Studies in our laboratory, using rat jejunum and descending colon have shown that PYY and NPY exert their inhibitory effects either via epithelial Y_2 receptors, or a combination of Y_1 and Y_2 subtypes on different cells, respectively.

The availability of the Y_1-selective antagonist, BIBP3226 has enabled better (albeit partial) classification of NPY/PYY responses in both *in vitro* preparations and epithelial cell monolayers. Human adenocarcinoma cell lines have been notably devoid of NPY receptors except for one line called Colony-6. This cell line expresses Y_1-receptors as well as a PP-sensitive population, which are insensitive to BIBP3226. Such receptor heterogeneity is becoming the rule rather than the exception. We have also stably transfected wild-type and mutant Y_1 receptor cDNA into human epithelial cells, including non butyrate-treated HT-29. These recent studies have provided an interesting insight into the functional consequences of expressing Y_1 receptors with a point mutation in the third intracellular loop, an area known to be important for G-protein coupling.

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SP AND VIP IMMUNOREACTIVITY OF RAT CEREBELLAR CORTEX IN THE ACUTE MODEL OF EPILEPSY

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Cerebellar functional involvement in the acute model of epilepsy was suggested by massive accumulation of neuropeptide Y in cerebellar cortex (Ćulić et al., *Pharmacol. Toxicol., 1995, 76, Suppl. IV, a.4*). The aim of the present study was to investigate the substance P (SP) and vasoactive intestinal peptide (VIP) immunoreactivity of Wistar male rat cerebellar cortex in the acute model of epilepsy induced by parenteral administration of penicillin (1000000 I.U/kg, i.p). Two hours after penicillin administration the treated and control (untreated) rats were sacrificed and cerebellar specimen were immunostained later by streptavidin-biotin technique using the prediluted polyclonal antibody against SP and VIP (Zumed, San Francisco, CA). SP immunoreactivity in cerebellar cortex, particularly in Purkinje cell layer was increased but there was no change of VIP immunoreactivity in rats treated by penicillin when compared to control ones. These changes in the SP and VIP immunoreactivity in cerebellar cortex may also reflect the cerebellar involvement in epilepsy.

NEUROPEPTIDERGIC INNERVATION OF MEYNERT'S BASAL NUCLEUS IN PRIMATES: FUNCTIONAL AND PATHOLOGICAL IMPLICATIONS

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Immunohistochemical studies of Meynert's basal nucleus of Macaca mulatta have proved presence of substance P, CGRP, somatostatin, NPY, parvalbumin and neurotensin-containing extrinsic axons; neurotensin- and somatostatinergic axons derive also from autochthonous nerve cells. CGRP- and parvalbumin-containing axons establish axo-somatic and axo-dendritic synapses with large cholinergic principal cells displaying choline acetyltransferase immunoreaction. Principal cells are equipped with numerous auto- or homeo-synapses, deriving from initial axon collaterals; these are closely related to synapses of CGRP-positive axons and to sites exerting immunoreaction with biotinylated α-bungarotoxin, labelling the α7 subunit of the neuronal nicotinic acetylcholine receptor (nnAChR). Binding sites of mAb-35 and mAb-74 are distinctly differing from that of the α7 subunit, providing the first structural proof for the pleiotropy of nnAChR. Failure of maintanance of the nnAChR by CGRP might result in lack of cholinergic activation of the prefrontal cortex, characterizing Alzheimer's disease.

^{3}H-SUBSTANCE P BINDING SITES IN HUMAN SKIN

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Substance P (SP) is an important inflammatory mediator that has been found to stimulate fibroblast and endothelial cells proliferation (1) and has been suggested to be involved in the pathogenesis of certain inflammatory skin diseases, such as psoriasis (2). Recent studies using cultured normal human keratinocytes revealed the presence of SP receptors (3). The present study was undertaken to examine the characteristics of ^{3}H-SP binding sites on biopsies from normal-appearing skin of seven healthy subjects. For the saturation experiments, aliquots of tissue homogenate were incubated for 20 min with a range of ^{3}H-SP concentrations (0.0625-0.5 nM) and the specific binding was defined as that displaceable by 10^{-4} M SP. Analysis of saturation curves with the non linear iterative curve-fitting program LIGAND gave a significantly better fit using two sites binding compared to the single site model. The low affinity sites (K_D=5.5x10^{-6} M) were more numerous than the high affinity sites (K_D=3.8x10^{-9} M). The binding characteristics of ^{3}H-SP in the human skin were further investigated in competition studies employed for NK1 the selective agonist [Sar^{9},Met $(O_2)^{11}$]-SP; for NK2 the peptide antagonist MEN 10,376 and NKA; MEPHE7NKB and NKB for NK3 receptor site.
The results of our experiments showed that only [Sar^{9},Met $(O_2)^{11}$]-SP was able to displace ^{3}H-SP binding, suggesting the presence of a NK1 receptor population.

1) Dalsgaard C.J., Hultgardh-Nilsson A., Haegerstrand A., Nilsson J. Regul. Pept. 25: 1-9, 1989.
2) FarberE.M., Nickoloff B.J., Rech B., Fraky J.E. J. Am. Acad. Dermatol. 14: 305-311, 1986.
3) Restoff B., Kemeny L., Michel G., Ruzicka T. J. Invest. Dermatol. 98: 510, 1992.

MICROVASCULAR REACTIVITIES IN ALOPECIA AREATA: POSSIBLE INVOLVEMENT OF SENSORY NERVES. A STUDY WITH LASER-DOPPLER FLOWMETRY

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Alopecia areata (a.a.) is a common dermatosis that shows a sudden occurrence of one or more bald patches on the scalp. Although aetiology of a.a. is unknown, numerous experimental data indicate an important role of cutaneous microcirculation: peri and interpapillary microvessels showed a reduction of their diameter and an evident vacuolisation of the endothelial cells (1). It is known that the skin is richly innervated by neuropeptidergic sensory nerves (2) that play an important role in the regulation of microvascular cutaneous circulation. The results of this study shows a significant reduction of substance P and calcitonin gene related peptide (CGRP) cutaneous levels but not of vasoactive intestinal polypeptide in scalp biopsy of patients affected by a.a. in comparison to healthy subjects tissues. Moreover we used Laser-Doppler Flowmetry to study cutaneous microcirculation of the scalp. Our experiments showed a lower basal blood flow in a.a. subjects. In order to evaluate if this vasoconstriction could be due to a sensory nerves disorder correlating with the loss of vasoactive neuropeptides detected, we studied the effect of intradermal injection of CGRP. In control subject we obtained an increase in blood flow that returned to basal values in 20-30 min; in subjects affected by a.a. the vasodilatatory reaction was much greater and after 30 min the flow remained at maximal levels.It is therefore supposable an hyperreactivities of peripheral vascular receptor for vasodilatatory substances, such as neuropeptides, probably because of the lack of these substances and/or a disorder of vasoconstrictor sympathetic reflexes. Then we analysed the possibility that also the second messenger nitric oxide, produced by endothelial cells, could be involved in the disregulation of vasomotion. Injecting CGRP intradermically after a previous injection of L-NAME, an inhibitor of NO synthase, in control subjects we obtained a reduction of the vasodilatatory effect of CGRP, indicating that CGRP at least in part acts stimulating the production of nitric oxide by endothelial cells. In a.a. we obtained an inhibition of CGRP effect, not so appreciable as in controls.

1) Rook GAW, WilkinsonJD, Ebling FJG: Textbook of Dermatology, Fifth Edition 1992: 2586-2594
2) Bjorklund H, Dalsgaard CJ, Jonsson CE and Hermansson A: Cell Tissue Res. 1986, 243:51-57

NOVEL IMMUNOHISTOCHEMICAL PROCEDURES FOR SIMULTANEOUS DETECTION OF NEUROPEPTIDES IN HUMAN BRAIN

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We propose two distinct novel procedures that are suitable for simultaneous detection of two antigens within the same sections of adult human brain. For immunofluorescence mapping, a series of pretreatments (1) was elaborated which suppresses the intense autofluorescence of adult human neural tissue. The other technique is a modification of the silver-gold intensification (2) used for amplification of diamino-benzidine (DAB) reaction end-product resulting in a more effective and sensitive labeling.
(1) For immunofluorescence, the free-floating sections were (a) dehydrated gradually in ethanol, (b) delipidated in acetone, (c) stained with 0.3% Sudan Black (M. W. Wessendorf, personal communication) solved in 70% ethanol, and (d) finally slightly differentiated in 70% ethanol. Thereafter, single and double immunofluorescent labelings were performed.
(2) In the improved silver-gold intensification procedure, the sections were treated with 10% thioglycolic acid for 30 min - to suppress endogeneous argyrophilia - prior to the application of the immunoreagents. The immunoreactions were developed with DAB in the presence of Ni^{2+} ion and the polymerized Ni-DAB was silver-intensified according to Liposits et al.(Neuroscience, 13: 513, 1984). This technique allows the use of four times higher antibody dilutions, and lowers the background, compared to conventional methods.
We demonstrate the capacities of these techniques by means of simultaneous revelation of different neuropeptides in adult human diencephalon.

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INVOLVEMENT OF CAPSAICIN - SENSITIVE AFFERENT NERVES IN THE MEDIATION OF REACTIVE CUTANEOUS HYPERAEMIA IN THE RAT

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Capsaicin-sensitive afferent nerves participate in the reflex and local regulation of vascular reactions in various organs. The aim of this study was to investigate the possible participation of capsaicin-sensitive nerves in the mediation of reactive cutaneous hyperaemia. The right and left sciatic nerves of adult male Wistar rats (n=6) were exposed and treated with capsaicin (1%, 100 μl) and its vehicle, respectively. One week later the animals were anaesthetized and the abdominal aorta was exposed by a midline incision. Placing a thread underneath the aorta permitted a transient occlusion of the vessel. Skin blood flow was measured with a Periflux 2-channel laser-Doppler flowmeter. The flow probes were placed over the lateral skin of the hindpaws. After obtaining stable baseline values aortic blood flow was stopped for 1, 2 or 3 min and blood flow was measured for another 10 min. In control skin areas skin blood flow showed a time-dependent increase after aortic occlusion; perfusion increased by $31.5\pm12.2\%$, $152.6\pm17.8\%$ and $297.9\pm32.2\%$ after occlusion periods of 1, 2 and 3 min, respectively. In contrast, in skin areas relating to the capsaicin treated nerve this hyperaemic response was slower in onset and reduced in magnitude; after a 3-min occlusion the increase in skin blood flow decreased by $51.8\pm3\%$ as compared to the control. The present findings provide further evidence for a significant role of capsaicin-sensitive afferent nerves in the mechanism of postocclusive cutaneous hyperaemia. Additional studies are needed to identify the transmitter(s) involved in the mediation of this response.
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PERINEURAL CAPSAICIN TREATMENT ALTERS THE INNERVATION PATTERN OF RAT HAIRY SKIN

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Direct application of capsaicin onto peripheral nerves results in a selective impairment of unmyelinated afferent nerve fibres. The aim of the present quantitative immunohistochemical study was to determine cutaneous fibre populations affected by perineural capsaicin treatment. The sciatic nerves of adult rats were exposed and treated with capsaicin (1%, 100 μl) 3, 14 or 42 days prior to an i.v. injection of a colloidal silver solution followed by painting the dorsal hindpaw skin with 5% mustard oil to elicit a neurogenic inflammatory response. Normally innervated areas showed small vessels labelled with silver, whereas areas innervated by the treated nerve did not. Cryostat sections of the skin were processed for immunohistochemistry using antisera against substance P (SP), calcitonin gene-related peptide (CGRP), somatostatin (SOM), protein gene-product 9.5 (PGP), growth associated protein 43 (GAP) and neurofilaments (RT97). The density of the innervation was expressed as immunopositive nerve fibres per mm^2 skin surface area.
Perineural capsaicin treatment resulted in a long-lasting, significant depletion (80-100%) of SP-immunoreactive (IR) fibres in all layers of the skin. In the epidermis a marked reduction of PGP- and GAP-IR fibres was found. The densities of CGRP-, SOM- and RT97-IR fibres were not significantly changed. The present results confirm and extend earlier findings by showing a selective loss of epidermal nerve fibres after perineural treatment with capsaicin. Although this group of fibres involves mainly SP-positive nerves, the present data demonstrate the existence of a large population of capsaicin-sensitive epidermal fibres which do not contain the peptides studied, but can be demonstrated with PGP- and GAP-immunohistochemistry.
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CHROMOGRANIN A AND B AND SECRETOGRANIN II IN CEREBROSPINAL FLUID

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The chromogranins, acidic proteins of large dense core vesicles, are found throughout the central nervous system. We have raised antibodies against synthetic peptides representing sequences in these molecules.In a previous study we have already characterized chromogranin A and secretogranin II in human cerebrospinal fluid (CSF) by molecular size exclusion chromatography followed by radioimmunoassay (RIA). We have now obtained analogous results for chromogranin B which is proteolytically processed to a high degree. Thus, in the CSF both secretogranin II and chromogranin B are characterized by nearly complete processing, whereas chromogranin A is processed less. For chromogranin B and secretogranin II there is no difference in the chromatographic pattern between lumbar and ventricular CSF. For chromogranin A the ventricular CSF apparently contains a higher amount of the free peptide GE-25. We are presently investigating possible changes in the levels and in the proteolytic processing of the chromogranins in various diseases. In cases of Parkinson proteolytic processing was unchanged compared to controls. In contrast to reports in the literature chromogranin A levels were not reduced. In multiple sclerosis no difference between controls and cases with the first attack could be observed.

ENDOTHELIN-INDUCED CGRP RELEASE FROM GUINEA-PIG AIRWAYS: ROLE OF NO.
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The ability of endothelin to potentiate the capsaicin-evoked release of substance P and CGRP has been shown from rat dorsal horn neurons in culture and guinea-pig airways. Here, we investigated the mechanism through which endothelin causes the release of sensory neuropeptides from slices of guinea-pig airways. Preparations were superfused with oxygenated Krebs solution at 37°C in the presence of thiorphan (1 μM). Superfusates were freeze-dried and peptide content was measured by an immunometric assay. The selective agonist for endothelin ET_B receptor, IRL 1620, potentiated the release of CGRP induced by capsaicin. The selective antagonists of ET_B receptor, BQ 788, and of ET_A receptor, FR 139317, produced a partial inhibition of the release induced by the combination of ET-1 and capsaicin. A mixture of BQ 788 and FR 139317 abolished the potentiation of CGRP release induced by ET-1. Indomethacin reduced partially the potentiation of CGRP release induced by ET-1. The nitric oxide (NO) synthase inhibitor, L-NAME, but not the inactive enantiomer D-NAME, reduced the ET-1 and capsaicin-induced CGRP release without affecting the release induced by capsaicin alone. ET-1 potentiates the release induced by capsaicin by stimulation of both ET_A receptors and ET_B receptors. This effect of ET-1 is partially mediated by prostanoid and NO release.

DISTRIBUTION AND FINE STRUCTURE OF THE DIFFERENT NEUROPEPTIDE-CONTAINING NERVE ELEMENTS IN THE GASTROINTESTINAL TRACT.
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Immunohisto- and immunocytochemical investigations were carried out to study the distribution and localization of different neuropeptide-containing /substance P (SP), somatostratin (SRIF), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and cholecystokinin (CCK)/ nerve elements in the wall of the gastrointestinal tract. Large number of SP and VIP immunoreactive (IR) nerve cell bodies were observed in the myenteric plexus. The SRIF IR perikarya were mainly found in the submucous plexus. A few CCK IR cell bodies were observed in the myenteric plexus of the proximal portion of the small intestine. However, no CGRP IR cell bodies were found in the intrinsic plexuses. Abundant IR nerve fibres were observed mainly in the inner circular muscle layer and in the tunica propria. In several cases they were in a close situation to the smooth muscle cells and to the epithelial cells. In additon, some IR nerve fibres were found around the blood vessels and in a very close contact to the immuno-competent cells (lymphocytes, plasmacells). Apparent synaptic contacts were observed between the IR nerve processes and IR negative nerve cell somata. These neuropeptide-containing fibres may participate in regulation of smooth muscle activity, the function of the epithelial cells and blood flow. In addition, they may play an important role in the neuroimmunological processes and they have direct effect on the other intrinsic nerve elements.

KININ MODULATION OF NEUROPEPTIDE RELEASE

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Kinins exert a broad spectrum of actions either by acting directly on effector cells or indirectly by activating the production or release of a number of mediators from intermediary cells. Primary sensory neurons are amongst these intermediary cells and the calcitonin gene-related peptide (CGRP) and the tachykinins substance P (SP) and neurokinin A (NKA) are amongst these mediators. Local application of kinins to certain tissues, including the airways, conjunctiva, skin, etc., causes an inflammatory response that is almost entirely mediated by tachykinins released from sensory nerves. Kinins are powerful stimulants of sensory neuropeptide release from various preparations of peripheral tissues and from dorsal root ganglion neurons in culture. A major role of prostanoids in the ability of kinins to release sensory neuropeptides has been demonstrated. More recently, evidence has been presented that endogenous kinins cause inflammation *via* sensory neuropeptide release: thus, inflammatory responses to acidic media, clyclophosphamide, cold air and antigen are mediated by kinins that subsequently stimulate sensory nerves and release tachykinins. In neutral endopeptidase (NEP) knock out mice, an increased baseline plasma extravasation was observed. This increase in plasma extravasation was abolished by a kinin B_2 receptor antagonist and by a tachykinin NK_1 receptor antagonist: thus, absence of NEP results in reduced degradation of kinins that increase plasma extravasation *via* a neurogenic inflammatory mechanism.

EFFECTS OF ARGININE-VASOPRESSINE (4-9) ON LEARNING AND MEMORY RETENTION PROCESSES IN LESIONED RATS USING WATER MAZE TASK

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The Vasopressine analogue Arginine-Vasopressine$_{(4-9)}$ (AVP_{4-9}) like Arginine-Vasopressine affect the learning and memory retention processes. The lesions of the infralimbic prefrontal cortex (ILPFC) disturb short term memory. The aim of this study was to obtain a data on the effects of AVP_{4-9} on learning and memory retention in rats with lesions of ILPFC using water maze. Six groups (8 rats per group) were used divided in two main groups: S=sham and L=lesioned. The lesions (or sham) were made under aneasthesia using stereotaxic technique, silver electrodes with (or without) electrical current 0.2mA, 20 sec. Two weeks after the operation and recovery the rats were injected 30 min before first testing i.p. with saline (0.1ml/100g) or with 0.1 or 0.01 μg/kg AVP_{4-9}. We used a standard circular pool (Morris maze). The rats received a block of 4 trials (intertrial interval of 15 min) for 5 days. Swimming patterns were registered by a video camera and analysed by computerized image analysis. The sham lesioned control rats learned the task. The sham lesioned injected with AVP_{4-9} rats learned the task also. Lesioned control rats failed to learn the task. The lesioned rats injected with AVP_{4-9} (in both doses used) showed improving efect on learning. The sham lesioned controls showed memory retention effect on last trial (without the platform) as compared with lesioned controls. The AVP_{4-9} treated animals (sham and lesioned) performed last trial as sham lesioned controls. The obtained data permitted us to suggest that AVP_{4-9} in both doses applied improve learning and memory retention in lesioned rats using water maze task.

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NOCICEPTIN MODULATES TACHYKININ RELEASE FROM PERIPHERAL ENDINGS OF SENSORY NERVES IN GUINEA-PIG RENAL PELVIS

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Nociceptin is a novel heptadecapeptide which share the NH_2 terminal tetrapeptide sequence common to all opioid peptides. The aim of this study was to ascertain whether nociceptin has any neuromodulatory action on tachykinin release from peripheral endings of capsaicin-sensitive primary afferent neurons in the guinea-pig isolated renal pelvis (GRP). GRP were electrically field stimulated (EFS) at 5 Hz for 10 s, 0.5 ms pulse width at 60 V in the presence of indomethacin (10 μM) under isotonic recording condition (Maggi et al., Neuroscience 46: 549-559, 1992). Nociceptin (10 nM-1 μM, 5 min before EFS) produced a concentration-dependent inhibition of the inotropic response to EFS without affecting the basal spontaneous contractile activity: at 1 μM the maximal inhibition of the response to EFS was 85±4% (n=8) with an EC_{50} of 28.4 nM (18-52 nM are 95% c.l.). Naloxone (0.3 μM, 15 min before EFS), naltrindole (3 μM) and Nor-binaltorphimine (1 μM) were ineffective in blocking the effect of a submaximally effective concentration (0.3 μM) of nociceptin. On the contrary, naloxone almost suppressed the inhibitory response to dermorphin (0.1 μM). Nociceptin (1 μM) did not change the inotropic response to exogenously applied neurokinin A (0.01 μM, n=5).

In conclusion nociceptin inhibits the release of tachykinins from peripheral endings of capsaicin-sensitive primary afferent neurons through a mechanism independent of activation of either μ -, δ - or κ - opioid receptors.

ARE SENSORY NEUROPEPTIDES INVOLVED IN OPIOID-INDUCED GASTROPROTECTION?

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Data of the literature on the effects of opioids on different types of experimental gastric damage are contradictory. We found that morphine inhibited the acidified ethanol-induced gastric mucosal damage in a dose-dependent manner in the rat, its ID_{50} value proved to be 0.07 mg/kg sc. - which is far below its analgesic dose (1). Our recent data suggest that both mu and delta receptors may be involved in the gastroprotective effect. How opioids are able to protect the gastric mucosa against the necrotizing effect of ethanol ? Recently, it was published that endogenous NO system is likely to have role in the gastroprotective effect of morphine, since N^G-nitro-L-arginine inhibited the protective effect of morphine, while pre-treatment with D-arginine resulted in restoration of morphine-induced gastroprotection (2). The question was raised whether the effect of morphine on the NO system is direct or indirect ? It is known that some of the sensory neuropeptides - e.g. CGRP - exert gastroprotective action (3), probably by stimulating the mucosal blood flow in the gastric mucosa and NO is likely to be involved in this action (4). In order to examine the role of sensory neuropeptides in the gastroprotective effect of morphine the rats were pre-treated with capsaicin (20, 30, 50 mg/kg sc.) for 3 consecutive days and 10 days later the effect of morphine on gastric lesions induced by 100% ethanol was studied. It was found that the gastroprotective effect of morphine highly decreased. Since this dose of capsaicin produced functional ablation of capsaicin-sensitive sensory neurones, the reduction of the protective effect of morphine might be due to the involvement of neuropeptides in its action.

References: 1. Gyires, K., Arch. int. Pharmacodyn. 306, 170, 1990. 2. Gyires, K., Eur. J. Pharmacol., 255, 33, 1994. 3. Lippe I.T. et al., Regul. Pept. 266, 35. 1989. 4. Lippe, I.T. and Holczer P., Regul. Pept. 34, 122, 1991.

CHEMOSENSITIVE C-FIBERS IN HUMAN SKIN NERVES: POSSIBLE CONTRIBUTIONS TO ITCH SENSATIONS AND FLARE REACTIONS.

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Microneurography techniques were used to study chemosensitivity of single afferent C - fibers in the superficial peroneal nerves of healthy volunteers. To get a bias free sample of units an electrical stimulation and a marking technique utilizing the slowing of nerve conduction were used for identifying individual C - fibers. In a sample of more than 200 afferent C-fibers sampled several distinct classes were found that were affected by histamine and / or by the irritants mustard oil and capsaicin. The most common class are the heat and mechanoreceptive units (CMH) of which about half were either excited or sensitized by mustard oil and capsaicin. Excitation and sensitization to subsequent mechanical and/or heating stimuli were not always coincident in individual units suggesting different membrane mechanisms. Excitation by histamine iontophoresis was observed only in a small subgroup of CMHs. Even in this small subgroup the responses to histamine were usually weak. Therefore, CMHs probably contribute little or nothing to itch sensations. However, two other classes of C-fibers were found to be sensitive to chemical irritants: CH units, responding to heating, but not to mechanical stimulation and "silent" CMiHi units unresponsive to both forms of physical stimulation. Of the latter about one third turnd out to be excited and / or sensitized by mustard oil and capsaicin. These mechano-insensitive units and in particular some CH units tended to show vigorous responses to histamine. By mapping of the receptive fields of these different fiber classes we tried to find out if they possibly can contribute to axon reflex flare reactions. It was found, that the receptive fields of many CMH units are too small to account for the extension of axon reflex flares induced by local application of histamine.

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CAPSAICIN SENSITIVE AFFERENTS MEDIATE CHRONIC COLD, BUT NOT MECHANICAL, ALLODYNIA-LIKE BEHAVIOR IN SPINALLY INJURED RATS

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We have developed an animal model of chronic pain after ischemic spinal cord injury which in some aspects mimics the characteristics of central pain in patients with spinal cord injury. The main symptom of this model is pain-like response to cold or innocuous mechanical stimuli applied to skin areas immediately rostral to the dermatome of the injured spinal segments. The aim of the present study was to evaluate the involvement of capsaicin sensitive afferents in mediating these responses. Resiniferatoxin (RTX), an ultrapotent capsaicin analogue, was used to produce long-term desensitization of capsaicin sensitive afferents. The responses to cold, innocuous mechanical or noxious heat stimuli were examined after either RTX or vehicle treatment in chronic allodynic rats. A single subcutaneous (s.c.) injection of 0.5 mg/kg RTX produced marked hypoalgesia to noxious heat as assessed with the tail flick test. The enhanced response to cold observed in allodynic rats were totally normalized by RTX. In contrast, the mechanical allodynia-like response was not influenced by RTX. The effect of s.c. RTX on heat or cold response recovered in parallel after three weeks. Vehicle produced no effect on responses of rats to stimuli of any modality. It is concluded that the enhanced response to cold, but not to light touch, observed in the chronic allodynic rats is mediated by capsaicin sensitive afferents. Capsaicin and related compounds may have a therapeutic potential for treating neuropathic pain elicited by some, but not all, modalities of stimulation.

Pharmacological profile of SB 223412, a novel, potent and selective, non-peptide NK-3 recepto antagonist.

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In this study we describe the pharmacological profile of SB 223412 a member of a novel class o potent and selective non-peptide NK-3 receptor antagonists based on the 2-phenylquinoline backbon (1).

SB 223412 ((S)-(-)-N-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide) had potent binding affinity for the human NK-3 (hNK-3) receptor (K_i = 1.0 ± 0.1 nM for displacement o [^{125}I]-[MePhe^7]-NKB binding from hNK-3-CHO membranes; n = 8). SB 223412 (10-1000 nM produced a concentration-dependent antagonism of NKB-induced Ca^{2+} mobilization in HEK 293 cell expressing the hNK-3 receptor, with a K_b of 3 nM; Schild plot analysis of the data revealed a slope o 1.2, which was not significantly different from 1, and therefore indicative of competitive antagonism SB 223412 (3-30 nM) also produced a concentration-dependent, surmountable antagonism o senktide-induced contractions in rabbit isolated iris sphincter muscle preparation with a pA_2 = 8.4; n = 4. Regarding selectivity for the human tachykinin receptors, SB 223412 had about 140-fold highe affinity for hNK-3 versus hNK-2 (K_i = 144 ± 22; n = 7), and at concentrations up to 10 μM wa devoid of binding affinity for the hNK-1 receptor. SB 223412 (1 and/or 10 μM) was without effect i a battery of receptor, ion channel and binding assays. SB 223412 was orally active (30 mi pretreatment) against behavioural responses induced by the selective NK-3 receptor agonist, senktid (1 mg/kg s.c), in mouse with an ID_{50} of 12 mg/kg.

In summary, SB 223412 is a member of a novel class of potent and selective, orally active non peptide NK-3 receptor antagonists which appears to possess the appropriate pharmacodynamic profil to assist in the elucidation of the functional and pathophysiological roles of the NK-3 receptor.

[1] Farina, C. *et al.*: Int. Pat. Appl. WO 95/32948 (07.12.95).

POSSIBLE ROLES OF TACHYKININS AS MODULATORS OF BASAL GASTRIC BLOOD FLOW AND ACID-EVOKED GASTRIC HYPERAEMIA

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Acid backdiffusion into the rat gastric mucosa causes a protective hyperaemic response via stimulation of primary afferent nerve fibres and release of calcitonin gene-related peptide (CGRP). Since the tachykinins neurokinin A (NKA) and substance P (SP) are likely to be coreleased with CGRP from these fibres, we investigated the effects of NKA and SP on gastric mucosal blood flow under basal conditions and after induction of acid backdiffusion.

Gastric mucosal blood flow in urethane-anaesthetized rats was assessed by the H_2 clearance technique, and gastric mucosal vascular conductance (GMVC) was calculated as blood flow divided by mean arterial blood pressure. Blood flow in the left gastric artery was measured with the ultrasonic transit time shift technique and left gastric arterial vascular conductance (LGVC) was calculated. Under basal conditions, with 0.05 M HCl being perfused through the stomach, NKA (1.3 nmol kg^{-1} min^{-1} infused intra-aortically) caused a 25-30 % decrease in GMVC, which was prevented by the NK-1 antagonist SR-140,333 (150 nmol kg^{-1}) but left unchanged by the NK-2 antagonist MEN-10,627 (100 nmol kg^{-1}) or the mast-cell stabilizer ketotifen (1 mg kg^{-1}). Similarly, the same dose of NKA reduced LGVC by 20%. Acid backdiffusion induced by disrupting the gastric mucosal barrier with 15 % ethanol in the presence of 0.05 M HCl increased GMVC by 80-90 % and LGVC by 200 %. Both NKA and SP (0.14-3.8 nmol kg^{-1} min^{-1}) caused a dose-dependent depression of the hyperaemic response to acid backdiffusion, an effect that was paralleled by aggravation of macroscopic lesions. The anti-vasodilator effect of NKA in the gastric mucosa (1.26 nmol kg^{-1} min^{-1}) was prevented by the NK-2 antagonist MEN-10,627 but left unaltered by the NK-1 antagonist SR-140,333 or ketotifen. These drugs themselves failed to significantly influence basal GMVC or the acid backdiffusion-evoked vasodilatation. Intra-aortic infusion of arginine-vasopressin, at a dose (0.1 nmol kg^{-1} min^{-1}) that reduced GMVC under basal conditions by 30-40 %, was unable to suppress the vasodilator response to acid backdiffusion.

The vasoconstrictor action of NKA seen under basal conditions is mediated by NK-1 receptors and is unlikely to account for the inhibition of the acid backdiffusion-evoked hyperaemia. The anti-vasodilator action of NKA is brought about by NK-2 receptors and might reflect a negative control by tachykinins of afferent nerve-mediated gastric vasodilation.

NEW PERSPECTIVES OF SOMATOSTATIN ANALOGS AS ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE DRUGS

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A variety of effects of somatostatin has already been described. These are mediated by five receptor subtypes. Many new somatostatin analogs have been synthesized to study the importance of specific substitutions in the selectivity of effects. We demonstrated earlier that local neurogenic inflammation has a systemic anti-inflammatory effect. Participation of somatostatin in this action has been assumed since this peptide is released from the activated capsaicin-sensitive afferent nerve endings. As the efferent function of the C-fibers is neurogenic inflammation and the afferent is nociception, somatostatin is possible to have an effect on pain as well. *The aim of the present study* was to examine the effect of somatostatin, octreotide (a somatostatin analog with prominent endocrine effect), RC-160, and three stable, cyclic analogs, TT-250, TT-248, and TT-232 on inflammation and nociception. For the assesment of ***neurogenic inflammation*** evoked by topical application of mustard oil after acute, bilateral denervation of the hindleg, plasma extravasation was measured by the Evans blue tracer method in the paw skin. ***Non-neurogenic inflammation*** was induced by subplantar injection of dextran in the chronically denervated hindleg. Oedema was measured by plethysmometry. For examining ***antinociceptive effects*** vegetative reflexes to nociceptive stimulations were recorded in anaesthetized rats. Somatostatin and its analogs except octreotide significantly inhibited both forms of inflammation. The vegetative responses to nociceptive stimulations were also decreased by pretreatment with somatostatin and its analogs except octreotide. TT-232, a somatostatin analog without endocrine actions, had the greatest inhibiting effect. *These data suggest,* that the receptors mediating the endocrine effects of somatostatin are different from those responsible for the anti-inflammatory and anti-nociceptive actions. In this respect TT-232 seemed to be the best and most promising compound.

INHIBITORY FUNCTION OF SOMATOSTATIN ON PRIMARY AFFERENTS IN NORMAL AND INFLAMED KNEE JOINTS OF THE RAT

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The neuropeptide somatostatin (SOM) is known as a substance with analgesic activity in the central nervous system. SOM and its receptors are also present in the peripheral nervous system. Therefore, in the present study we examined the effect of SOM on the sensory activity of primary afferent nerve fibres during non-noxious and noxious movements in normal and inflamed rat knee joints.
Fine afferent nerve fibres (conduction velocity: 1.4 - 11 m/s) were recorded as single units. In some rats, an acute inflammation was induced using kaolin and carrageenan. All nerve fibres tested responded to local mechanical stimulation, movements of the joint and i.a. injection of KCl close to the joint. Mechanosensitivity was tested by non-noxious and noxious outward and inward rotations before and after i.a. application of SOM (0.1 mM, 0.2 ml) close to the joint.
SOM caused no direct response of the units. In normal joints, SOM did not change discharges evoked by non-noxious joint movements. Responses during noxious movements, however, were reduced to about 50%. In inflamed joints, SOM markedly reduced the responses evoked by non-noxious and noxious movements to 60% and 50%, respectively. A second injection of SOM led to further decrease of the responses in some units. A recovery to nearly control level was achieved after applications of substance P or bradykinin.
These data show that SOM reduces the mechanosensitivity of fine primary afferents in normal and inflamed rat knee joints. The presence of SOM receptor mRNA in primary afferents (Señaris et al., Mol. Brain Res. 29, 185-190, 1995) supports the idea of a direct function of somatostatin on the peripheral nerve fibres.

CHANGES OF CHEMICAL CODING OF PIGEON DRG NEURONES AFTER AXOTOMY

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It has been recently demonstrated that the neuronal markers of cervical DRG neurones of pigeons diverge from those of other vertebrates. Similar to mammals substance P (SP) and calcitonin gene-related peptide (CGRP) containing neurones, constituting 50-60%, are the most frequent population in pigeon DRGs, but the relation between SP and CGRP is 3 to 2 in contrast to 1 to 2 in mammals. Consequently, about 40% of SP positive neurones are not co-localized with CGRP. Strikingly, more than 20% of pigeon cervical sensory neurones contain galanin (< 5% in mammals) while somatostatin appears to be completely absent; the number of NPY containing neurones is only small (< 5%). SP and CGRP appear to be mainly located in small and medium sized neurones in the distal part of the ganglion while galanin appears in a belt-like central area.
Axotomy increased the number of both SP and CGRP containing neurones by approximately 40% in the ipsilateral DRG. The number of NPY neurones was also increased while the number of galanin positive neurones appeared to be slightly diminished. Remarkably, about 12% of the DRG neurones were somatostatin positive after axotomy; they were restricted to the distal part of the ganglion. Six weeks after axotomy the topographical organization of SP and CGRP neurones had disappeared ipsilateral as well as contralateral to the axotomy, indicating a special bilateral reorganization, whereas the topographical distribution of galanin positive neurones was not changed. The present observations suggest functional differences in mammals and pigeons which appear to have consequences on reactions to pathological events or repair mechanisms.

CHIMERIC AND RETRO-INVERSO SYNTHETIC STRATEGIES FOR NEUROPEPTIDES AND PEPTIDE HORMONES: PSEUDOPEPTIDIC ANALOGUES OF BRADYKININ AND VASOPRESSIN.

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Structural epitopes can be combined in the synthesis of chimeric peptide ligands which specifically interact with pharmacologically distinct G-protein-coupled receptors (GPRs). We have prepared a series of bradykinin/vasopressin chimeras synthesized as single peptides, amino-hexanoic acid-linked dipeptides, and cross-linked dimers. These chimeric peptides bind selectively and with high affinity to GPRs. Functional studies confirm that the structure/activity relationships of small peptide hormones and neuropeptides can be used as a template for the design of both chimeric agonists and antagonists.

The reversal of one or more peptide bonds with appropriate configurational compensation (partial retro-inverso transformation) is a common pseudopeptidic modification compatible with the synthesis of conventionally-active peptide ligands. We have used a total retro-inverso strategy to synthesize protease-resistant analogues of bradykinin and vasopressin from mostly D-amino acids. Our data indicate that this strategy is congenial to the design of pseudopeptides which bind to GPRs for bradykinin and vasopressin. The unique properties of retro-inverso peptides also allow them to distinguish main-chain and side-chain interactions in the molecular recognition of ligands by GPRs.

ROLE OF SUBSTANCE P AS A NEUROTRANSMITTER IN THE RESPIRATORY CHEMOREFLEX TO HYPOXIA

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The solitary tract nucleus (NTS) is considered to be the the primary relay station for respiratory chemoreflex to hypoxia. This area is one of the highest in substance P (SP) content among nuclei in the brain stem. The SP-containing primary afferent projections to the NTS are mainly from glossopharyngeal and vagal nerves. This nerves include afferents from peripheral chemoreceptors. A high density of SP binding sites has been also demonstrated in the NTS. In anesthetized rats microinjections of 10^{-12}-10^{-4} M SP into the NTS induced an increase in tidal volume and peak integrated activity of inspiratory muscles. 10^{-4} M SP additionally induced an increase in respiratory frequency. After SP microinjections the respiratory response to increasing hypoxia (we used rebreathing test with CO_2 absorption) was inhibited with dose-dependent way. Together with preceding data regarding increase in the SP release in the NTS during hypoxia our results demonstrate that SP is a likely neurotransmitter in the respiratory chemoreflex to hypoxia.

MECHANISMS OF "CAPSAICIN DESENSITIZATION"

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Repeated applications at low concentrations or administration of a single large dose of capsaicin render sensory neurons insensitive to nociceptive stimuli and to subsequent administrations of the drug. This permanent and selective action of this compound is known as "capsaicin desensitization". Although this is a common phenomenon observed after the administration of capsaicin by different routes, the mechanisms of desensitization are not well understood. The studies summerized here were initiated in an attempt to reveal possible morphological and/or histochemical changes which might contribute to the development of capsaicin desensitization. Systemic administration of capsaicin or resiniferatoxin to adult animals (rat, guinea pig, cat) resulted in a dose-dependent degeneration of small, type B primary sensory neurons associated with an increase in intracellular free calcium. Perineural application of capsaicin or resiniferatoxin produced an impairment of polymodal nociceptor afferents which was associated with a selective inhibition of axoplasmic transport in, a depletion of sensory neuropeptides from and a delayed irreversible degeneration of sensory ganglion neurons. In different in vitro organ preparations application of capsaicin at concentrations producing characteristic functional responses produced rapid degeneration of unmyelinated axons. These findings indicate that cellular events implicated in the progression of capsaicin desensitization may be interpreted in terms of changes in cellular calcium metabolism, neuropeptide depletion, inhibition of axonal transport processes and neuronal degeneration and/or structural alterations which may potentially disrupt cellular mechanisms involved in neurotransmitter release.
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THE EFFECTS OF REPEATED COCAINE ADMINISTRATION ON THE THYROTROPIN-RELEASING HORMONE LEVEL AND RECEPTORS IN THE RAT BRAIN

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Recent evidence indicates that an endogenous thyrotropin-releasing hormone (TRH) system is involved in the adaptive response to the drugs of abuse (Jaworska-Feil et al., Neuropeptides 1995; 29: 171,343) In the present study the effects of single and repeated administration of cocaine on the TRH level and receptors in discrete rat brain structures were evaluated.
Male Wistar rats received saline or cocaine (15 mg/kg i.p., once an hour throughout 3h, for 1 or 8 days). The animals were killed by decapitation at 45 min and 72h (chronic group only) after the last injection.
As shown by a specific radioimmunoassay, a single dose of cocaine increased the TRH level in the striatum (by ca 67%) but had no significant effect on the peptide content in nucleus accumbens, hippocampus, amygdala, septum, frontal and prefrontal cortex at 45 min after the drug injection. Repeated cocaine increased the TRH level in the striatum by 89% at 45 min, and in the hippocampus by 26% at 72h after the last dose. No changes in the TRH level in other brain structures were found. Acute cocaine had no effect on the density and affinity of TRH receptors. Repeated cocaine evoked a long-lasting decrease in the Bmax of TRH receptors in the striatum (by ca 30%), whereas in the frontal cortex an increase in that parameter was observed. The Bmax and affinity of TRH receptors in the nucleus accumbens following repeated cocaine remained unchanged.
The obtained results indicate that cocaine affects the TRH system mainly in the striatum, and to a lesser extent in the cortex and hippocampus.

BRADYKININ-EVOKED CALCIUM-DEPENDENT RELEASE OF EXCITATORY AMINO ACIDS FROM SCHWANN CELLS

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It has been well established that glia have multiple roles in the nervous system which include the regulation of external potassium and the uptake of the excitatory amino acids (EAA) glutamate and aspartate which are released from neurons into the extracellular space. This study demonstrates an additional process in the regulation of external EAA through the neuroligand-activated, calcium-dependent release of EAA from Schwann cells. The release of EAA from Schwann cell cultures derived from dorsal root ganglia was assayed using high-performance liquid chromatography. The neuroligand bradykinin caused a receptor-mediated, dose-dependent release of the EAA glutamate and aspartate from Schwann cell cultures.

To ask whether calcium might play a role in bradykinin-induced excitatory amino acids release, we used Fura-2 AM (4μM) to monitor Schwann cells calcium levels. Bradykinin increased the cytoplasmic level of Schwann cells free calcium. The calcium mobilizing action of bradykinin was impaired by thapsigargin (1μM), but not by caffeine (10mM), ryanodine (10μM) nor by removal of extracellular calcium. To determine whether the bradykinin-induced calcium mobilization stimulates excitatory amino acids release, cells were loaded with BAPTA-AM (50μM). Addition of this calcium chelator blocked BK-induced excitatory amino acids release. Addition of ionomycin (5μM) caused calcium-dependent excitatory amino acids release. Taken together these data demonstrate that calcium is both necessary and sufficient for stimulating the release of excitatory amino acids from Schwann cells culture.

INTRACEREBROVENTRICULAR INJECTIONS OF GROWTH HORMONE AFFECTS THE SERUM LEVELS OF ß-ENDORPHIN IN THE MALE RAT.

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Recent studies suggest that growth hormone (GH) can cross the blood-brain-barrier and reach the central nervous system (CNS), where the hormone may produce profound effects. In adult GH-deficient patients subjected to GH-therapy, the hormone was found to affect the levels of various neurotransmitters and neurohormones in the cerebrospinal fluid. Moreover, the psychological improvements seen in these patients were suggested to result from direct action of the hormone on GH-responsive areas in the brain. The presence of specific binding sites for GH in the CNS has been confirmed in many laboratories.

In the present studie the effect on serum ß-endorphin after intracerebroventricular injections of GH in the male rat was investigated. The hormone was found to produce a significant increase in the levels of the circulating opioid. This elevation was found to be dose-dependent.

REGULATION OF NEURONAL CAPSAICIN SENSITIVITY *IN VIVO*: POSSIBLE ROLE OF NERVE GROWTH FACTOR

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Previous *in vivo* investigations revealed that axotomized sensory neurons loose their selective vulnerability to the neurotoxic action of capsaicin. *In vitro* studies showed that capsaicin sensitivity of sensory ganglion cells is regulated by nerve growth factor (NGF). The aim of this study was to explore the possible role of NGF in the *in vivo* regulation of neuronal capsaicin sensitivity. Cultures of sensory neurons were prepared from the 4th and 5th lumbar spinal ganglia taken from intact adult male Wistar rats (control cultures; n=10) or from animals whose sciatic and saphenous nerves were ligated and transected 4 days prior to sacrifice (axotomized neuron cultures, AXOX; n=22). Neurons were cultured for 7 days either in the presence of NGF (200 ng/ml, NGF^+) or without NGF with NGF-antiserum added (NGF^-). Capsaicin-sensitive (CAP^+) neurons were detected with a new technique which demonstrates histochemical changes associated with an increased level of intracellular free calcium in response to application of capsaicin (10 μM). In control NGF^+ cultures CAP^+ neurons amounted $35.3\pm4.7\%$ In control NGF^- cultures the percentage of CAP^+ neurons decreased to $15.8\pm2.2\%$. Similarly, the number of CAP^+ cells was very low ($13.3\pm4.1\%$) in AXOX NGF^- cultures. In contrast, in AXOX NGF^+ cultures, the proportion of CAP^+ nerve cells reached control levels ($39.5\pm6.25\%$). These findings strongly indicate that NGF may play an important role in the *in vivo* regulation of neuronal capsaicin sensitivity. It is suggested that axotomy-induced loss of capsaicin sensitivity resulted from the lack of retrogradely transported NGF and that the microenvironment of sensory nerve endings may significantly influence neuronal capsaicin sensitivity.

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IS THERE AN ANP-LINK IN CARDIOGENIC REFLEXES? - A REVIEW OF EXPERIMENTS

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While the diversity of neural pathways makes it difficult to separate the divergent functions of cardiac reflexes, it is even more difficult to determine contributions of accessory humoral agents involved in them. These studies were undertaken to characterize, using chloralose anesthetized dogs, the possible role played by the atrial natriuretic peptide (ANP) in some well-defined cardiac reflex patterns with special reference to coronary blood flow (CBF) regulation. CBF was measured with electromagnetic probes and was also estimated by computer-assisted thermography. A 10 cm H_2O elevation of left atrial pressure (LAP) by inflating a balloon in the atrium (Henry-Gauer volume regulatory reflex, n=8) increased heart rate (197±10 vs 167±7 bpm, $p<0.001$), CBF (75±9 vs 57±6 ml/min, $p<0.01$), and epicardial heat emission. Atropine did not block this pattern, but bilateral cervical vagotomy prevented both tachycardic and flow components. The reflex was accompanied by a rise of IR-ANP plasma concentration and an eightfold increase of ANP secretion rate through the coronary sinus (8.22±1.98 vs 0.99±0.24 ng/min). Vagotomy significantly ($p<0.05$) reduced the secreted amount of ANP during LAP increase (2.96±0.85 vs 0.77±0.48 ng/min). Activation of ventricular C-afferents (Bezold-Jarisch reflex, n=14) by administering veratrine (2μg/kg/min for 10 min) induced, as expected, opposite heart rate responses, it failed to elicit lasting coronary dilation except when combined with regional ischemia, but similarly increased ANP secretion which was totally prevented by vagotomy. Several types of tests with intracoronary ANP (n=10) make debatable whether the released amounts of the peptide are sufficient to elicit short-term CBF actions. However, the results indicate that neurally mediated mechanisms may play a role in ANP release in response to atrial distension and ventricular mechanoceptor activation.

MODULATION BY NO OF CHOLINERGIC NEUROTRANSMISSION IN THE GUT

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Inhibitors of nitric oxide (NO) synthase facilitate the electrically evoked release of acetylcholine (ACh) from myenteric neurones (Kilbinger and Wolf, Naunyn-Schmiedeberg's Arch Pharmacol 349: 543-545, 1994) which suggests that endogenous NO inhibits the release. We have studied the effects of the NO donors S-nitroso-N-acetylpenicillamine (SNAP), SIN-1, and sodium nitroprusside (SNP) on basal and electrically evoked release of ACh from myenteric plexus-longitudinal muscle preparations of the guinea-pig ileum preincubated with [^{3}H]choline and subsequently stimulated electrically at 10 Hz (1 min).

SNP (EC50: 10 μM), SNAP (26 μM) and SIN-1 (110 μM) caused transient and concentration-dependent increases in basal [^{3}H]release. The release caused by 300 μM SIN-1 was measured in the presence of neostigmine and consisted to 92±10 % (N=3) of [^{3}H]ACh. The effects of the NO donors were calcium-dependent and were prevented by 300 nM tetrodotoxin. The increases in basal release were paralleled by increases in smooth muscle tone. The electrically evoked [^{3}H]ACh release was inhibited by the NO donors. SNAP (EC50: 30 μM) was more potent than SIN-1 (370 μM) and SNP (440 μM).

8-Bromo cyclic GMP and the cyclic GMP-specific phosphodiesterase inhibitor, zaprinast, (both 0.01 - 1.0 mM) caused increases of basal release, but did not inhibit the electrically evoked release of [^{3}H]ACh. In the presence of 0.1 mM zaprinast the effect of 10 μM SNAP on basal [^{3}H]ACh release was significantly enhanced. The selective inhibitor of guanylyl cyclase, ODQ (0.1 μM) antagonised the effects of SNAP (100 μM) on basal release but not on the evoked release of [^{3}H]ACh.

The results suggest that the increase in basal [^{3}H]ACh release by NO donors is due to activation of guanylyl cyclase whereas other mechanisms are probably involved in the inhibition of the electrically evoked [^{3}H]ACh release.

INCREASE OF VIP IN THE INNERVATION OF THE PAROTID GLAND AFTER IRRADIATION

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Vasoactive intestinal polypeptide (VIP) is involved in nerve-mediated secretion in the parotid gland. VIP has also marked trophic and growth regulatory effects. We have previously observed that the expression of certain neuropeptides, but not VIP, is enhanced in submandibular and laryngeal glands in response to irradiation. In the present study, rat parotid glands were examined ten days after cessation of a five-day treatment with high energy photons. Immunohistochemistry and radioimmunoassay (RIA) were applied. There was a marked increase in the number and intensity of VIP-immunoreactive nerve fibres in the parotid gland parenchyma after irradiation. RIA analysis revealed a more than threefold increase in the content of VIP-like peptide in the gland. The observations show that a marked up-regulation of VIP occurs in the parotid gland after irradiation. This up-regulation may be related to retrograde mechanisms occurring in this experimental situation and suggests that VIP markedly potentiates the effects of other secretagogues in the parotid gland after radiotherapy.

EFFECT OF ELECTRICAL STIMULATION OF THE GASSERIAN GANGLION ON CGRP IMMUNOREACTIVITY AND C-FOS EXPRESSION IN THE CAUDAL TRIGEMINAL NUCLEUS IN AN EXPERIMENTAL MIGRAINE MODEL

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Electrical stimulation of the Gasserian ganglion induces decrease of CGRP immunoreactivity in the caudal trigeminal nucleus. Densitometric studies, performed with the help of a Nikon Macintosh Quadra computer running the NIHImage 1.55 image analysis software, prove that significant CGRP decrease occurs ipsilaterally; however, at the same time, partial and topographically restricted decrease was observed also contralaterally. At the level of electron microscopy, affected terminals exhibit decreased CGRP reactivity suggesting release of the peptide; in addition, signs of incipient transganglionic degenerative atrophy were noted. Electrical stimulation of the Gasserian ganglion also induces increase in the number of secondary sensory nerve cells expressing the oncoprotein *c-fos* in the caudal trigeminal nucleus in the medulla and cervical spinal cord (1, 2). We found that this is not prevented by i.v. administration of Sumatriptan probably because it does not pass the blood-brain-barrier. Apparently, immunohistochemical studies might facilitate analysis of central and peripheral effects of prospective anti-migraine drugs.

1. Nozaki *et al* (1992) *Br. J. Pharmacol.* **106**: 409-415
2. Shepheard *et al* (1995) *Neuropharmacology* **34**: 255-261

ANXIETY-RELATED EFFECT OF ICV ADMINISTRATION OF AMYLIN IN ELEVATED PLUS-MAZE IN RATS

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Amylin is a 37 amino acid peptide of the calcitonin gene-related peptide (CGRP) family originally isolated from pancreatic amyloid deposits of type II diabetic patients. Amylin is sharing 45 % homology with CGRP. Previously we have shown that intracerebroventricular (ICV) injection of amylin increased the latency of passive avoidance behavior and facilitated the extinction of active avoidance behavior of rats in a dose-dependent manner. Amylin administered ICV significantly reduced exploratory activity and increased the grooming behavior of animals in novel enviroment.

The aim of the study presented here to further characterize the effect of amylin on behavior of rats in elevated plus-maze test, which is one of the most widely used models in anxiety. Wistar adult male rats were used in all expriments. Two doses (500 ng, 1 μg) of amylin were injected ICV and entries into and time spent on the aversive open arms were tested 30 min after the treatment. Data were expressed as percentages or ratios of total entries and total arm time.

Amylin reduced both parameters, but only 1 μg of peptide caused significant decrease in the entries into the open arm and in time spent on open arm. The present data suggest that amylin has anxiogenic effect.

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CORRELATIVE CHARACTERISATION OF PULMONARY NEUROENDOCRINE SYSTEM IN CHILDREN WITH ACUTE AND CHRONIC LUNG DISEASE.

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Distribution of neuroendocrine cells (NC) and neuropeptide-containing nerves may change during the inflammation in the lung of humans. Until this time still unclear are correlations in the occurrence of elements of diffuse neuroendocrine system (DNES) in children with acute and chronic process in the lungs. There were 4 children (2 with bronchopulmonary dysplasia (BPD) and 2 with acute pneumonia in the phase of reparation) lung biopsies under our investigation. General neuroendocrine markers and putative messengers of DNES were detected in tissue samples immunocytochemically.

No NC were detected in the epithelium of bronchi and bronchioles in children with BPD. Numerous protein gene peptide 9.5 (PGP 9.5)-, vasoactive intestinal peptide-, neuropeptide Y (NPY)-containing nerve fibers were seen in smooth muscle layer, around seromucous glands and blood vessels in the subepithelial layer. Also fine few helospectin and substance P (SP) immunoreactive nerves were detected in the above mentioned location. PGP 9.5-positive nerve trunks were detected in the close relation to glands. Additionally, NPY and PGP 9.5 positive nerves innervated blood vessels in the lung parenchyma. No NC were observed in the epithelium in bronchi of children with pneumonia. Moderate PGP 9.5-, NPY- immunoreactive nerves were seen around blood vessels, among the smooth muscle layer in the outer layer of perichondrium in the bronchial wall and around blood vessels in the lung parenchyma. Few serotonin, calcitonin, gastrin releasing peptide - NC were detected in the epithelium of respiratory bronchioles. It can be speculated, that blood vessels displayed more rich immunoreactive nerve - containing innervation in a case of chronic process to compare with acute inflammation, probably, due chronic hypoxia, characterising the BPD.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) INDUCES FLARE REACTION BUT INHIBITS HISTAMINE INDUCED PLASMA EXTRAVASATION IN THE PIG SKIN

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The contribution of CGRP and tachykinins to the flare reaction in the pig skin was investigated in animals anaesthetized by halothane/N_2O. Flare was induced by application of heat (55°C; 10 s) through the edge (15 x 0.3mm) of a Peltier operated thermode or intradermally injected capsaicin (300 µM, 20 µl) between two skin strips injected with antagonists to CGRP, NK1 or NK2 receptors 10 min previous to the flare induction. The CGRP antagonist $CGRP_{8-37}$ totally prevented the propagation of heat induced flare at the injected skin strips while the inhibition was not complete immediately after injection of capsaicin. The appearance of a jumping flare reaction behind the pretreated skin strips indicated that the antagonist did not block nerve conductivity and that the flare reaction is due to an axon reflex. Both, the NK1 receptor antagonist SR 140, 333 and the NK2 antagonist SR 48968, did not inhibit flare propagation but might cause a slight reduction in flare intensity. Similar to the human skin neurogenic plasma extravasation cannot be induced by electrical stimulation or capsaicin application in the pig but can be produced by histamine. Using the blister base perfusion method the total histamine induced plasma extravasation was reduced to 40% by the NK2 antagonist and to about 60% by the NK1 antagonist; the CGRP antagonist did not reduce plasma extravasation but slightly increased the later phase of the reaction (not significant). However, CGRP (1 µM) itself completely blocked the plasma extravasation when pre- and co-perfused with histamine. Since this inhibitory effect was not observed on the non-neurogenic plasma extravasation in the denervated skin we suggest that CGRP exerts its action on the primary afferents.

SENSORY RECEPTOR PROPERTIES AND PEPTIDE CONTENT IN PRIMARY AFFERENT NEURONES: SUBSTANCE P AND CGRP.

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We have examined the relationship between the sensory receptor properties of neurones in guinea pig dorsal root ganglia, and substance P- and CGRP-like immunoreactivty (LI) of the somata. Intracellular recordings were made in the L6 and S1 DRGs of anaesthetised young guinea pigs and cells were classified as having C, Aδ or Aα/β fibres. Receptive field location (including depth) and sensory receptor properties were examined. Dye was electrophoretically ejected into the soma. Avidin-biotin complex (ABC) immunocyto-chemistry was used to demonstrate peptide-LI on sections of these cells.

Nociceptive C fibre units that expressed substance P-LI or CGRP-LI included a) less than half the polymodal nociceptor unit and b) some C-fibre high threshold mechanoreceptors (HTM) units, particularly those with non-epidermal receptive fields. Non-nociceptive C-fibre neurones (cooling or mechanoreceptor units) were negative except one cooling unit with weak SP-LI. Aδ mechanoheat units, and HTM units with non-epidermal receptive fields expressed the peptides. No A-fibre low threshold mechanoreceptor (LTM) units showed SP-LI, although a small subgroup of non-nociceptive Aα/β fibre units showed CGRP-LI. Expression of both peptides was related to sensory receptor properties, depth of the receptive field and also to the conduction velocity of the fibres.

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CAPSAICIN-SENSITIVE MECHANISMS IN THE MODULATION OF RAT COLONIC VASCULAR PERMEABILITY

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Inflammatory bowel disease (IBD) causes a prolonged life-quality reduction of patients and high costs for health services. The aim of the present study was to explore the possible involvement of peptidergic capsaicin-sensitive afferent nerves (CSNs) in the pathomechanism of IBD. For defunctionalization of colonic CSNs, the lower part of the colon (1-4 cm from the anus) was exposed through a midline incision and small pieces of gelfoam moistened with capsaicin (1%, 100 μl) was applied onto the serosal surface for 30 min. Colonic vascular permeability was assessed by measuring the extravasation of [^{125}I] human serum albumin (2 μCi/kg, i.v., 2 h prior to sacrifice). Two months after treatment a significant increase in albumin extravasation was found in the lower ($\triangle 82 \pm 10$ μl plasma/g dry tissue, $p < 0.005$, $n=8$) but not in the upper (5-8 cm from the anus) part of the colon as compared to the control.One week after treatment intrarectal (8 cm from anus) administration of trinitro-sulphonic acid (TNBS, 30 mg/rat) induced similar plasma leakage in the lower and upper colon of control (CSN-intact) rats ($\triangle 152 \pm 26$ and $\triangle 127 \pm 24$ μl/g, respectively, $p < 0.001$, $n=5$). TNBS + ethanol (50%) produced marked extravasation throughout the colon of CSN-intact animals (lower: $\triangle 942 \pm 149$ μl/g, upper $\triangle 799 \pm 181$ μl/g, $p < 0.001$, $n=5$). In the lower colon of capsaicin pretreated rats TNBS provoked an increase in plasma extravasation similar to that caused by TNBS + ethanol in CSN-intact rats ($\triangle 999 \pm 163$ μl/g, $p < 0.001$, $n=5$). In the upper colon there was no difference in the effect of TNBS on plasma extravasation between control and capsaicin pretreated rats. The results suggest that capsaicin-sensitive nerves may play a significant protective/anti-inflammatory role in the rat colon under normal and pathological conditions.

NITRIC OXIDE IN GASTROINTESTINAL MOTILITY

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Nitric oxide (NO) serves as a non-adrenergic non-cholinergic inhibitory neurotransmitter throughout the gastrointestinal tract. At the level of the proximal stomach, it is involved in receptive relaxation. Vasoactive intestinal polypeptide (VIP) is also involved in gastric receptive relaxation but the interaction between NO and VIP differs from species to species. First, NO and VIP can be colocalized in the same neurones or not. Second, the contribution of NO to sustained relaxation differs. Whereas in the rat and ferret stomach NO is mainly important for initiating the relaxation and VIP for sustaining the relaxation, NO also contributes in an important way to sustained gastric relaxation in the guinea-pig, cat and pig. Doubt has raised about the nature of the nitrergic neurotransmitter in the stomach, as substances such as 6-anilino-5,8-quinolinedione (LY83583) and hydroquinone nearly abolish relaxations induced by exogenous NO but hardly influence relaxations due to the endogenous nitrergic neurotransmitter, released upon electrical field stimulation in the rat and pig gastric fundus. Irreversible inhibition of endogenous Cu/Zn-containing superoxide dismutase induced some inhibitory effect of LY83583 versus electrically induced contractions in the rat stomach, but this inhibitory effect was not reversed by exogenous superoxide dismutase. Furthermore, Cu/Zn-containing superoxide dismutase inhibition did not abolish the discrimination between exogenous NO and the endogenous nitrergic neurotransmitter by hydroquinone. These results therefore leave open the possibility that the endogenous neurotransmitter in the rat gastric fundus is not free NO but NO linked to a carrier.

Processing of chromogranin B in developing and adult rat brain

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Chromogranin B is an acidic protein found in large dense core vesicles of neuroendocrine tissues. We have developed a radioimmunoassy against a synthetic peptide (PE-11) representing 11 amino acids in the C-terminal part of chromogranin B. PE-11 in the chromogranin B sequence is flanked by potential proteolytic cleavage sites. In adult rats a concentration of 46.9 fmol PE-11-IR/mg w.w. of rat brain was detected. Molecular sieve chromatography revealed an almost complete proteolytic processing of chromogranin B to PE-11 and to a peptide of about 20 to 25 amino acids. During ontogenesis the concentration of PE-11 rises gradually (from 0.5 to 20 fmol/mg wet weight) from embryonic day 14 to postnatal day 10. A steep increase was found to occur between P10 and P15 when PE-11-IR reaches adult levels (46.9 fmol/mg w.w.). Processing of chromogranin B commenced early during ontogenesis. Already at embryonic day 16 processing to PE-11 appeared complete.

These data indicate that chromogranin B in rat brain is synthesized early in embryonic live and becomes immediately processed to small peptides. Such properties are consistent with the concept that chromogranins represent precursors of functional peptides.

PREABSORPTIVE SATIETY: EFFECTS OF BOMBESIN IN THE AMYGDALA

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It has been suggested that bombesin (BN)-like peptides are involved in the mechanisms of preabsorptive satiety. Namely, in rats the most characteristic action of BN is the short latency transient inhibition of feeding. The effects of BN have been examined on the central feeding regulatory processes by i.c.v. applications with the exception of the lateral hypothalamus (LH), where the direct injection of BN inhibited food intake. Although in both the LH and the amygdala (AMY) BN immunoreactive neural elements were found, the possible effects of BN on AMY feeding mechanisms have not been studied so far. In the present experiments food intakes of 24 hr food deprived CFY rats were examined after BN microinjections into the central (CA) or the basolateral (BLA) AMY nuclei. Bilateral chronic guide cannulae were implanted stereotaxically to the CA or BLA. BN was injected in two different doses (10 ηg or 40 ηg in 1 μl). In both AMY nuclei direct application of BN resulted in transient, dose-dependent inhibition of food intake. In the CA 40 ηg BN caused a significant decrease in food consumption for 20 min that was followed by significant food intake increase 40 min later. While it was ineffective in the CA, the 10 ηg BN inhibited feeding for 20 min in the BLA. By 40 ηg BN a significant food intake reduction was caused for 60 min in the BLA and no "compensatory overeating" was observed later. Effects of BN in both AMY structures were prevented by prior application of BN antagonists. Although the intensity and characteristics of BN effects are somewhat different in the AMY hunger (CA) and satiety (BLA) "centers", our results show that BN may act as a preabsorptive satiety signal in the AMY,

DUAL EXCITATORY AND INHIBITORY EFFECT OF NITRIC OXIDE ON PERISTALSIS IN THE GUINEA-PIG INTESTINE

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Nitric oxide (NO) is a messenger of enteric motor pathways in the intestine. The aim of the present study was to investigate the effect of NO donors and inhibitors of endogenous NO synthesis on intestinal peristalsis.

The experiments were carried out with gut segments isolated from the guinea-pig ileum. Peristalsis was triggered by fluid distension of the intestinal wall, and the pressure threshold for eliciting peristaltic waves was used to quantify facilitation (decrease in threshold) or inhibition (increase in threshold) of peristalsis.

The NO donor sodium nitroprusside (SNP, 0.1-100 μM) caused a concentration-related facilitation of peristalsis. When SNP was tested in the presence of a threshold concentration of atropine (10 nM) which caused only minor depression of peristalsis, the SNP-induced facilitation of peristalsis was followed by a concentration-related inhibition of peristaltic motor activity. Further analysis showed that SNP (10 and 100 μM) first relaxed and then contracted the longitudinal muscle of the guinea-pig isolated ileum, the contraction being markedly attenuated by atropine (1 μM). Blockade of endogenous NO synthesis by N^G-nitro-L-arginine methylester (L- NAME, 100 - 300 μM) or N^G-nitro-L-arginine (30 μM) facilitated peristaltic motor activity, an effect that was antagonized by L- arginine (1mM) which by itself caused some depression of peristalsis. Atropine (10 nM) failed to attenuate the facilitatory influence of L-NAME on peristaltic motility. Peristalsis was also stimulated by apamin (0.5 μM) which is known to block inhibitory neuromuscular transmission through fast inhibitory junction potentials. Successive exposure of the ileum to apamin and L-NAME (300 μM), in this or reverse order, disrupted the coordinated pattern of peristaltic motor activity and caused irregular non-propulsive contractions of the circular muscle.

It is concluded that exogenous NO derived from SNP has a dual excitatory and inhibitory effect on intestinal motility. The excitatory effect of SNP involves cholinergic motor neurons, interference with which reveals a pronounced inhibitory influence of SNP-derived NO on intestinal peristalsis. Combined blockade of apamin-sensitive and NO-dependent inhibitory neurotransmission in intestinal muscle abolishes peristaltic motor activity, which attests to an essential role of enteric inhibitory motor neurons in the coordination of propulsive motility in the intestine.

DIFFERENT EFFECTS OF NEUROKININ A AND [ßAla^8]-NKA(4-10) ON VASOCONSTRICTION AND MOTILITY OF THE RAT STOMACH IN VITRO

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Enteric and extrinsic afferent nerve fibres containing neurokinin A (NKA) innervate the gastric musculature and vasculature and are likely to play a regulatory role in gastric function. The present study examined as to how the vasoconstrictor effect of the naturally occuring NKA and the more specific NK_2 receptor agonist [ßAla^8]- NKA(4-10) is related to their contractile effect on the gastric muscle and whether similar or different tachykinin receptors mediate the two actions of the peptides.

The rat isolated stomach was perfused via the left gastric artery with oxygenated Krebs solution containing 3% dextran by means of a peristaltic pump. Vasoconstrictor responses were recorded by an increase in intravasculare pressure. The lumen of the stomach was filled with 5 ml saline, gastric contraction being recorded by an increase in intraluminal pressure.

Vascular perfusion of NKA (1 nM - 10 μM) or [ßAla^8]-NKA(4-10) (1 nM - 10 μM) caused dose-dependent vasoconstriction and contraction of the gastric musculature. The maximal vasoconstriction caused by 10 μM of either substance amounted to about 50% of the maximal vasoconstriction caused by a 20 nmol bolus of noradrenaline which served as an internal standard. The NK_1 receptor agonist SP methylester (100 μM) and the NK_3 agonist senktide (100 μM) were only weakly active in causing vasoconstriction and gastric contraction. The NKA-induced vasoconstriction remained essentially unchanged by the NK_1 receptor antagonist SR-140,333 (0.1 μM), the NK_2 receptor antagonist MEN-10,627 (1 μM) and the NK_3 receptor antagonist PD-161,182 (1 μM). However a combination of all 3 antagonists was able to block the vasoconstriction due to NKA. The NKA-induced contraction of the gastric musculature was prevented by MEN-10,627 but left unaltered by SR-140,333 or PD-161,182. In contrast both the vasoconstrictor response and gastric contraction evoked by [ßAla^8]-NKA(4-10) were blocked by MEN-10,627.

It is concluded that NKA and [ßAla^8]-NKA(4-10) contract the gastric musculature by activating NK_2 receptors. Although the vasoconstrictor response to [ßAla^8]-NKA(4-10) is also mediated by NK_2 receptors, the vasoconstriction caused by NKA seems to involve an atypical tachykinin receptor, a combination of tachykinin receptors or a particular mode of agonist-receptor interaction.

COEXISTENCE OF SEROTONIN AND FMRFAMIDE-LIKE IMMUNOREACTIVITIES IN THE NERVOUS SYSTEM OF THE EARTHWORM, *LUMBRICUS TERRESTRIS*

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Serotonin is a widely distributed neurotransmitter in invertebrates, including the nervous systems of annelids as well. FMRFamide is a member of a molluscan neuropeptide family, supposedly serving as neuromodulator and/or neurotransmitter substances and its related peptides occur in all invertebrate species. The neurons containing either serotonin or FMRFamide-like substances, have their own characteristic distribution patterns. In many regions of the earthworm nervous system several types of immunopositive neurons occur, leaving the possibility that some neurons contain more than one neuropeptide. It is now well established, that classical neurotransmitter and neuropeptides co-exist in both vertebrate and invertebrate species. In the present study the co-localization of serotonin and FMRFamide immunoreactivity was investigated in the nervous system of the earthworm, *Lumbricus terrestris* (Annelids, Oligochaeta).

Alternate serial sections of the earthworm, *Lumbricus terrestris* were stained with antisera against serotonin and FMRFamide, respectively, using peroxidase-antiperoxidase method. The immunostained serial sections were reconstructed with the aid of Neurolucida and NIH Image program.

A number of nerve cells in the cerebral-, subesophageal-, and ventral cord ganglia seem to be immunoreactive for both antisera. Considering their location, the cells of the cerebral ganglion are probably interneurons, while those of the subesophageal and ventral cord ganglia are motoneurons as well. Our results are in accordance with the proposed role of FMRFamide-related peptides modulating the serotonin induced muscle contraction. Further investigations are to be carried out to verify the importance of these coexisting substances in *Lumbricus terrestris.*

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C-FIBRE AFFERENTS RESPONSIBLE FOR ANTIDROMIC VASODILATATION AND FLARE REACTIONS IN DIFFERENT SPECIES (HEAT VERSUS POLYMODAL NOCICEPTORS)

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Antidromic vasodilatation (ADV) and flare are thought to be due to release of neuropeptides from the terminals of nociceptive C-fibre afferents. Recent studies in anaesthetized pigs have shown that in this species C-heat nociceptors, with high heat sensitivity and minimal pressure sensitivity, cause ADV while C-polymodal nociceptors do not (Lynn, Shütterle & Pierau, 1996, J Physiol (Lond), in press). We have now used the same technique of antidromic stimulation of fine filaments containing identified afferent C-fibres to investigate the situation in anaesthetized rats and rabbits. In these species there are very few heat-only nociceptors. However, one such unit has been stimulated in the rat and gave a clear vasodilatation in the skin. Out of 24 polymodal units simulated in the rat, 4 have given vasodilator responses. In the rabbit, no heat units have been found and, as in the rat, a minority of the polymodal units tested have given vasodilator responses. In all species, the extent of single unit vasodilator zones has closely matched the extent of afferent receptive fields and has adequately accounted for the spread of flare around localised skin injuries. In the rat, and probably in other species, the proportion of C-fibre afferents containing vaso-dilator peptides such as CGRP substantially exceeds the proportion of C-fibres producing detectable ADV. Perhaps some C-afferents do not terminate close enough to arterioles to produce vasodilation and the released peptides have other functions.

ARE NEUROPEPTIDE Y-IMMUNOREACTIVE (NPY-IR) PERIFOLLICULAR NERVE FIBRES IN THE PORCINE OVARY CHOLINERGIC IN NATURE?

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Previously (*Majewski & Heym, 1991, Cell Tissue Res 266:591-6*) we have reported that the porcine ovary is innervated by noradrenergic (NA^+) and non-noradrenergic (NA^-) NPY-IR axons supplying different ovarian compartments. However, we were not able to disclose whether NA^-/NPY^+ ovarian axons are cholinergic or NA^-, non-cholinergic in nature. As recently an antiserum towards ChAT has been raised (*Schemann et al., 1993, Am J Physiol 265:G1005-9*), we aimed the present study at disclosing whether 1) NA^-/NPY^+ ovarian axons are cholinergic, 2) they originate from neurons located in the inferior mesenteric (IMG) or paracervical ganglia (PCG), 3) axotomy of IMG NA^-/NPY^+ "ovarian" neurons (*Ov*) induces the upregulation of ChAT-synthesis and, 4) affected NA^+/NPY^+ neurons undergoes the transmitter switch from the noradrenergic to the cholinergic trait. Six juvenile gilts were injected with the retrograde tracer Fast Blue (FB) into the ovary and subsequently, three of them were subjected to the transection of the plexus nerve. Tissues were processed for double-immunofluorescence using antisera to TH, ChAT, NPY, VIP and SOM. ChAT-IR ovarian axons were primarily related to the secondary and tertiary follicles, while the perivascular ChAT-IR axons were infrequent. Colocalization study revealed that the vast majority of ChAT-IR perifollicular nerves exhibited simultaneously different combinations of NPY-, VIP- and/ or SOM-IR, however, they were TH-immunonegative. As revealed by the retrograde tracing, such chemically coded *Ov* neurons were never found within the IMG, even though they were axotomized. Contradictory, many PCG *Ov* neurons exhibited transmitter combinations identical to those of perifollicular nerves. Thus, the present study provides immunochemical evidence that 1) a large proportion of NA^- perifollicular nerve fibres is cholinergic and originates from the PCG, 2) NA^- IMG ovarian neurons are non-cholinergic, 3) axotomy of NA^- IMG neurons does not induce the upregulation of the ChAT synthesis in injured perikarya, and, 4) axotomy does not induce the transmitter switch of NA^+ IMG *Ov* neurons from the noradrenergic to the cholinergic trait.

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Secretoneurin in the human forebrain

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Secretoneurin is a novel 33 amino acid neuropeptide produced by endoproteolytic processing from secretogranin II which is a member of the chromogranin/secretogranin family. In this immunocytochemical study we compared the distribution pattern of secretoneurin-like immunoreactivity with that of calbindin, substance P and enkephalin in the human forebrain.

A high density of secretoneurin immunostaining was found in the medial part of the nucleus accumbens. All subdivisions of the bed nucleus of the stria terminalis displayed a high density of secretoneurin-like immunoreactivity, but the appearance varied between the different subdivisions. A strong similarity in the type of secretoneurin-like immunoreactivity was found between the lateral bed nucleus of the stria terminalis and the lateral part of the central amygdaloid nucleus. In the pallidal areas a strong secretoneurin immunostaining was found in the area of the ventral pallidum and in the inner pallidal segment with a ventromedial orientation.

This study demonstrates that secretoneurin helps to differentiate the different parts of the human forebrain. It is a neuronal marker of the human extended amygdala forming a continuum from the bed nucleus of the stria terminalis to the centromedial amygdala, thereby supporting the existence of this system not only in rodents but also in man.

TACHYKININS AND ALCOHOL INTAKE IN ALCOHOL-PREFERRING RATS

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Tachykinins and their receptors are widely distributed in limbic structures that are known to be targets for the action of drugs of abuse. Substance P has been reported to induce conditioned place preference in rats, suggesting that it may influence the brain reward mechanisms. Finally, in the rat central nervous system tachykinin agonists have been reported to release serotonin and dopamine, neurotransmitters which have been implicated in alcohol intake control.

The present study evaluated the effect of agonists, selective for different tachykinin receptor subtypes, on alcohol intake in genetically selected alcohol-preferring rats. Water and food sated rats were offered 10 % ethanol 2 hr/day. The agonists were given by intracerebroventricular injection just before access to ethanol. The NK3 selective agonists [$Asp^{5,6}$,$MePhe^{8}$]substance P(5-11), Suc-[Asp^{6},$MePhe^{8}$]substance P(6-11) and [$MePhe^{7}$]neurokinin B markedly reduced alcohol intake. The NK1 selective agonist [Sar^{9},$Met(O_2)^{11}$]substance P and the NK2 selective agonist GR 64349 did not modify alcohol intake. Also, the amphibian tachykinin PG-KII, endowed with potent agonist activity at NK3 receptors, potently inhibited ethanol intake. NK3 receptor agonists, however, did not modify solid food intake in food deprived rats, or water intake in water deprived rats.

Suc[Asp^{6},$MePhe^{8}$]substance P(6-11), but not [$Asp^{5,6}$,$MePhe^{8}$]substance P(5-11), reduced ethanol intake also following subcutaneous injection, but doses about 1000 times higher than those effective following intracerebroventricular injection were required, suggesting a central site of action.

Direct injections of [$Asp^{5,6}$,$MePhe^{8}$]substance P(5-11) were given in discrete brain areas to evaluate their sensitivity to the effect of NK3 agonists on alcohol consumption. While injections into the medial amygdala and the CA1 region of the hippocampus at doses up to 50 ng/site were devoid of effect on ethanol intake, administration into the BNST markedly inhibited ethanol intake at doses of 0.5-25 ng/site. Experiments are under way to evaluate the sensitivity of the NAC and of the VTA.

Since NK3 receptors can be involved in gustatory processes, at least for the salty taste, further experiments are being carried out in order to understand whether the inhibitory effect of tachykinins is exerted on the appetitive or consummatory aspects of ethanol intake.

DO SUBTYPES OF NK-2 RECEPTORS EXIST IN THE RAT?

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Tachykinins have been implicated in pain and inflammation and contract smooth muscle in the gastrointestinal and urogenital tracts. Subtypes of the NK-2 receptor have been suggested, based on differences in antagonist potency between species. However there is little evidence for within-species subtypes of the NK-2 receptor. In the present study, competition binding studies were used to examine the heterogeneity of tachykinin NK-2 receptor subtypes in the rat fundus, colon, bladder, vas deferens and spinal cord. The NK-2 selective radioligand [^{125}I]-[Lys^{5},$Tyr(I_2)^{7}$,$MeLeu^{9}$,Nle^{10}]-NKA(4-10) exhibited a very high specific binding (80-95% of total) to membranes from rat fundus, colon, bladder and vas deferens, whereas no specific binding was observed in spinal cord. 'Hot' saturation studies were carried out in membranes from rat colon, bladder, fundus and vas deferens, and analysed by LIGAND. Binding occurred to one site only in each tissue. In rat colon (K_D 0.44 nM) and bladder (K_D 0.42 nM), binding was of higher affinity than in fundus (K_D 1.3 nM) and vas deferens (K_D 1.9 nM). In competition binding studies, the NK-2 ligands NKA, neuropeptide γ, [Lys^{5},$Tyr(I_2)^{7}$,$MeLeu^{9}$,Nle^{10}]-NKA(4-10), SR48968, GR94800 and MEN10627 displayed similar high affinity (pIC_{50} 8.4-9.5) in membranes from colon, bladder, fundus and vas deferens. There was no significant difference observed in the binding potency of any competitor in the four tissues. In functional studies, the NK-2 selective antagonists SR48968 and MEN10627 were of equally high affinity in fundus (pA_2 7.9 and 8.2) and in bladder (pA_2 7.9 and 7.5). In summary, these results do not provide evidence for NK-2 receptor heterogeneity expressed on smooth muscle tissues in the rat. In the spinal cord, no specific binding of the radioligand [^{125}I]-[Lys^{5},$Tyr(I_2)^{7}$,$MeLeu^{9}$,Nle^{10}]-NKA(4-10) was seen, suggesting either that spinal cord NK-2 receptors have no affinity for this radioligand, or are expressed in very low, undetectable numbers.

NEUROKININ 1 RECEPTOR EXPRESSION BY A SUB-POPULATION OF YOUNG RAT DORSAL ROOT GANGLION NEURONS

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The tachykinin substance P (SP) found in the small diameter sub-population of dorsal root ganglion (DRG) neurons has been regarded as one the most important neuropeptide involved in nociception. Substance P released from the terminals of DRG cells acts on neurokinin 1 (NK1) receptor, expressed by spinal cord neurons and different cells of mesenchymal origin. Recent findings suggest, however, that some DRG cells may also express NK1 receptor. The aim of the present study was to investigate the type of DRG neurons expressing NK1 receptor.

Lumbar 4 DRGs from 14 day old rat pups anaesthetised by ether were removed and put either into 4% paraformaldehyde or ice cold modified Krebs solution saturated by oxygen. Paraformaldehyde fixed DRGs were processed for immunohistochemical staining for neurokinin 1 receptor and substance P while survival DRGs were used for intracellular recording of the effect of SP superfusion to DRG neurons.

Neurokinin 1 receptor immunostaining was observed in about 30% of the total cell population belonging predominantly to the large diameter sub-population. Double immunostaining with the NK1 receptor and SP antibodies revealed that NK1 receptor and SP are never co-expressed in DRG cells. Intracellular recordings showed that cells posessing axons with a conduction velocity in Aδ and Aβ fibre range may respond with firing or depolarization while C-cells showed no response to SP superfusion.

As NK1 receptor has not been observed in central terminals of DRG cells our result suggest that they may be transported selectively to the periphery and involved in peripheral nociceptive mechanisms.

TOPOGRAPHICAL CORRELATIONS BETWEEN CGRP AND THE α7 SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN THE RAT BRAIN

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In restricted areas of the rodent neocortex, a well-defined population of local circuit neurons (basket cells) displays CGRP immunoreactivity. Another set of interneurons (stellate cells) exhibit specific binding for α-bungarotoxin. Similar micro-topographical correlation between the localization of CGRP and α-bungarotoxin binding sites characterizes Meynert's basal nucleus, cerebellar Purkinje cells and the hippocampus. It has been shown that α-bungarotoxin-binding sites and theα7 subunit of the neuronal nicotinic acetylcholine receptor (Barrantes et al, 1995) are co-existent or identical. Close areal correlation between CGRP and α-bungarotoxin binding suggests that, in a manner similar to that prevailing in the neuromuscular junction (New and Mudge, 1986; Fontaine et al, 1986; Csillik et al, 1991, 1993), CGRP might be involved in the maintenance of the α-subunit of the neuronal nicotinic acetylcholine receptor in numerous regions of the rodent brain.

NEUROGENIC INCREASES IN MENINGEAL ARTERIAL BLOOD FLOW: INVOLVEMENT OF NEUROPEPTIDES, NITRIC OXIDE AND SEROTONIN RECEPTOR (5-HT_1) AGONISTS

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Neurogenic inflammation of the meninges, in particular the dura mater encephali, is thought to play a key role in the cascade of nociceptive processes that lead to migraine pain and other severe headaches. Hyperemia is an important response of neurogenic inflammation. It can be experimentally elicited in the rat dura mater through local electrical stimulation and is monitored by recording of the blood flow in arterial vessels with a laser Doppler flowmeter.

Increases in blood flow following electrical stimulation were found to be mainly mediated by the release of calcitonin gene-related peptide (CGRP), but not substance P (SP), since these increases could be blocked by the CGRP antagonist CGRP(8-37) but not by the SP antagonist RP 67580. Serotonin receptor (5-HT_1) agonists such as sumatriptan and a specific 5-HT_{1B} agonist in low doses, which have been shown to inhibit plasma extravasation, had little influence on the neurogenic blood flow increases, whereas in high doses they attenuated both the basal dural blood flow (without stimulation) and the evoked increases in flow. The nitric oxide synthase inhibitor L-NAME affected the evoked increases in flow only at high doses after systemic administration.

We conclude that neurogenic increases in meningeal arterial blood flow which represent an element of neurogenic inflammation may be largely independent of the release and action of SP, i.e. the mechanisms that cause plasma extravasation. Different types of peptidergic afferent nerve fibers may be involved in these two neurogenic inflammatory responses. Nitric oxide does not significantly contribute to the neurogenic increases in flow.

KININS AND HYPERALGESIA

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Kinins are small peptides produced locally in response to tissue injury or trauma and, particularly, during inflammation. The two most studied kinins are bradykinin and kallidin.

Bradykinin and kallidin mediate their effects via two main classes of receptor, B_1 and B_2 with bradykinin and kallidin acting preferentially on the B_2 receptor and their only active metabolites, des-Arg^9bradykinin and des-Arg^{10}kallidin, respectively, are the preferred agonists for the B_1 receptor. Bradykinin has two main effects on nociceptive neurones, primarily via the B_2 receptor, namely activation and sensitisation. In behavioural models *in vivo*, selective B_2 receptor antagonists have been shown to be antinociceptive. Recently, studies have shown that during inflammation there is an increasing contribution of B_1 receptors to the accompanying hyperalgesia and in such circumstances B_1 receptor antagonists have been shown to be antinociceptive.

The B_1 receptor appears to be expressed or synthesised *de novo* in tissues following trauma or when inflammatory mediators such as cytokines are present. A close interaction has been shown between kinins other inflammatory mediators particularly cytokines. In particular, interleukin-1β up-regulates B_1 and B_2 receptors as well as enhances responses to bradykinin and desArg9bradykinin. The potential exists, therefore, for a powerful reinforcement between kinins and other inflammatory mediators leading to a sustained hyperalgesic state.

NEUROPEPTIDES IN GASTRIC MUCOSAL PROTECTION

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It is well established that stimulation of afferent neurons protects against damage caused by various noxious agents. Afferent nerve stimulation is associated with release of calcitonin gene-related peptide (CGRP) and tachykinins. CGRP and neurokinin A (NKA) analogs but not substance P have protective activity that involves nitric oxide (NO). In the rat stomach, capsaicin-induced afferent nerve-mediated protection is prevented by CGRP immunoneutralization and CGRP receptor blockade. Furthermore, NK2 receptor blockers attenuate capsaicin-evoked protection indicating that endogenous CGRP and NK2 receptor-activating tachykinins are involved. We have recently shown that a number of gut peptides such as gastrin-17, cholecystokinin-8, bombesin, urogastrone, corticotropin-releasing factor protect the rat gastric mucosa against ethanol-induced injury. The protective activity of gut peptides is lost after afferent nerve defunctionalization or after pretreatment with anti-CGRP antibodies, the CGRP receptor antagonist $CGRP_{8-37}$ or inhibitors of NO biosynthesis. For exogenous gastrin-17 and endogenous gastrin released by peptone perfusion of the gastric lumen we could demonstrate that NK2-selective tachykinins, prostaglandins and cholinergic neurons also contribute to the protective effect. The afferent nerve-, neuropeptide- and NO-related mechanism is not found with other types of gastroprotective agents such as SH-modulating compounds or salicylate. With some peptides such as gastrin the protective activity is inhibited by antisecretory drugs and hence seems to be related to the stimulated acid secretion, while no such association is found with bombesin or NKA analogs. Taken together, the findings show that certain exogenous and endogenous gut peptides exert potent gastroprotective effects that involve activation of afferent neurons, release of neuropeptides and NO biosynthesis. Peptide-evoked increase in mucosal resistance may represent an important endogenous mechanism supporting the maintenance of gastric mucosal integrity e.g. during food intake.

DIFFERENT RESPONSES OF SENSORY NEURONS TO LOW AND HIGH CONCENTRATIONS OF CAPSAICIN ON THE CELLULAR LEVEL

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It has been generally assumed that capsaicin, like resiniferatoxin, acts on sensory neurons via the vanilliod receptor. However, anomalies in potency and specificity between both substances suggest that there is more than one binding site.
We investigated the response to a prolonged application of capsaicin of different concentrations in an external solution with different ion compositions in isolated rat dorsal root ganglion cells with the whole-cell patch-clamp technique. Capsaicin can evoke up to three distinct current components with maxima at about 10, 40 and 70 sec., respectively. The first and second component could be activated independently, the third component occurred only together with the second one. The activation of the first and/or second component depended on the concentration of capsaicin. A low concentration predominantly elicited the second component, while a high concentration activated the first and suppressed the second one. The third component seems to be a secondary response. The first and the second current component showed a strong tachyphylaxis. However, after tachyphylaxis, if the extracellular medium was then acidified to a pH of 6.3, the second component alone could then be elicited by capsaicin.
These results support the hypothesis of distinct binding sites of capsaicin which may account for the variety of physiological responses evoked by capsaicin and the variations in these responses between species. Supported by DFG Pe/3-1.

PARTICIPATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND SUBSTANCE P (SP) ON THE FLARE REACTION AND PLASMA EXTRAVASATION IN THE PIG SKIN

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The unpigmented skin of domestic pigs appears to be an ideal model for investigating the neurogenic inflammation of the human skin. This is particularly true for the flare reaction which can be induced by antidromic nerve stimulation, heat, various endogenous and exogenous algesic substances and strong mechanical stimuli. Recently, it has been demonstrated (Lynn et al., this symposium) that only the stimulation of a particular set of chemo-heat receptors can induce antidromic vasodilatation and that the flare reaction is the consequence of the activation of axoncollaterales of these particular nociceptors. Antidromic and chemical stimulation induces the release of CGRP and SP but the flare reaction is preferentially induced by CGRP; SP and NKA might contribute to the maintenance of the reaction. With respect to plasma extravasation pig skin closely resembles human skin in that antidromic nerve stimulation and local application of capsaicin cannot induce detectable oedema. In both species, however, histamine produces plasma extravasation which is in the pig partly neurogenic and mediated by NK2 and to a lesser extent by NK1 receptor activation. Pretreatment with CGRP, however, inhibits the histamine induced plasma extravasation. This anti-inflammatory action of CGRP might result from an interaction of CGRP with receptors on C-fibre afferents, either by a negative feedback mechanism for a neuropeptide release, or the release of an ant-inflammatory mediator such as somatostatin.

TYPES OF DISTRIBUTION OF NEUROENDOCRINE ELEMENTS IN BRONCHI OF HUMANS WITH AND WITHOUT LUNG DISEASE.

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Several putative messengers and general markers of diffuse neuroendocrine system (DNS) of bronchi were investigated in 87 humans with bronchectasis, chronic bronchitis, Chernobyl liquidators immunocytochemically. Biopsies were taken during bronchoscopy from the large bronchi in the region of subcarinae. Control material was obtained from 22 people who died from non-respiratory disease. Two types of distribution in neuroendocrine elements were revealed in both - patients and control samples. The first type showed bronchial wall with non-changed and metaplastic epithelium. Elements of DNS occurred practically in all bronchial wall from mucosa to adventitia. There were protein gene peptide 9.5 (PGP 9.5), gastrin releasing peptide, calcitonin gene related peptide (CGRP), chromogranin A, chromogranin A and B, serotonin detected in neuroendocrine cells (NC). Nerve fibers of bronchial wall contained PGP 9.5, vasoactive intestinal peptide (VIP), neuropeptide Y, pituitary adenylate cyclase activating peptide, helospectin, substance P, CGRP, galanin. The second type of distribution in neuroendocrine elements showed bronchial wall without any immunoreactive NC in the epithelium. This type was observed in 6 patients and 6 control samples. No immunoreactive nerves were observed in all bronchial wall. Exception was 2 cases, when occasional fine VIP - containing nerves were detected around the seromucous glands. Also bronchial wall with the second type of distribution in neuroendocrine elements displayed both - non-changed and metaplastic epithelium of mucosa. Although non-changed epithelium dominated in the above mentioned cases. We suggest, that these two distribution types of neuroendocrine elements seems to be a biological phenomena in human bronchi.

ANTI-INFLAMMATORY MEDIATORS RELEASED BY ACTIVATION OF CAPSAICIN-SENSITIVE SENSORY NERVE FIBRES

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Neuropeptides released by electrical or chemical stimulation from peripheral endings of the capsaicin-sensitive primary afferent neurons evoke local neurogenic inflammation in the innervated skin and mucosal areas. It has been established by previous studies that a local neurogenic inflammation (primary reaction) induced by antidromic as well as orthodromic stimulation prevents the development of a subsequent neurogenic or non-neurogenic inflammation on a distant part of the body (secondary reaction). A pure non-neurogenic inflammation evoked by dextran had no anti-inflammatory activity.

The aim of the present study was to determine the neurotransmitter background of this phenomenon. Following the primary reaction induced by 1% mustard oil application on acutely denervated leg skin of the rats, the secondary plasma protein extravasation on the contralateral leg evoked also by mustard oil was inhibited by 50 percent. Pretreatment with an opiate antagonist naloxone (1 mg/kg), almost totally abolished this anti-inflammatory effect of the primary reaction. Neurogenic inflammation produced by 1% mustard oil smearing in the skin of acutely denervated leg (primary reaction) significantly decreased the oedema induced by subcutaneously injected dextran in the contralateral leg after chronic denervation (secondary reaction). Although naloxone preteatment diminished the anti-inflammatory effect, the oedema developed in the mustard oil treated animals was significantly smaller than it was in the paraffin oil treated animals. *Conclusions*: A primary local neurogenic inflammation prevents the development of a secondary inflammatory reaction. If the secondary reaction has neurogenic component, the opioidergic transmission plays key-role in mediation of the anti-inflammatory effect. If the secondary reaction is a non-neurogenic inflammation participation of other anti-inflammatory mediators (somatostatin, galanin?) are also important besides opioids.

IMMUNOHISTOCHEMICAL VISUALIZATION OF SUBSTANCE P AND NEUROKININ-1 RECEPTOR IN THE RAT SPINAL DORSAL HORN AFTER ULTRAVIOLET IRRADIATION

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The neurokinin substance P (SP) and its high affinity receptor, neurokinin 1 receptor (NK1-R), have been shown to play an important role in the spinal nociceptive information processing. Recent findings suggest that both SP and NK1-R expression may undergo plastic changes in prolonged hyperalgesia. The aim of the present study was to investigate these alterations in ultraviolet (UV) irradiation induced inflammatory hyperalgesia in the rat spinal dorsal horn. The plantar surface of the right hind paw was irradiated with a UV light source on 3 consecutive days. Heat hyperalgesia was measured by hot plate test and on day 4 when the hyperalgesia proved to be the highest animals were perfused transcardially by fixative. Vibratome sections from the L4 segment were processed for dual pre-embedding immunohistochemical reaction to visualise SP and NK1-R immunoreactive (IR) neuronal elements. A significant decrease of the intensity of SP- and NK1-R-IR could be observed on both ipsi- and contralateral side of the spinal dorsal horn in the UV irradiated animals compared to that of the control rats. At the electron microscopic level modification of synaptic profiles could be observed. The present data indicate that inflammation of the peripheral tissues may induce complex changes in the SP-NK1-R involved synaptic transmission. Our findings, compared to previous studies on other types of prolonged inflammatory hyperalgesia models, suggest that alterations in SP and NK1-R expression and their synaptic contacts may depend on the type of inflammation.

CENTRAL EFFECTS OF THE AMPHIBIAN TACHYKININ PG-KII IN CONSCIOUS RATS.

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PG-KII, a new kassinin-like tachykinin of amphibian origin, has been reported to be a potent agonist at NK3 receptors and a weak agonist at NK1 receptors in in vitro tests. The present study evaluated the pharmacological profile of PG-KII in in vivo tests for central effects in conscious rats.

Previous studies have shown that NK3 receptor agonists inhibit alcohol intake in rats. Both NK1 and NK2 receptor agonists are devoid of effect. PG-KII was tested by intracerebroventricular (i.c.v.) injection on alcohol intake of food and water sated, genetically selected alcohol-preferring rats, offered 10% ethanol 2 hr/day. PG-KII inhibited ethanol intake at 10 ng/rat; 100 ng/rat produced more than 50% inhibition; 1000 ng/rat completely suppressed the intake for about 30 min.

Central injection of tachykinins inhibits gastric acid secretion in rats. Selective agonists and antagonists provide evidence that the effect is mediated by NK3 receptor activation. PG-KII, given by i.c.v. injection at doses of 1-20 μg/rat in pylorus-ligated rats, elicited a marked inhibition of gastric acid secretion, similar to that induced by NKB.

PG-KII was also tested on drinking induced by central injection of angiotensin II, 100 ng/rat. Both selective agonists and antagonists indicate that the inhibitory effect of tachykinins on angiotensin II-induced drinking is mediated by central NK1 receptors. PG-KII did not modify drinking to angiotensin II at 10 or 100 ng/rat; but only at 1000 ng/rat. However, the NK1 receptor antagonist SR140333, at the i.c.v. dose of 1 μg/rat (that completely suppresses the effect of other tachykinins on angiotensin-induced drinking) only partially blocked the effect of PG-KII, 1000 ng/rat. The same dose of PG-KII inhibited also food intake in food deprived rat, but its effects on the ingestive behaviour might be non-selective since it induces also a marked increase in locomotor activity

In conclusion, the present study shows that also in tests for central activity PG-KII behaves as a potent NK3 receptor agonist. On the other hand, its agonistic activity at NK1 receptors is evident only at higher doses, which, however, may evoke more general behavioural alterations.

CENTRAL NK3 RECEPTORS: A ROLE IN TASTE MECHANISMS

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Central injections of tachykinins inhibit salt intake induced by a variety of natriorexigenic stimuli in rats. The order of potency of agonists suggests that NK3 receptors mediate the effect.

Substance P and tachykinin receptors are present in gustatory pathways in the rat central nervous system. The amygdala and the bed nucleus of the stria terminalis (BNST), which are terminals of gustatory pathways in the prosencephalon, show high density of NK3 receptors, high levels of PPT-A mRNA, and are very sensitive to the inhibitory action of tachykinins on salt intake.

The present study evaluated the effect of intracerebroventricular administration of the NK3 selective agonist [$Asp^{5,6}$,$MePhe^{8}$]substance P(5,11), also referred to as NH_2-senktide (NH_2-SENK), on the taste evaluation of NaCl solutions (0.03, 0.15, 0.25 and 0.5 M), water, 1% sucrose and 0.01 M quinine. The taste reactivity test was employed: a small amount (0.8 ml/rat) of tastant was infused in 1 min into the oral cavity through an oral catheter; during infusion the rat's oral facial region was videotaped and the number of ingestive (mouth movements, tongue protrusions, lateral tongue protrusions) and aversive (gapes, chin rubs, forelimb flails, head shakes, passive dripping) reactions was measured under slow motion.

NH_2-SENK, 31-125 ng/rat, consistently induced passive dripping 20-45 sec after the beginning of the intraoral infusion of isotonic or hypertonic NaCl solutions (0.15, 0.25 and 0.5 M). Other aversive reactions were not modified. The number of ingestive reactions was reduced when passive dripping occurred. Taste reactions to water, 0.03 M NaCl, sucrose or quinine were not modified.

The finding that NH_2-SENK modifies taste reactivity within a few sec of intraoral infusion of NaCl solutions supports the idea that the drug elicits a prompt alteration of taste mechanisms for salt. The influence of NH_2-SENK on taste mechanisms is rather selective, since it modified taste reactions only to isotonic and hypertonic NaCl solutions.

In keeping with these behavioural findings, in situ hybridization studies indicate that in a variety of conditions under which there is increased intake of hypertonic NaCl solutions, the expression of PPT-A mRNA is markedly reduced in several prosencephalic regions.

REGULATION OF PREPROTACHYKININ-A MESSENGER RNA IN GENETIC HYPERTENSIVE AND NORMOTENSIVE RATS

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Central administration of tachykinins (Tks) inhibit salt intake in rats. Recent studies have shown that conditions that arouse salt appetite, such as adrenalectomy, deoxycorticosterone acetate (DOCA) administration and sodium depletion induce a decrease in preprotachykinin-A (PPT-A) mRNA in discrete regions of the rat brain, suggesting that reduced levels of PPT-A mRNA in the brain may have a permissive role on the expression of salt appetite.

Spontaneously hypertensive rats (SHR) show higher avidity for salty solutions than their normotensive control Wistar Kyoto (WKY) rats. In this regard, the present study tested whether SHR and WKY rats differ in expression of the gene coding for PPT-A. Using quantitative in situ hybridization histochemistry, we examined the level of PPT-A mRNA of SHR and WKY rats under no treatment and after three days of Na^+ depletion in discrete brain regions in which PPT-A mRNA is expressed: the olfactory tubercle (Tu), the lateral olfactory tubercle (LOT), the dorsal and ventral caudate putamen (d/v CPu), the medial preoptic area (mPOA), the bed nucleus of the stria terminalis (BNST), the habenula (Hb), and the postero-dorsal part of the amygdala (MePD).

Quantitative analysis of silver grains revealed about 30% lower expression of the PPT-A mRNA levels in SHR opposite to WKY rats under no treatment in v-CPu, mPOA, BNST and Hb. A 35% lower level of PPT-A mRNA in SHR, with respect to WKY rats, was detected after three days of Na^+ depletion in d-CPu, v-CPu and mPOA. A lower gene expression, even though not statistically significant, was also found in Tu, LOT, MePD in both experimental conditions.

These findings show a consistent difference of PPT-A mRNA levels in discrete regions of the SHR brain opposite to WKY rats. Since SHR are notoriously more salt-avid than WKY rats and Tks are potent inhibitors of salt intake, the down-regulation of PPT-A mRNA may contribute to the higher natriophilia and to the etiology of the hypertensive disease.

THE ROLE OF CAPSAICIN/RTX SENSITIVE NEURONES IN THE REGULATION OF MICROCIRCULATION OF SKIN AND STRIATED MUSCLE

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It has been shown, that antidromic stimulation of L4-L5 dorsal roots elicites an increase in blood flow in ipsilateral skin and muscles of hindleg in rats. This antidromic vasodilatation is mediated by capsaicin/RTX sensitive primary afferents. The aim of the present paper is to test the effect of orthodromic stimulation of these nerve endings by capsaicin or resiniferatoxin. In order to rule out the interfering effect of systemic blood pressure changes evoked by drug administration a blood perfused hindleg preparation was developed.

Male Wistar rats weighing 250-350 g were used and anaesthetised with thiobutobarbital (Inactin) i.p. Continuous pulsative blood perfusion of the hindleg was achieved by connecting the right carotid artery to the right iliac artery through a peristaltic pump (flow rate 3 ml/min). The perfusion pressure of the efferent arch of the iliac cannula and the systemic blood pressure from the carotid artery were recorded. The drugs were administered through the perfusion system intraarterially. Laser Doppler probes for monitoring microcirculatory changes were placed on the glabrous skin of the hindpaw and on the flexor muscles of the thigh. A group of animals was submitted to crossection of femoral, genitofemoral and the sciatic nerve a week before the experiment.

Capsaicin (0.1-3.0 μg, i.a.) enhanced the blood flow of the skin of hindpaw while in the striated muscle an initial short rise was followed by a fall in microcirculation. The capsaicin evoked vasodilatation in the skin and the lasting vasoconstriction phase in the muscle were dose related. The higher dose of capsaicin (1, 3 μg) caused a rise in perfusion pressure and a fall in systemic blood pressure. All these effects were abolished by i.v. administration of 1 μg/kg resiniferatoxin (RTX) and strongly inhibited by ruthenium red (2 mg/kg) indicating a site of action on the capsaicin-gated cation channels. Involvement of neural elements in the vasoconstrictor effect of capsaicin is also indicated by the significant reduction (78-89 %, $p<0.001$) of the evoked rise in perfusion pressure by chronic denervation. Atropine (1 mg/kg), phentolamine (0.3 mg/kg) or propranolol (0.3 mg/kg) given i.v. induced no alteration of the vascular responses. The present study provides the first evidence that sensory neural mechanisms participate in the vasoconstrictor response of capsaicin, which manifest itself in the muscles but not in the skin.

Sensory neurone activation by kinins

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Activation of primary afferent nerve endings by bradykinin has two aspects: (a) slowly but inactivating excitation of about half of the polymodal nociceptors by relatively high concentrations of BK and (b) non-inactivating slowly deactivating sensitisation to heat stimulation of almost all polymodals and part of other nociceptor types. If present, both BK effects are irreversibly blocked by the B_2-receptor antagonist HOE140 (10^{-8} M). Neurones sensitised but not excited by BK exhibit selective and reversible B_1-receptor antagonism alternative to B_2 (50%).
In different models, BK-induced sensitisation also applies to responsiveness upon low-pH stimulation that appears as physiological basis of sustained nociceptor discharge. In cultivated DRG cells of the adult rat, a sustained cationic inward current evoked by low pH is augmented by BK which by itself is as ineffective as 5HT, histamine and PGE_2. A combination of these inflammatory mediators, however, is much more effective in DRG cells and also increases pH-induced nociceptor discharge in rat and pain in human skin. This sensitizing synergism does not depend on histamine, and PGE_2 appears to play a role only in visceral but not rat cutaneous tissue. BK alone, however, is not sufficient to increase pH-induced pain nor nociceptor discharge. Serotonin seems to be the crucial adjuvant that facilitates the actions of BK. Sensitisation rather than excitation may be the true physiological function of BK in inflammatory pain and hyperalgesia
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NEUROPEPTIDES AND NEUROTROPHINS IN INFLAMMATORY BOWEL DISEASE AND EXPERIMENTAL COLITIS

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Sensory neuropeptides like Substance P (SP) and Calcitonin Gene-related Peptide (CGRP) are released from sensory enteric nerves during the timecourse of experimental colitis (Gastroenterology 101,1211-1219, 1991). We have shown that sensory nerves containing sensory neuropeptides exert an protective effect in an acute (Gastroenterology 106,1208-1214, 1994) and chronic model of experimental colitis (Am.J. Physiol. 270, G79-G86, 1996). Inhibition of CGRP but not SP endogenous activity increased the severity of the inflammation in the colon indicating that CGRP is the protective neuropeptide in the gut (Gastroenterology 108, A676, 1995). SP-receptors (SP-R) are highly upregulated in the inflamed gut of patients with Crohn's disease and ulcerative colitis. Im Crohn's disease there is even a significant upregulation of SP-R in the noninflamed gut of patients in remission. Neurotrophins regulate neuropeptide expression in the sensory nervous system of the gut. Since lymphocytes and other inflammatory cells produce Nerve Growth Factor (NGF) neurotrophins may provide an important link between the enteric nervous system and the immune system in the gut. Immunoneutralisation of NGF or Neurotrophin-3 (NT-3) significantly worsened the chronic colitis (Gastroenterology 110,1996).We could show recently an significant increase in NGF and NGF prohormone content in the inflamed gut of patients with inflammatory bowel disease and in experimental colitis (Soc. Neurosci. Abs. 21, 23.3, 1995).
These data provide increasing evidence that sensory neuroeptides and neurotrophins play an important role in the regulation of experimental colitis and inflammatory bowel disease.

NEUTRAL ENDOPEPTIDASE INHIBITION MODULATES CAPSAICIN INDUCED MESENTERIC VASODILATION IN DIABETIC RATS

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Previous studies have reported that the vasodilator function of capsaicin sensitive afferent nerves (C afferent) in rat mesenteric arteries is severely impaired in diabetic rats. In recent years, it has become increasingly clear that neutral endopeptidase (NEP) is a potentially important enzime capable of regulating the biological activity of sensory neuropeptides. NEP is present in the rat gut. Aims: Therefore, we examined the effect of a new orally active NEP inhibitor KC 12792 on intestinal blood flow (IBF) in diabetic rats after stimulating C afferent with capsaicin. Methods: Wistar rats were treated with streptozotocin (65 mg·kg^{-1}i.p.) to induce diabetes. Subgroups of age-matched controls and streptozotocin-treated rats were given 30 mg·kg^{-1}·day^{-1} of KC 12792 orally. Eight weeks later, the animals were anesthetised and the IBF was recorded by transit-time flowmeter;arterial blood pressure (MAP) and heart rate (HR) were also measured. After 20 min baseline recordings intrajejunal administration of 1.6·10^{-4}M capsaicin effect was assessed. Results: Resting IBFs from diabetic rats were significantly lower compared to the controls and to the NEP inhibitor treated rats. IBFs at rest were 9±1.7 (n=10), 15±2.8 (n=10) and 14±1.9 (n=10) ml·min^{-1} for diabetic, control and diabetic + NEP inhibitor treated rats, respectively. Treatments failed to alter MAP or HR. Furthermore, the capsaicin-elicited vasodilator response of IBF was 31±4% in controls which was significantly smaller (- 68±6%) in diabetic rats. Whereas the capsaicin-induced increase of IBF was significantly elevated in NEP inhibitor treated controls (29±5%) and in the NEP inhibitor treated diabetic rats (+54±7) vs controls and diabetic rats. Conclusion: 1., Diabetes induces impairment of efferent vasodilator function of visceral C afferent nerves. 2., This local vasodilator function of C afferent nerves is markedly potentiated by chronic NEP inhibition. 3., The results suggest that this effect of NEP inhibition is due to reduced degradation of sensory neuropeptides of the gut.

EFFECTS OF LESIONS OF THE GASSERIAN GANGLION ON THE CGRP IMMUNOREACTIVITY OF THE CAUDAL TRIGEMINAL NUCLEUS

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In striking contrast to the generally accepted doctrine of exclusively ipsilateral representation of primary afferents, immunohistochemical and neuropathological studies performed in this laboratory raised the possibility of a restricted contralateral representation of primary nociceptive axons. This question has been studied by means of stereotactical lesions of the Gasserian ganglion and by surgical transection of the sensory root of the trigeminal nerve. We found a well-defined decrease of CGRP immunoreactivity of the primary axon terminals in the caudal trigeminal nucleus, accompanied by a restricted focal contralateral decrease, supported by Fink&Heimer's and Eager's silver staining and Csillik's protargol impregnation. A review of the pertinent literature proves that contralateral representation of trigeminal afferents has been surmised already by Torvik (1956) and supported also path-tracing experiments (Marfurt, 1981, Pfaller&Arvidsson, 1988). Partial contralateral representation of primary nociceptive afferents has been noted earlier also in the sacral spinal cord (Knyihár-Csillik and Csillik, 1981). It is concluded that contralateral representation of primary nociceptive afferents in restricted regions of the neuraxis might be involved in the spreading of potentially painful impulses, like in generalization of migraine attacks.

PARTICIPATION OF PEPTIDERGIC AFFERENT NERVE FIBRES IN VISCERAL NOCICEPTION AND INFLAMMATION

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Visceral organs are innervated by at least two types of primary afferent nerve fibres which are involved in visceral nociception: 1.) Low threshold intensity-coding afferents which respond to physiological mechanical stimuli but are also able to encode noxious stimuli and 2.) visceral nociceptors that respond to noxious mechanical and chemical stimuli. In most viscera, visceral nociceptors make up 20-40% of the mechanosensitive innervation. In the guinea-pig ureter, however, more than 90% of the afferents can be considered as nociceptors. These fibres are capsaicin sensitive and contain both substance P (SP) and calcitonin gene-related peptide (CGRP). In the rat ureter, these SP/CGRP-containing afferents represent the dominant innervation and make up more than 90% of the nerve fibres in the subepithelial plexus. Insertion of an artificial "stone" in the rat ureter *in vivo* induced colic-like crisis and resulted in a strong depletion of SP/CGRP-containing fibres.

Due to their local-efferent functions the peptidergic visceral nociceptors are also involved in neurogenic inflammatory mechanisms (vasodilatation and plasma extravasation). To analyse the involvement of the ureteric SP/CGRP-containing afferents in plasma extravasation, direct labelling of permeable blood vessels was achieved using the colloidal silver technique and local application of capsaicin. The study revealed that in the guinea-pig ureter 50-66% of the leaky vessels are innervated by SP/CGRP-containing nerve fibres. Using the same technique significant capsaicin-induced plasma extravasation could be demonstrated in the rat small intestine (predominately in the smooth muscle layer), but not in the stomach and the colon. However, only less than 15% of the leaky vessels were directly innervated by CGRP-positive fibres. In addition, peptidergic sensory fibres in the gastrointestinal tract can induce vasodilatation and might have direct protective functions. Although in rodents there is good evidence for the involvement of SP/CGRP-containing fibres in visceral nociception and inflammation, the role of these afferents in other species, including man, is less clear.

PERIPHERAL NERVE SECTION PREVENTS CAPSAICIN - INDUCED DEGENERATION OF ADULT RAT DORSAL ROOT GANGLION NEURONS

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A particular group of mammalian primary afferent neurons is characterized by their specific sensitivity to capsaicin. A striking manifestation of neuronal capsaicin sensitivity is the degeneration of a morphologically well characterized population of sensory ganglion cells following a systemic injection of this neurotoxin. The aim of this study was to investigate the effect of peripheral nerve injury on the neurodegenerative action of systemically injected capsaicin. In 11 adult male Wistar rats the right sciatic nerves were exposed and transected distal to a ligature. After a survival period of 1-4 days the animals were given a single subcutaneous injection of capsaicin (100 mg/kg) 3-4 hours prior to sacrifice. The lumbar dorsal root ganglia relating to the sciatic nerve were removed and processed for light microscopic examination. In dorsal root ganglia relating to the intact nerve 14-19% of all neurons displayed alterations characteristic of capsaicin-induced degeneration. In contrast, significantly fewer neurons amounting only 2-6% of the total neuronal population showed degenerative changes ipsilateral to the sciatic nerve which had been transected 4 days before capsaicin treatment ($p<0.05$, $n=6$). In rats whose nerves were transected 1 day before a systemic injection of capsaicin, there was no significant difference in the proportion of degenerating nerve cells between the ganglia of the control and experimental sides. These results are in line with our earlier findings showing a significant reduction of the neurotoxic action of capsaicin on nerve cells of ganglia related to a nerve which had been treated previously with capsaicin. We conclude that capsaicin sensitivity is critically dependent on the integrity of the peripheral branch of the primary sensory neuron and peripherally derived trophic factor(s) may play a crucial role in the maintenance of neuronal capsaicin sensitivity.

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ENKEPHALIN-ARG^6-MEL^7 - A NOVEL POTENT IRREVERSIBLE KAPPA OPIOID LIGAND

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Met-enkephalin-Arg^6-Phe^7 (MERF) was modified with the methyl ester of melphalan (Mel) on the C terminus (Tyr-Gly-Gly-Phe-Met-Arg-Mel) and its opioid binding property to guinea pig and rat brain membranes was characterized. It was expected that the nitrogen mustard reactive group of the Mel will interact covalently with a nucleophilic center of the target protein. In the rat brain the ligand did not show any specificity, but the peptide exhibits high affinity in displacement experiments for the [^{3}H]ethylketocyclazocine (EKC) and [^{3}H]norbinaltorphimine (NBI) binding sites in guinea pig with K_i values $K_{i\ high}$=5.53 ($K_{i\ low}$=564) and 25.3 nM respectively. Moreover the ligand shows very low potencies in inhibiting [^{3}H]DAGO (μ) or [^{3}H]TIPPψ (δ) binding in both species. The rank order of affinities to a variety of labeled subtype specific ligands suggested a κ binding profile, and can be expressed as κ>> μ = δ selectivity, although on an isolated GPI tissue the peptide showed weak affinity (IC_{50}=1117 nM). The Mel derivative of MERF showed similar K_i value with the parent compound when tested in displacement experiments using [^{3}H]EKC as a radioligand as well as in physiological assay on GPI preparation. Preincubation of the brain membranes with 1-10 μM of MERF-Mel produced an apparent irreversible inhibition of [^{3}H]opioid ligand binding. Irreversible binding was optimal incubating the membranes with the peptide for 45 min at 24°C at the concentration 10 μM (45% of control binding). The effect of sodium ions on MERF-Mel competition against [^{3}H]naloxone binding showed mixed agonist/antagonist nature of the compound. These results suggest that the new derivative of MERF in tritiated form can be used as a relatively high affinity covalent label for the κ opioid receptor.

ENTERIC PHYSIOLOGY OF NEUROPEPTIDES AND TRANSMITTERS

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The enteric nervous system (ENS) organises the behaviour of various gastrointestinal effector systems, like motility and secretion. Combination of electrophysiological, immunhistochemical and retrograde tracing methods were used to identify enteric circuits involved in the complex control mechanisms of muscle and mucosa activities. In the stomach we identified a polarized reflex within the myenteric plexus of the ENS which consisted of ascending cholinergic and descending NO/VIP/NPY- (nitric oxide,vasoactive intestinal polypeptide and neuropeptide Y) containing neurones. This projection pattern and neurochemical coding did apply for both motor neurones and interneurones. These cell populations might be involved in the coordination of oral contraction and aboral relaxation during a peristaltic wave. Most of the ascending cholinergic neurones, but only a few descending NO/VIP/NPY neurones, could be excited by serotonin through 5-HT3 receptors. This indicated the significance of serotonergic mechanisms for stimulating cholinergic-mediated motor responses.

For the innervation of the mucosa in the colon, we also identified a polarized reflex in the submucosal plexus which consisted of local short projecting cholinergic neurones and long projecting, descending VIP neurones. Both are seceto-motorneurones and responded to inflammatory mediators. The sensory neurones in this reflex pathway are very likely calbindin-positive neurones which use acetylcholine and substance P as transmitters. Some myenteric neurones did also project to the mucosa and probably function also as sensory neurones relaying informations coming from the mucosa to the muscle. The vast majority of these myenteric neurones were also calbindin-positive.

The results indicate that the control mechanisms within the ENS are organized as polarized reflexes. There projection patterns are characterized by distinct neurochemical codes which appeared to be specific for the function of a certain cell population.

KINETICS OF PLASMA EXTRAVASATION AND NEUROPEPTIDE CONCENTRATION IN HUMAN SKIN AS MEASURED BY A MICROFILTRATION TECHNIQUE

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Plasma extravasation is a main event in neurogenic inflammation. In this study we used the technique of microfiltration to measure plasma extravasation and substance P (SP) concentration after induction of a neurogenic inflammation by capsaicin and by histamine.
Linear microfiltration probes (filtration coefficient for albumine 1.0, outer diameter 0.3 mm, Asahi Co., kindly gifted from Diamed GmbH, Köln) were inserted intracutaneously by a surgical suture needle (outer diameter 0.35 mm) and perfused with Hanks solution at a flow rate of 4 µl/min (Harvard Microdialysis pump 22). Total protein concentration was measured photometrically (Biolinx, MRX reader) after addition of Coomassie blue reagent in probe volumes of 10µl. Substance P was measured by Elisa technique (Cayman, USA) in probe volumes of 50 µl. Total protein and SP were measured in the perfusate of the stimulated probe and in a second probe which was located in the flare zone about 1.5 cm apart. Mean total protein concentration after insertion was 0.75 mg/ml (n=12) and declined rapidly to reach 0.2 mg/ml by 100 minutes. Application of histamine and capsaicin to the skin induced a local wheal and an axon reflex flare. Plasma extravasation increased in the dialysate from the treated site. However, in the flare area no increase in total protein concentration could be measured while SP concentration increased from 25 to 33 pg/ml.
It is concluded that the axon reflex mechanism induces a release of SP from axon collaterals in the surroundings of the stimulated site without a measurable increase of plasma extravasation.

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EFFECTS OF ARGININE-VASOPRESSINE (4-9) ON EXPLORATION AND ANXIETY IN LESIONED RATS

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The lesions of the infralimbic prefrontal cortex (ILPFC) disturb the spatial orientation. The aim was to study the effects of Arginine-Vasopressine$_{(4-9)}$ (AVP_{4-9}) on exploration in open field and on anxiety using shock prod bury test and elevated plus maze in ILPFC lesioned rats. Six groups (8 rats per group) were used divided in two main groups: S=sham and L=lesioned. The lesions (or sham) were made under aneasthesia using stereotaxic technique, silver electrodes with 0.2mA, 20 sec. The rats were injected 30 min before testing i.p. with saline (0.1ml/100g) or with 0.1 or 0.01 µg/kg AVP_{4-9}. The rats received a block of 10 min training for 3 days in open field arena, 2 days 10 min training in shock prod bury test and 1 day 5 min training in elevated plus maze. The exploration was registered using video computerized image analysis system. The animals were observed for both anxiety tests using "Behaviour"computer programme. The S control rats as wel as the rats injected with AVP_{4-9} showed habituation in open field. The L control rats showed less habiuation. The L rats injected with AVP_{4-9} decreased the locomotor acivity as compared with control rats. The number of shocks and the sniffing of prod in S rats were less as compared with L control rats. The rats (S or L) injected with AVP_{4-9} at low dose incresed the number of shocks The L rats visited less open arms of elevated plus maze as compared with S rats. The rats injected with AVP_{4-9} did not show any effect in elevated plus maze. AVP_{4-9} decrease the exploration and show antianxiety effect.

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BINDING CHARACTERISTICS OF CONFORMATIONALLY RESTRICTED RADIOLABELED DELTORPHIN ANALOGS IN RAT BRAIN MEMBRANES

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In an attempt to enhance δ receptor affinity and/or selectivity of deltorphins, conformationally restricted analogs were designed. We describe novel deltorphin analogs using modification in side chain at positions 3, 5 and 6, which caused changes in hydrophobic and stereoelectronic properties. The new analogs were obtained from substitution of Phe at position 3 with 2-amino-tetralin-2-carboxylic acid (Atc) and of Val at position 5 and 6 with Ile in deltorphins I and deltorphins II. These peptides were synthesized in tritiated form by catalytic dehalogenation. They were tested for opioid receptor affinity and selectivity by ligand binding assays in rat brain membrane preparations. The binding was saturable and time-dependent. The δ specific agonist peptides labeled a single class of opioid sites with a maximal number of binding sites (B_{max}) of 120-130 fmol/mg protein and high affinity (K_d: 0.3 nM). The ligands selective for δ receptors, such as naltrindole, TIPP[ψ], DPDPE, DSLET, inhibited the binding of the new tritiated compounds with Ki values in the nanomolar range. Ligands selective for μ and κ receptors were found to be weak inhibitiors. Due to their high δ receptor affinity, δ selectivity and very low non-specific binding (<15%), the new conformationally restricted deltorphin analogs are very useful tools for the identification and characterization of δ opioid receptors and also in the pharmacological studies on opioid receptors. (This work was supported by OTKA T-16991 research grant.)

[^{3}H]TICP[ψ]: A HIGHLY POTENT AND STABLE PSEUDOPEPTIDE δ OPIOID RECEPTOR ANTAGONIST

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Substitution of the Phe^3 aromatic ring in H-Tyr-Ticψ[CH_2-NH]Phe-Phe-OH (TIPP[ψ]), with cyclohexylalanine (Cha), led to a compound H-Tyr-Ticψ[CH_2-NH]Cha-Phe-OH (TICP[ψ]) with substantially increased δ antagonist potency and high δ selectivity. TICP[ψ] was radiolabeled by catalytic tritiation of its precursor Tyr(3',5'-I_2)^{1}TICP[Ψ]. Binding characteristics of the new tritiated pseudopeptide were determined in rat brain membranes. On the basis of the saturation binding studies at 25°C an equilibrium dissociation constant (K_d) of 0.35 nM and a receptor density (B_{max}) of 112 fmol/mg protein were calculated. This new tritiated ligand exhibits high affinity for the δ opioid binding sites, whereas its binding to the μ and κ type is weak, displacement studies by various receptor-selective opioids showed the following rank order of potency: δ>>κ≈μ. These characteristics of the ligand, together with its high specific radioactivity (41.3 Ci/mmol) makes it an useful tool for labeling of the δ receptors, both *in vitro* and *in vivo*. (This work was supported by OTKA T-16991 research grant.)

THE ROLE OF CCK AND SP IN TEMPERATURE REGULATION OF RATS I. CENTRAL THERMOREGULATORY EFFECTS OF THE EXOGENOUS PEPTIDES

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To learn if CCK or SP could take part in central body temperature control, female Wistar rats were equipped with chronic intracerebroventricular (ICV) guide cannulae to facilitate lateral ICV microinjections. In conscious, slightly restrained rats ICV injections of CCK, ceruletide or SP were given and their effects on heat production, body core and tail skin temperatures were compared with those of prostaglandin E_1 microinjected similarly. When tested in thermoneutral as well as in slightly cool or warm environments a dose dependent co-ordinated thermoregulatory response of short latency and duration was observed after ICV injection of either of these peptides, each time the response consisting of rises in core temperature and metabolic rate and a fall in skin temperature. This rise in core temperature appeared to be, therefore, a fever-like response, which could be significantly attenuated by central or peripheral injection of the type-B antagonist of CCK (L-365,260) but not by the type-B CCK antagonist (L-364,718) or by a peptide or non-peptide antagonist of SP, for CCK- or SP-induced fevers, respectively. Since the well known hypothermic effect of CCK given peripherally could be attenuated by the type-A CCK-antagonist, but not by the type-B one, it is concluded that the fever-like response of rats elicited by ICV injection of CCK is probably a specific one. A similar conclusion can also be drawn for the SP-induced fever-like response; thus both neuropeptides might also be implicated in the central mechanism of fever iduced by pyrogens such as endotoxin.

THE ROLE OF CCK AND SP IN TEMPERATURE REGULATION OF RATS II. POSSIBLE ROLE FOR THE ENDOGENOUS NEUROPEPTIDES

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The neuropeptides (NPs) cholecystokinin (CCK) and substance P (SP), upon central administration, have been demonstrated to induce co-ordinated thermoregulatory responses resulting in elevations of core temperature, which were preventable by using the appropriate antagonists. In order to see whether or not any endogenous role can be ascribed to these NPs, their possible participation in the fever elicited by lipopolysaccharide (LPS) in rats was analyzed. Cold-adapted female Wistar rats were preimplanted with a jugular and an intracerebroventricular (ICV) cannula for LPS and antagonist injections, respectively. On the day of the experiment, they were equipped with a subcutaneous (SC) cannula and thermocouples into the colon and the tail skin. All injections were given in a metabolic chamber at thermoneutrality (25°C). The antagonists were applied prior to the LPS either SC or ICV. Parameters of body temperature and metabolic rate were recorded continuously. LPS induced a characteristic biphasic fever. The CCK type-B-receptor antagonist (L-365,260) in a SC dose that effectively antagonized the central CCK-action modified the course of LPS-fever: the first, but not the second, phase was inhibited. The antagonism was stronger with small ICV doses of the substance. The inhibitory influence of the CCK type-A-receptor antagonist (L-364,718) was more pronounced during the second phase. ICV injections of both peptide- and non-peptide-antagonists of SP strongly attenuated the whole fever course. Accordingly, these neuropeptides appear to have some role in the mediation of fever, independent of the known role of prostaglandins.

CENTRAL AND PERIPHERAL MECHANISMS UNDERLYING VAGAL DEPENDENT PROTECTION OF THE GASTRIC MUCOSA AGAINST INJURY.

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The vagus was reported to prevent adaptive gastric protection in rats. Previous studies indicate that thyrotropin-releasing hormone (TRH) in the medulla play a physiological role in activating vagal outflow to the stomach (*Peptides*, 16: 431-435, 1995). TRH analog injected intracisternally or into the dorsal motor nucleus of the vagus at low dose prevents gastric lesions induced by 60% ethanol in conscious or anaesthetized rats. Using injection of specific TRH antibody into the cisterna magna or bilaterally into the dorsal motor nucleus of the vagus, we showed that TRH antibody blocked adaptive gastric protection induced by low concentration of ethanol or acid and gastric protection induced by low dose of kianic acid microinjected into the raphe pallidus. Control antibody had no effect as well as TRH antibody microinjected outside of the dorsal vagal complex. Exogenous or endogenous TRH-induced gastric protection is mediated by vagal cholinergic activation of prostaglandin, calcitonin gene raleted peptide (CGRP) and nitric oxide (NO) dependent mechanisms. CGRP originating from capsaicin sensitive afferents, and NO, unlike gastric prostaglandins, are involved in low doses TRH analog-induced gastric hyperemia. Ketotifen prevents low dose of TRH analog-induced gastric hyperemia and activation of multi-unit in splanchic afferents. These results suggest that the activation of „efferent function" of capsaicin sensitive afferents by vagal cholinergic stimulation is mediated by mucosal mast cells.

EFFECT OF SUMATRIPTAN ON THE CGRP-INNERVATION APPARATUS OF THE RAT CEREBRAL DURA MATER IN AN EXPERIMENTAL MIGRAINE MODEL

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The supratentorial cerebral dura mater displays a rich innervation apparatus subserving nociception *via* an axonal plexus of trigeminal nerve fibers containing calcitonin gene-related peptide (CGRP). Electrical stimulation of the Gasserian ganglion, regarded as an experimental migrain model, induces 3-4 x size increase of CGRP-immunopositive perivascular axon terminals, followed by their disintegration. Present studies were aimed to analyse the effect of the anti-migraine drug Sumatriptan (Imigran, Glaxo) by means of immunohistochemical techniques. It has been shown that i.v. administration of Sumatriptan, prior to electrical stimulation of the Gasserian ganglion, prevents disintegration of CGRP-immunopositive axon terminals while CGRP is accumulated in preterminal portions of the axons, resulting in an increased number and size of CGRP-immunopositive varicosities. Since plasma CGRP levels are increased during migraine attacks, and since the anti-migraine effect of Sumatriptan is due to its 5-hydroxy-triptamin agonist activity, it is assumed that at least one of the prerequisits for vasodilatation-inducing CGRP release is the presence of free serotonin receptors. Accordingly, the therapeutical effect of Sumatriptan seems to be related to binding of 5-HT-1D receptors which inhibits release of CGRP.

INVERTEBRATE TYPE NEUROPEPTIDES IN EARTHWORM GASTROINTESTINAL TRACT

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The occurence and distribution of two invertebrate type neuropeptides, proctolin and FMRFamide were studied by immunocytochemical methods in the stomatogastric nervous system of earthworm (Eisenia fetida). The stomatogastric system consists of a chain of six paired ganglia on the outer wall of the pharynx and the enteric nerve plexus. The stomatogastric ganglia contained both peptides in cell bodies and fibres, FMRFamide immunoreactivity being more pronounced in them. The enteric nerve plexus was examined in the whole length of the gastrointestinal tract and generally occupied a subepithelial position. The organisation of this system is polarised along the rostrocaudal axis of the gut. The rostral portion (pharynx, esophagus, ingluvies and stomach) was rich in immunoreactive cell bodies and fibres while the midgut and hindgut contained fibres only and were less densely innervated. The number of immunoreactive elements decreased gradually towards the caudal end of the alimentary tract. In the pharynx and esophagus both neuropeptides were present in cell bodies and fibres. The FMRFamide-immunoreactive nerve elements were more numerous in both parts. In the esophagus, contrary to other portions of the gut plexus, the nervous elements could be located at both sides of the calciferous glands (i.e. below the epithelium as well as outside of the glands, towards the muscles). From the midgut to the end of the alimentary tract nerve cells containing either substance could not be observed; FMRFamide-immunoreactive system was found to be substantially richer than the proctolin-containing nerve plexus. In the gut epithelium, especially in the forgut, proctolin-immunoreactive cells were present. The results suggest that the organisational centre of the stomatogastric system can be found in the foregut area.

TRITIUM LABELLING OF NEUROPEPTIDES

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Investigation of neuropeptide receptors requires biologically active peptide analogues containing radioactive labels. In vitro, biochemical receptor analyses are usually based on tritiated receptor ligands. There are several possibilities to label neuropeptide with ^{3}H using halogenated (dil-Tyr, pI-Phe) or unsaturated (ΔPro, ΔLeu) amino acids in the peptides as precursors. Catalytic dehalogenation or saturation with tritium gas gives the radioactive peptide with high specific radioactivity. Examples will be presented from the new opioid agonist (Deltorphins) and antagonist (TIPPΨ), β-amiloid fragments (β-amiloid(25-35), β-amiloid(31-35)) and other peptides with disulphid bridge. The tritiated ligands will be qualified as tools in the radioreceptor assays.

This work was supported by grants from Hungarian Research Fundation (OTKA) T 16991 and from European Community Copernicus (CIPA-CT94-0226).

EFFECT OF PSYCHOSTIMULANTS ON OPIOID RECEPTORS IN THE RAT BRAIN - AN AUTORADIOGRAPHIC STUDY

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An involvement of endogenous opioids in mechanism of dependence to psychostimulants has been postulated. Biochemical data showed that chronic treatment with drugs of abuse evoked increase in prodynorphin and decrease, or no changes in proenkephalin biosynthesis. On the other hand, little data are available on adaptive changes in characteristic of specific opioid receptors following chronic psychostimulants administration. In order to address this issue in a time-course study, we estimated effects of single and repeated amphetamine injections on density of μ and κ opioid receptors in nucleus accumbens and striatum of the rat. Experiments were carried out on male Wistar rats. Amphetamine was injected once in a dose 5mg/kg i.p., or twice daily for 5 days. The rats were killed at 3 h after the single dose and 3, 24, and 48 h after the last dose of chronic treatment. Autoradiography of opioid receptors was performed using [^{3}H]DAMGO and [^{3}H]U69,593 as ligands for labelling μ and κ receptor, respectively and quantified using a computer image analysis system (MCID).

Results showed that single amphetamine administration increased density of κ opioid receptor in both structures, whereas the density of μ receptors was decreased only in the striatum. Repeated amphetamine administration evoked decrease of κ opioid receptor density in nucleus accumbens at all time point studied. In contrast, chronic amphetamine treatment decreased the μ receptor density only in the striatum at 3 and 24h. These results indicate that chronic amphetamine treatment differentially affect the μ and κ opioid receptors. Furthermore, the chronic amphetamine-induced down-regulation of κ opioid receptor may be an adaptive response to increased availability of endogenous ligands for this receptor, due to increase of prodynorphin biosynthesis.

This paper is supported by grant for statutory activity,KBN Warsaw and European Comission grant C.I.P.A.CT 94-0226

EVOLUTIONARY UNIVERSALITY OF PEPTIDES FUNCTIONS ON THE EXAMPLE OF BEHAVIOR

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Comparison of the neuropeptides effects in vertebrate and invertebrate animals is important for understanding universality of peptides origin and their regulative role in the metabolism. Influence of delta sleep peptide (DSP) (1 and 10mM water solution after 1h exposition) on motor activity in infusoria and fishes in „the open field" has been studied. The motor activity in infusoria was evaluated per number of crossing of magnifier ocular within 5 min. DSP diminished ($p<0.05$) motor activity both in infusoria and fishes. ACTH 4-10 in the same conditions manifested the same effect in the infusoria. TSH (10mM) stimulated the motor activity in the infusoria. Thus our experimental data point out that peptide regulation of animal behavior originated early in evolution and is preserved in more developed animals. It allows to raise a question about evolutionary universality of peptides regulation of behavior.

ACTIONS OF GALANIN AND ITS ANALOGUES ON ISOLATED HUMAN AND RAT JEJUNUM. I. Kisfalvi Jr., *E. Fehér, **A. Bálint, , B. Burghardt, **M. Ihász, E.S. Vizi, G. Varga. (Inst. Exp. Med., HAS; *1st Dept. Funct. Anat. and **3rd Dept. Surg. , SUM; H-1083 Budapest, Hungary)

The present study was designed to examine the effect of galanin, and its putative antagonists M15 (galanin$_{1-12}$-Pro-substance-P$_{5-11}$) and M35 (galanin$_{1-12}$-Pro-bradykinin$_{2-9}$-amide) on longitudinal jejunal smooth muscle isolated from humans and rats. Human tissue samples were received from surgeries due to benign and malign diseases. Longitudinal jejunal muscle strips isolated from humans and from rats were set up in organ baths filled with modified Krebs solution. Isometric contractions were recorded. Immunohisto- and immunocytochemistry was accomplished using primary galanin antiserum. Galanin and acetylcholine (ACh) were equally effective in stimulating contractions of the isolated jejunal muscle: sigmoid curve fitting showed that maximal contractile response to galanin and ACh were 25.7±11.1 mN and 23.7±9.7 mN in humans, while 8.0±0.6 mN and 8.1±0.3 mN in rats, respectively. These effects of galanin were not inhibited by either atropine ($5x10^{-6}$ M) or tetrodotoxin ($3x10^{-6}$ M). EC_{50} values for the contractile action of galanin were similar in humans and rats ($1x10^{-8}$ M and $4x10^{-8}$ M, respectively). Interestingly, neither M15 nor M35 (up to 10^{-7} M) were able to modify responses of isolated jejunal smooth muscle to galanin. However, both putative galanin receptor antagonists showed agonistic effects in our experimental models. In humans, calculated EC_{50} values for the contractile actions of M15 and M35 were $1x10^{-7}$ M and $2x10^{-7}$ M, respectively. In accordance with the functional studies, both the longitudinal and the circular muscle layers were abundant in nerve fibers and varicosities showing galanin immunoreactivity.
Our data suggest that galanin is a potent physiological regulator of jejunal contractions in humans and rats. Its action on the jejunum, however, is mediated by receptors that are different from M15- and M35-sensitive galanin receptors located in the central nervous system.

EFFECT OF PALLIDAL BOMBESIN INJECTION ON FOOD INTAKE IN THE RAT
J. Vígh[1], L. Lénárd[1,2], I. Hernádi[1] and É. Fekete[1] ([1]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University, Pécs, and [2]Neurophysiology Research Group of the Hungarian Academy of Sciences at Institute of Physiology, Pécs University Medical School, Pécs, Hungary)

The lateral hypothalamic area (LH) and globus pallidus (GP) are basically involved in the regulation of feeding and metabolic processes. Neurochemical lesions of the LH or the GP result in aphagia, adipsia and sex-dependent body weight decrease. Both in the LH and GP glucose sensitive neurons were found which are influenced by various feeding associated neurochemical signals. Since they influence food intake, the Bombesin (BN)-like peptides could also belong to those neurochemical signals involved in the GP feeding mechanisms. Namely, it is well known that the intraperitoneally or intracerebroventricularly (i.c.v.) applied BN reduces food intake and it was shown recently that direct microinjection of BN into the LH inhibits feeding. Since BN immunoreactive neural elements were found in the GP, in the present experiments it was examined whether the direct pallidal microinjections of BN result in similar effects to those seen in the LH.

Ten or 40 ηg BN (in 1 μl) were applied through formerly stereotaxically implanted bilateral chronic guide cannulas into GP of male CFY rats after 24 hr food-deprivation and solid food intake was measured. BN injection into the GP caused transient, dose-dependent inhibition of food intake. Significant food intake reduction occurred during the first 20 min after 40 ηg BN application, and it was followed by a long "compensatory overeating" from 80 min till 120 min. These pallidal effects of BN were eliminated by prior i.c.v. application of a BN antagonist. In conclusion, our experiments demonstrated that locally applied BN effectively inhibits feeding in the GP. However, the confirmation of pallidal BN effect as a main satiety signal rests upon future research.

CONTROL BY ANGIOTENSIN II OF GASTRIC BLOOD FLOW

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Angiotensin II (ATII) is involved in the regulation of arterial blood pressure (MAP), but its role in the control of vascular tone in the stomach has not yet been investigated. This study assessed the effects of ATII and blockade of angiotensin AT_1 receptors on gastric hemodynamics.

In anesthetized rats, blood flow (BF) in the gastric mucosa was determined with the clearance of hydrogen and in the left gastric artery with the ultrasonic transit time shift technique. Vascular conductance (VC) was calculated as BF divided by the corresponding values of MAP.

Intravenous infusion of ATII (0.3-10 nmol min^{-1} kg^{-1}) dose-dependently increased MAP and led to a sustained reduction of BF and VC in the gastric mucosa. Bolus injections of ATII (1 nmol kg^{-1}, i.v.) likewise evoked pressor responses and reduced VC in the left gastric artery with no change in BF. The AT_1 receptor antagonist telmisartan (BIBR 277 SE; 1 mg kg^{-1}, i.v.) markedly reduced MAP and increased VC both in the gastric mucosa and in the left gastric artery but had no effect on BF. Pretreatment with telmisartan reduced the systemic pressor effects of ATII as well as the vasoconstriction caused by ATII in the gastric mucosa and left gastric artery. The specificity of telmisartan as an ATII antagonist was proved by its inability to alter the systemic hypertensive and the vasoconstrictor effect of arginine vasopressin (0.3 nmol kg^{-1}, i.v.) in the left gastric artery.

These findings indicate that ATII constricts the vasculature of the rat stomach as shown both in the gastric mucosa and left gastric artery. This effect is mediated by angiotensin AT_1 receptors since it is effectively blunted by telmisartan. Furthermore, endogenous ATII appears to be involved physiologically in the regulation of gastric vascular tone, suggested by the vasodilation observed after blockade of angiotensin AT_1 receptors.

RELEASE OF PREPROTACHYKININ-A mRNA FROM RABBIT IRIS UPON C-FIBRE STIMULATION

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It is usually said that axons and nerve terminals do not contain messenger RNA (mRNA) and that peptide transmitters are packaged in granules in the Golgi area and transported towards the periphery of the neuron. In the present study, mRNA coding for preprotachykinin-A (PPT-A) was demonstrated in the rabbit iris by Northern and Southern blot analysis and sequencing of PCR-fibre transmitters substance P and neurokinin A in the aqueous humor after application of noxious stimuli, such as infrared irradiation of the iris or retrobulbar application of the C-fibre excitant capsaicin. β-Actin mRNA was detected in the iris but not in the aqueous humor. The results suggest that PPT-A mRNA is released in response to noxious stimuli. Previous observations have suggested that neurons are capable of taking up and expressing foreign mRNA. Conceivably, one set of neuronal projections may secrete specific mRNAs that can be taken up and expressed by another group of neurons (or by immune cells). this mode of cell-to-cell communication might explain instances when neurons transiently express novel neuropeptides.

BIOTINYLATED ANALOGUES OF OXYTOCIN AND VASOPRESSIN: PROBES FOR VISUALISING RECEPTORS AT THE CELLULAR LEVEL IN THE CNS.

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The mammalian neurohypophysial peptide hormones oxytocin (OT) and [Arg^8]vasopressin (AVP) generate a large range of physiological responses. Both peptides function in the CNS as neurotransmitters/neuromodulators. In addition, OT stimulates lactation/uterine contraction and AVP has pressor/antidiuretic actions. These hormones have homologous structures composed of nine residues, an intramolecular disulphide bond and a sequence which only differs at positions 3 and 8. AVP and OT are full agonists at both oxytocin receptors (OTR) and vasopressin receptor (VPR). For a cell to be responsive to OT/AVP, it must express an appropriate receptor. The cellular localisation of these receptors therefore, is a key element to understanding physiological role of these hormones.

The distribution of receptors has been mapped hitherto by autoradiography. However, this lacks the resolution required for precise cellular localisation. As an alternative strategy, we have synthesised and chracterised biotinylated antagonists of OT and AVP. These bind with high affinity and subtype-selectivity to OTRs or VPRs. These selective molecular probes have subsequently been used to visualise receptors using avidin-conjugated reportors. OTR probes have been used to visualise human OTRs expressed by COS-7 cells. In addition, we have used this methodology to localise V_{1a} receptors on neurones and vasculature in the spinal cord and hippocampus.

This work was supported by the BBSRC and the BHF.

THE BINDING OF AN ENDOGENOUS NEUROPEPTIDE MET^5-ENKEPHALIN-ARG^6-PHE^7 TO OPIOID AND SIGMA RECEPTORS IN RAT AND FROG BRAIN

Wollemann,M.[1], Farkas,J.[2], Tóth,G.[2] and Benyhe,S.[1]
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In previous meetings (MIT 1993 and 1995) we reported the characteristics of the binding of an endogenously occuring heptapeptide (3H)met5-enkephalin-arg6-phe7 (MERF) to opioid receptors in frog and rat brain membrane fractions.It has been confirmed that in both preparations the affinity to the opioid receptor subtypes decreased in the following order kappa2>delta>mu>kappa1.

The one binding site was more probable than the two calculated from the Ligand programme in homologous displacement experiments but using heterologous competitors as opioid ligands only the high affinity site was displaced by most of the applied drugs. In contrary other endogenous opioid peptides like dynorphin, leu-enkephalin and met-enkephalin were effective competitors at both sites.

Among alkaloids the highest affinity was displayed by the benzomorphan (-)-N-allyl-normetazocine. The Ki value was in the rat brain fraction 0.25 nM and in the frog brain 0.2nM, whereas the Ki of the (+)-N-allyl-normetazocine was 1870 nM in rat brain and 2600 nM using frog brain membrane fractions. The Ki of haloperidol in the rat brain was 1750 nM.

These data let us to conclude, that the high affinity site is of opioid character, probably a new subtype of MERF-binding opioid receptor and the low affinity site could be one of the sigma-receptor subtypes (sigma2).

TACHYKININ NK2 RECEPTORS MEDIATE NON-ADRENERGIC NON-CHOLINERGIC EXCITATORY JUNCTION POTENTIAL AND CONTRACTION IN THE GUINEA-PIG STOMACH

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Using selective tachykinin antagonists and agonists excitatory non-adrenergic non-cholinergic (NANC) transmission was studied in the circular muscle from the guinea-pig fundus by the sucrose-gap method. After elimination of inhibitory junction potentials by apamin and L-nitroarginine, electrical field stimulation (EFS) (10Hz for 2s) in the presence of TEA (10mM) evoked pure biphasic NANC excitatory junction potentials (e.j.p.). Selective NK2 receptor antagonist, MEN 11420 dose dependently inhibited NANC e.j.p. (IC_{50}=0.09μM) and contraction (IC_{50}=0.05μM) evoked by EFS while selective NK1 receptor antagonist GR 82334 (3μM) only slightly (by 30%) inhibited e.j.p. leaving contraction unaffected. Selective NK1 and NK2 receptors agonists, [βAla^8]NKA (4-10) and [Sar^9]SP sulfone both produced dose-dependent depolarization (EC_{50}=0.03μM and EC_{50}=0.02μM, respectively) and contraction (EC_{50}=0.04μM and EC_{50}=0.05μM, respectively) of the circular muscle, the maximum values were 6.2±0.5mV and 5.8±0.6mN and 3.2±0.5mV and 0.4±0.04mN, respectively. These data provide evidence that NK2 receptors predominantly mediate NANC excitatory transmission in the circular muscle of the guinea-pig fundus.

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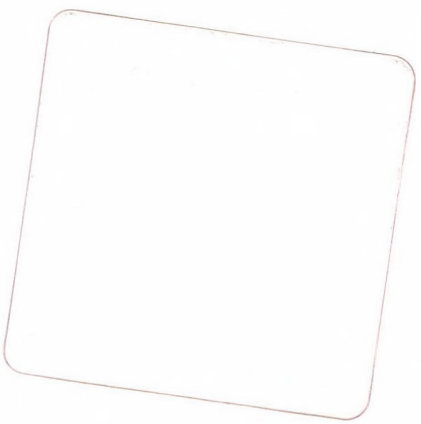
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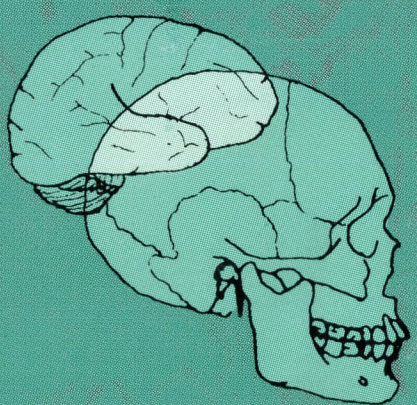
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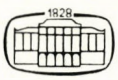
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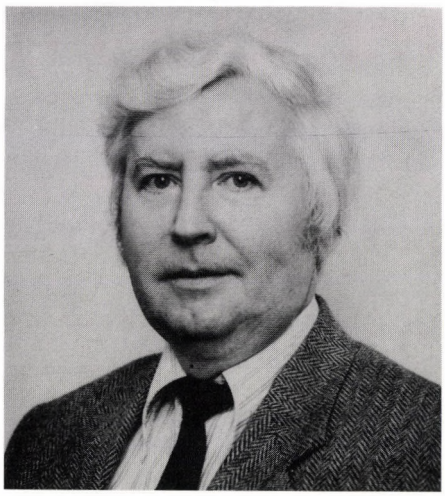
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Neurobiology; 4 (1996) 183-184

Obituary

Ferenc Joó (1938–1996)



It was a great shock to lose someone who should still have been with us. Ferenc Joó, one of the best-known Hungarian neurobiologists, and one who made such a large contribution to the unraveling of the mystery of certain aspects of the blood-brain barrier system, died on 25th February 1996. The son of a watch-craftsman, Ferenc Joó was born on 12 June, 1938. While still a medical student, he started his scientific career at the Department of Anatomy, Histology and Embryology of the Medical University in Szeged. He received his M.D. in 1963, his Ph.D. in 1976 for work on the "Significance of some enzymes in the regulation of permeability in brain microvessels", and his D.Sc. in 1990 on the "Regulation of the blood-brain barrier permeability".

As a young and enthusiastic researcher, he realized at the very onset of his scientific career that in his field he must compete not only with Hungarians, but also with many other scientists all over the world. Despite the fact that his research circumstances were much more modest than those of others, he very quickly, made his way to rank among the scientists widely acknowledged in his field.

"Frici" (the nick-name which his friends, associates and students all affectionately knew him) was not only an eminent investigator, but also a dedicated teacher. In 1988, he was appointed as Titular Professor by the University Council of Albert Szent-Györgyi Medical University. In appreciation of his scientific and educational activities, József Attila University in Szeged promoted him and likewise recognized him as Titular Professor. As the Head of the Laboratory of Molecular Neurobiology in the Institute of Biophysics at the Biological Research Center in Szeged, he attracted many students and postgraduate students to his laboratory, where high-level work went on in a calm, cheerful and thoughtful atmosphere. His

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way of thinking in the scientific sense was "Think big and win big". It was not his fault, but his circumstances that explain why many of his brilliant ideas could not be accomplished.

After successful years (1970-1971) in the Department of Anatomy at University College, Cardiff (U.K.), which provided him with firm ground for his further development, he visited and worked at several other scientific institutes abroad, among them the Department of Neurobiology, Max-Planck Institute of Biophysical Chemistry, Göttingen, F.R.G. (1977-1978); Zentrum Anatomie, Universität Göttingen, Göttingen, F.R.G. (1983-1984, 1986-1987; and the National Institutes of Health, LNNS, NINCDS, Bethesda, Maryland, U.S.A. (1987-1989).

In collaboration with many Hungarians and a large number of researchers from abroad, Ferenc Joó published numerous papers on a great variety of themes: the blood-brain barrier, brain edema, neuronal plasticity, transmitter systems, etc. He pioneered a technique on the separation of blood capillaries from the rat brain. He published more than 200 scientific papers, which were cited more than 1500 times.

Frici was always optimistic and remained very active throughout his short, but successful life. A fatal illness took him from us when he had reached the top of his career.

He received his students and coworkers at his bedside in the clinic, discussed their scientific results and made plans as to how to publish the more important ones. All the results and his plans must now be completed without him, a person who enjoyed life so much.

Ferenc Joó was a member of a number of scientific societies in Hungary and abroad (among others IBRO, ENA and ISN). He was also a member of the Editorial Board of the Journal of Neurochemistry, the Journal of Neurocytology, the Journal of Neurochemical Research and Neurobiology.

His work, and especially his results on the blood-brain barrier, made him an expert acknowledged worldwide. It is our hope that his much-loved research will be continued by his students both abroad and in Hungary.

All who had the pleasure of knowing or working with Frici can only regret that he has died so prematurely. He will be sorely missed by all who had the privilege of his acquaintance.

Péter Kása

Alzheimer's Disease Research Laboratory
Szeged, Hungary

Neurobiology; 4 (1996) 185-201

Research report

THE SUPRA-EPENDYMAL INNERVATION IS NOT RESPONSIBLE FOR THE REPRESSION OF TIGHT JUNCTIONS IN THE RAT CEREBRAL EPENDYMA

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Summary: Classically, the contiguous epithelial cells show intercellular tight junctions (TJ) positioned as a continuous belt of cell-cell contacts at the boundary of apical and basolateral plasma membranes. Among the epithelia, the "typical" cerebral ependyma is very peculiar due to the absence of TJ, in contrast to the ependyma of the circumventricular organs (e.g. choroid plexus and the subcommissural organ) which shows well differentiated TJ. Since the "typical" ependyma is covered in the rat with a plexus of intraventricular nervous fibres not present at the surface of the circumventricular organs, we hypothesized local repression of TJ by molecules (serotonin, GABA, etc.) released by the supra-ependymal varicosities. Neither the denervation of the "typical" ependyma nor the *ex vivo* activation of protein kinase C (which increases the transepithelial resistance as it has been reported in other epithelia) produced junctional fibrils as shown by freeze-fracture. As the protein ZO-1 was not detectable in the "typical" ependyma by immunocytochemistry, there is probably repression of the genes responsible for TJ biosynthesis by unidentified endogenous factors.

Keywords: supra-ependymal fibres, ependymal cells, GABA, serotonin, tight junctions, rat (Sprague-Dawley)

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INTRODUCTION

In the central nervous system (CNS) of mammals, the interface between the ventricular cerebrospinal fluid (CSF) and the cerebral parenchyma is formed by a mainly unistratified epithelium, the ependyma, derived from the fetal ventricular zone. For many years, precise information on intercellular junctions between contiguous ependymocytes was lacking (Brightman and Palay, 1963). The use of molecular tracers administered in the ventricles or in the blood compartment now allows various types of junctional complexes in the cerebral ependyma to be displayed at the ultrastructural level.

Unlike the ependyma of the circumventricular organs (CVO), most of the ependyma is permeable to macromolecules introduced into the ventricles. Today, it is clearly demonstrated that in the cerebral regions devoid of a blood-brain barrier (BBB), i.e. the regions with microvessels permeable to macromolecules such as the CVO, the ependyma constitutes the site of the blood-aqueous barrier (Kimble et al., 1973; Brightman et al., 1975; Madsen and Møllgård, 1979; Monroe and Holmes, 1982). However, in the greater part of the brain, the ependyma (named in this paper "typical" ependyma) is leaky (reviews in Bouchaud and Bosler, 1986 and Risau and Wolburg, 1990) and the barrier is located in the endothelium of the microvessels. These different barriers localizations, are mainly dependent on the presence of tight junctions (TJ) or *zonulae occludentes* (Brightman and Reese, 1969) between contiguous ependymocytes or endothelial cells.

So, the "typical" ependyma is a quite peculiar epithelium since the absence of a barrier is linked to the absence of TJ closing the intercellular space at the top of the junctional complex near the apical face of the epithelium (Privat, 1977). We hypothesized the existence in the CNS of a mechanism of repression preventing the formation of TJ in the "typical" ependyma. We were interested in the possible role of the release of serotonin (5-hydroxytryptamine, 5-HT) (Richards et al., 1973; Lorez and Richards, 1973; Bouchaud and Arluison, 1977; Tramu et al., 1983; Matsuura et al., 1985) and GABA (Harandi et al, 1986) by the supra-ependymal fibres that cover the apical plasmalemma of the "typical" ependymocytes and which they are almost totally absent from the CVO ependyma (Calas et al., 1977). These fibres are CSF-contacting axons originating from the raphe nuclei (Aghajanian and Gallager, 1975) and it has been shown that some raphe neurons are clearly CSF-contacting neurons (Vigh-Teichmann and Vigh, 1989). Since these fibres are in the immediate vicinity of the junctional

complexes, they could release repressive factors. This hypothesis was tested in the adult rat by destroying the supra-ependymal fibres by intraventricular administration of neurotoxic amines (5–6 or 5–7 dihydroxytryptamine, DHT) (Baumgarten et al., 1972, 1982; Matsuura et al., 1985).

Previously, it has been demonstrated that incubating MDKC cells (an epithelial cell line from dog kidney) in low Ca^{++} medium and placing then in a normal calcium medium ("Ca-switch") triggers the assembly and sealing of TJ (Gonzalez-Mariscal et al., 1985). G-proteins, phospholipase C (PLC) and protein kinase C (PKC) have been proposed as participating in the chain of events that results in the formation of TJ (Balda et al., 1991). 1,2-dioctanoylglycerol (diC8), an activator of PKC, stimulates development of transepithelial electric resistance (TER) and promotes the TJ formation in low extracellular Ca^{++} medium (Balda et al., 1993). Using this drug, we tried to induce TJ between "typical" ependymocytes in brain samples placed in a physiological medium. In parallel, immunocytochemistry was used to search for the presence of ZO1, — a 221 kDa cytoskeleton protein closely associated with TJ (Stevenson et al., 1986) and with F-actin (Madara, 1987) — and its possible ependymal redistribution at "typical" ependyma and CVO ependyma, as a consequence of the diC8 treatment.

MATERIALS AND METHODS

Animals

Adult Sprague-Dawley rats weighing 200 g were used (n=9); they had free access to food and water. All animal experiments were carried out in compliance with the principles of good laboratory animal care and the French laws on the protection of animals.

The studied cerebral areas were the regions of thalamus including lateral ventricles covered with choroid plexus or "typical" ependyma.

Electron microscopy

For transmission electron microscopy, brain samples were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 30 minutes, postfixed in 1% OsO_4 in the same buffer, and embedded in Araldite resin after dehydration. 120 nm ultrathin sections were double contrasted with aqueous uranyl acetate and lead citrate for 20 minutes in each medium. In some

procedures, lanthanum nitrate was added as tracer in the fixative solutions. For scanning electron microscopy, samples of fixed brains were dehydrated with ethanol and immersed in isoamylacetate. The critical point method was used for drying. Samples were observed using a Jeol JSM-840A scanning electron microscope at 20 kV.

Freeze-fracture

Glutaraldehyde-fixed cells and brains were fractured under high vacuum (10^{-7} Torr) in a freeze-fracture device (Cryofract, Reichert-Jung). Fracture faces were shadowed with platinum and coated with carbon (45° and 90° respectively). After cleaning overnight and rinsing, replicas were examined using a Philips EM201 electron microscope operating at 80 kV.

Co-localization of ZO-1 and actin

Rats were anaesthetized as described above. Brains were quickly removed, immediately frozen at -30 °C using isopentane, cooled by liquid nitrogen, and 20 µm sections cut in a cryostat (Reichert-Jung). Sections containing "typical" ependyma or choroid plexus were collected on glass slides and fixed by immersion for 5 minutes in acetone at -20 °C. Sections were incubated 1 hour in 3 % normal goat serum in 0.01 M phosphate buffer and then overnight at 4 °C in the primary antibody (anti-ZO1 rat monoclonal antibody kindly provided by D. Cassio). After three washes in phosphate buffered saline, sections were incubated for 45 minutes with fluorescein-conjugated anti-rat IgG (1/100) (Sigma chemicals). To reveal the cytoplasmic microfilaments, the actin-containing microfilaments were simultaneously labelled with rhodamine-conjugated phalloidin (40 µl/ml, Sigma chemicals). After washing three times, coverslips were mounted with Mowiol. Sections were observed with a Leitz Diaplan and photographed using TMAX film (Kodak).

Ependymal denervation using 5-7 dihydroxytryptamine

The rats were injected stereotaxically by the ventricular route under deep anaesthesia with pentobarbital sodium (0.1 ml/100g i.p.) with 5–7 dihydroxytryptamine (5–7 DHT, 10 µl of a solution containing 7.7 µg/ml 5–7 DHT in saline with 0.2 mg/ml L-ascorbic acid per rat; Sigma chemicals)

using a Kopf-type apparatus. Three days after the injection, animals were anaesthetized and perfused intracardially with a solution of 4% paraformaldehyde in 0.05 M Sorensen buffer pH 7.4. Brains were removed and postfixed for 2 hours in the same solution, then 50 µm sections cut in a Vibratome-Lancer® sectioning system. Sections were incubated with a primary rabbit antibody against serotonin, kindly provided by G. Tramu, followed by a biotinylated mouse anti-rabbit IgG (Pasteur Diagnostics, France). Finally, the avidin-biotine complex was applied according to Hsu et al. (1981) for serotonin fibers. Samples were processed for semithin or ultrathin-sections.

diC8 treatment

Rats were killed with an overdose of pentobarbital; then the brains were removed. Small samples containing the third ventricle were transferred into Eagle's basal medium with Earle's salts, supplemented with 10 % foetal calf serum, 1 % glutamine, 1% antibiotics. 1 mg/ml of 1,2-dioctanoylglycerol (diC8, Sigma chemicals) was added to the medium which was bubbled with air for 1 hour at 37 °C. The samples were fixed, cryoprotected and frozen as described below, then stored in liquid nitrogen until freeze-fracture.

RESULTS

In the CNS, the "typical" ependyma of the ventricular wall devoid of liquido-tissular barrier is characterized by a digited apical membrane with microvillosities and cilia (Fig. 1a–c). The curved microvillosities seems hooked with fine varicose amyelinic fibres at the apical surface of ependymocytes. The supra-ependymal fibres form a plexus covering the ventricular wall. These varicosities devoid of synaptic contacts contain mainly a population of small clear vesicles, often pleomorphic, and a few large granular vesicles, the ratio of small to large vesicles being approximately 10:1. Previously, these fibres had been identified by fluorescence, radioautography and immunohistochemistry as mainly "serotoninergic" (Fig. 1c). These fibres probably release their mediators in the ventricular cerebrospinal fluid; they are difficult to observe with the scanning electron microscope, a technique nevertheless well-adaptated for

studying cell surfaces; in fact, they are generally hidden by tufts of cilia and microvillies. However, in rare poorly ciliated locations, the supra-ependymal fibres can be observed (Fig. 1d). Note that the surface of the CVO are almost devoid of cilia and of supra-ependymal innervation.

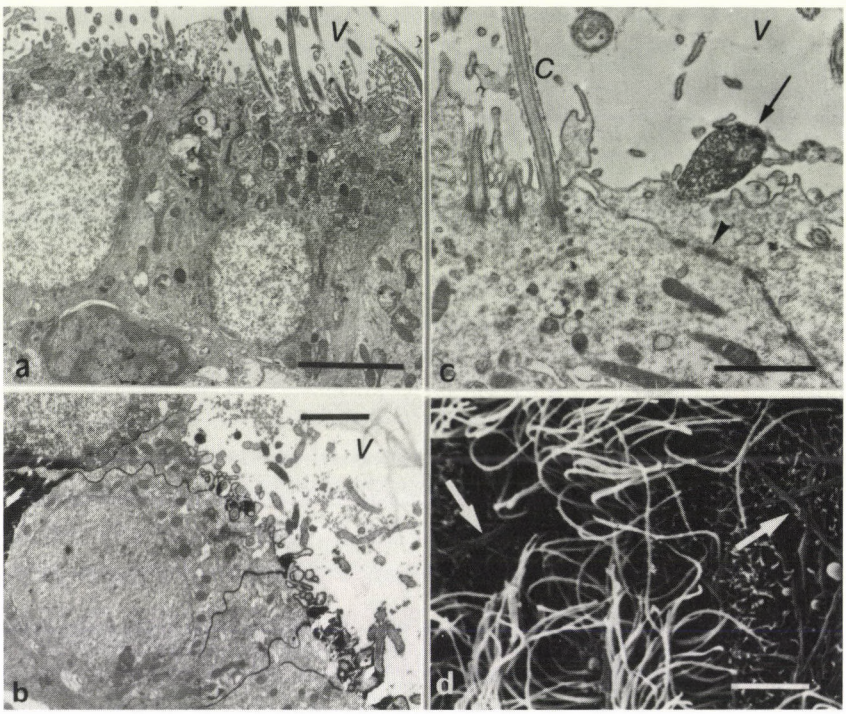
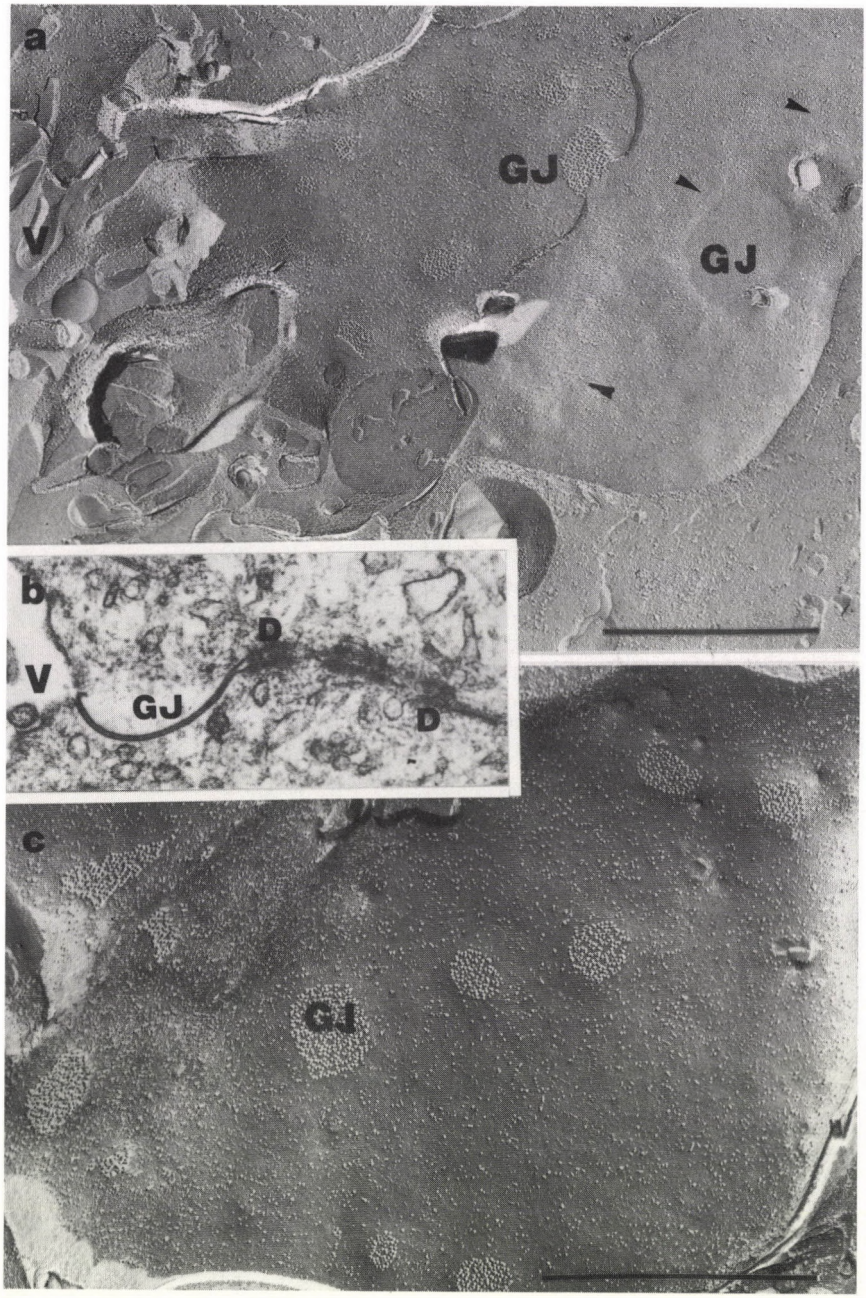


Fig. 1: 1a) Low magnification electron micrograph of "typical" ependyma of the rat. (X 7,000). 1b) Section of « typical » ependyma. The lanthanum nitrate has penetrated the spaces between contiguous ependymocytes three days after 5–7 DHT administration. 1c) Apical part of the "typical" ependyma showing a 5-HT immunoreactive supraependymal varicosity (*arrow*) and the junctional complex devoid of TJ (*arrowhead*). (X 16,000). 1d) Scanning electron micrograph showing the supraependymal fibres (*arrows*) at the surface of cerebral "typical" ependyma between the tufts of cilia and microvillosities. (X 6,000). V=ventricle. C=cilium. *Bar:* 2 µm

→

Fig. 2: Electron micrographs of "typical" ependyma. 2a, 2c) Replicas showing the absence of TJ in the plasmalemma in control animals. *Arrowheads:* orthogonal arrays (a, X 60,000; b, X 80,000). 2b) Ultrathin section of "typical" ependymal cell intercellular junctions showing an incomplete junctional complex. Note the succession of gap junction (GJ), intermediate junction and desmosomes (D). (X 80,000). *Bar:* 0.5 µm


a
GJ
V
GJ
b
V
GJ
D
D
c
GJ

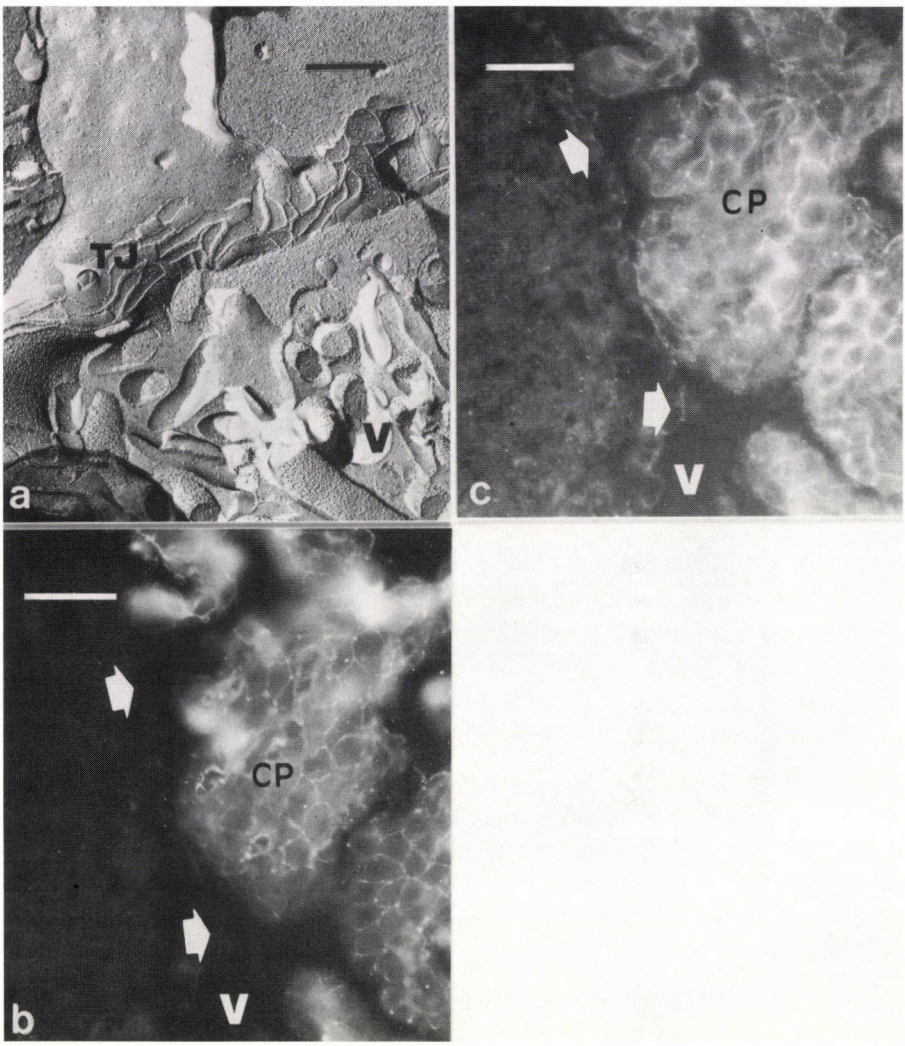
In the "typical" ependymal layer, three types of cell junctions are present in the vicinity of the apex: gap junctions, intermediate junctions (*zonulae adhaerentes*) and desmosomes but TJ are absent. Freeze-fracture replicas obtained from animals intoxicated by 5–7 DHT did not display networks of fibrils indicating the absence of TJ (not illustrated). Gap junctions appear in ultrathin sections as a rigid curved line (Fig. 2b). On freeze-fractured membranes, the gap junctions sometimes associated with orthogonal arrays are especially small but numerous at the most apical part of the cells, while the lateral surfaces of ependymocytes show gap junctions of various sizes, generally larger (Fig. 2a).

In contrast, the CVO possess an ependyma where the junctional complexes are characterized in freeze-fracture by a network of TJ fibrils where the E-face grooves are opposite the P-face ridges, for instance in the choroid plexus. In the adult rat, no gap junction is visible (Fig. 3a). In addition, apical and lateral membranes exhibit few orthogonal arrays, a special arrangement of intramembranous protein particles.

The intracellular localization of ZO-1, an ubiquitous component of TJ in epithelial and endothelial cells, and of F-actin present both in the cytoskeleton and in the junctional membrane contact points, was sought on brain sections. The epithelial cells of the choroid plexus exhibited ZO-1 immunofluorescence along the TJ forming continuous belts at the cell-cell contact sites. Filamentous materials identified as F-actin after phalloidin-rhodamine treatment were detectable at the site of ZO-1 immunoreactivity, perhaps bound to the intermediate junctions underlying the TJ. In contrast, the "typical" ependymocytes which do not have TJ, appeared immunonegative for the ZO-1 protein even in the cytoplasm were F-actin is abundant (Fig. 3b–c).

→

Fig. 3: 3a) Freeze-fracture in the epithelial cells of the choroid plexus. Note the presence of the network of ridges on fracture face P and the complementary grooves on fracture face E, also the absence of gap junctions in the choroid plexus of the adult rat (X 30,000). *Bar:* 3 µm. 3b) and 3c) Fluorescence micrographs from a section of brain at the level of a lateral ventricle. 3b) immunofluorescent localization of ZO-1. The "typical" ependyma (arrow) is negative while the antibody activity is localized to the area of the junctional complex in the choroid epithelial cells, CP (X 120). *Bar:* 100 µm. 3c) Direct fluorescence with phalloidin-rhodamine labelling the F-actin in the choroid epithelial cells (CP) and in the "typical" ependyma (*arrow*) (X 120). *Bar:* 100 µm. TJ=tight junction. V=ventricle



Immunoreactivity of supraependymal fibres to 5-HT antibodies revealed the serotoninergic plexus in the control rat (Fig. 4a). Intraventricular administration of the serotonin neurotoxin, 5,7-DHT, caused degeneration of 5-HT axons and terminals (Fig. 4b) which was evident after three days.

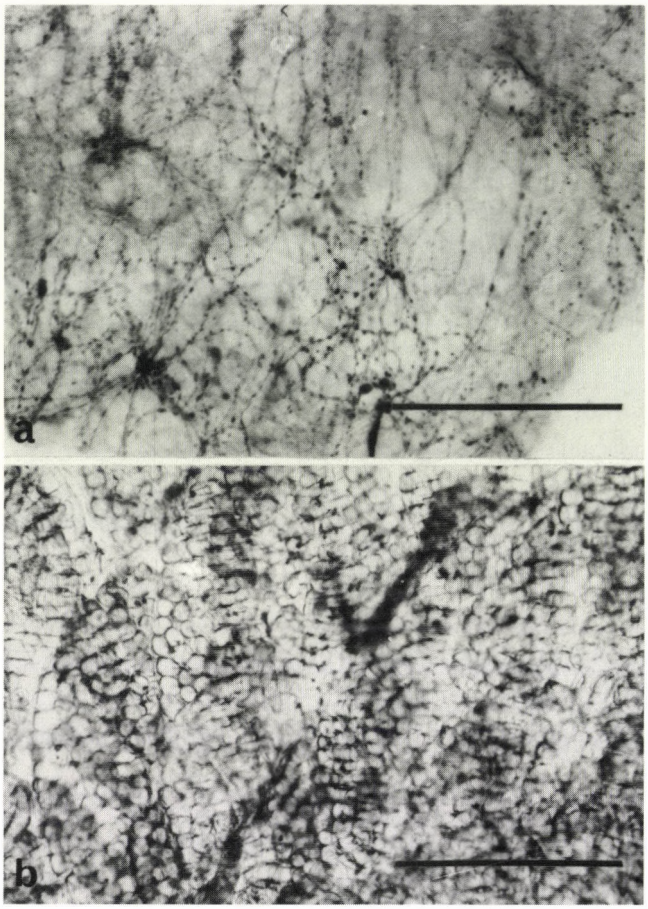


Fig. 4: Tangential sections of the ependyma in a lateral ventricle treated by immunochemistry for localizing serotoninergic supraependymal fibres (X 200) *Bar:* 50 µm. 4a). The varicose immunoreactive fibres are distributed over the ependymal surface. Photomicrograph focused of fibres. 4b) Three days after intraventricular administration of 5,7-DHT, the fibres have disappeared. Photomicrograph focused on the ependymal surface

Effect of diC8

Since immunochemistry failed to detect the presence, even diffuse, of the ZO-1 protein in the "typical" ependymocytes, we tried to induce TJ between neighbouring ependymal cells by treatment of brain slices with diC8. In our

ex vivo experiments, diC8 did not induce formation of TJ although high concentrations of this PKC activator were used (not illustrated).

DISCUSSION

Tight junctions of polarized cells create a barrier in the intercellular spaces. Spontaneously, within a few hours of isolation, epithelial cells or pairs of hepatocytes recreate intercellular spaces sealed by TJ (Cassio et al., 1991). Among epithelia, the "typical" ependyma of the CNS is very peculiar since the "terminal" bar or junctional complex is devoid of TJ (Brightman and Reese, 1969) as demonstrated by the passage of lanthanum nitrate, the barrier system being located at the level of the endothelial cells constituting "continuous" capillaries (Reese and Karnovsky, 1967; Brightman and Tao-Cheng, 1993). We thus tested a possible local mechanism of TJ repression that could originated in the supra-ependymal plexus covering the apical plasma membrane of the "typical" ependyma. The varicosities of the fine amyelinic fibres forming the plexus could release various factors including serotonin and during development serotonergic neurons have a tropism for ependymocytes (Voutsinos et al., 1994). The origin of these fibres is the dorsal and median raphe nuclei (Aghajanian and Gallager, 1975), the largest serotonin-containing cell groups as demonstrated by a variety of methods (formaldehyde-induced fluorescence, radioautography, cytochemistry and immunohistochemistry) (Richards et al., 1973; Bouchaud and Arluison, 1977; Richards and Tranzer, 1975; Arluison et al., 1982, respectively). Numerous neurons project onto the ventricular surface forming the supraependymal plexus (Arluison et al., 1982). It is generally admitted that stimulation of these neurons provokes release of serotonin as well as other molecules, the best characterized being gamma-aminobutyric acid (GABA) (Voutsinos et al., 1994). As emphasized recently, no reported experiments have addressed whether serotonin specifically affects ependymal functions although the destruction of supraependymal axons can influence the shape of ependymocytes (see review in del Bigio, 1995). In other models, 5-HT innervation has been shown to be involved in the biosynthesis of molecules such as the GABA-carrier in the apical plasmalemma of the subcommisural ependymocytes in rat brain (Didier-Bazes et al., 1989), and in the control of the expression of the S100 protein during postnatal development in the same cells (Didier-Bazes et al., 1991).

Our hypothesis that factors released by the supra-ependymal fibres might repress the differentiation of TJ in "typical" ependyma is supported by the fact that the ependyma of the CVO, a classical epithelium as regards the junctional complexes, is virtually devoid of this supra-ependymal innervation (Calas et al., 1977; Tramu et al., 1983 and review in Bouchaud and Bosler, 1986). Thus, the subcommissural organ (SCO) ependymocytes which are devoid of supra-ependymal innervation (Bouchaud and Arluison, 1977) possess TJ (Madsen and Møllgärd, 1979). At the border of the SCO, the contiguous « typical » ependymocytes which lack TJ are covered by a dense supra-ependymal plexus. The possible repressive role of molecules released by the supraependymal fibres was tested on adult rats injected intraventricularly with 5–7 DHT, a neurotoxic amine. After three days, this innervation disappeared (Stagaard et al., 1987). Replicas of the "typical" ependyma did not show strands and grooves in aldehyde-fixed samples. The absence of 5-HT did not induce the appearance of TJ. Moreover, in the apical plasmalemma of the "typical" ependymocytes we observed some orthogonal arrays often associated with gap junctions. In the CVO ependymocytes provided with TJ as the choroidal cells, orthogonal arrays are absent confirming the non-coexistence between TJ and orthogonal arrays except in the transitional zone between the "typical" ependyma and the choroid plexus in the rat (Mack et al, 1987).

Our immunohistochemical studies using an antibody against ZO-1, the first of the proteins associated with TJ to be characterized (Stevenson et al., 1986), showed highly immunoreactive belts in the choroid plexus, the CVO being used as control. This immunofluorescence is comparable to that described in various CVO by Smith and Shine (1992) and Petrov et al (1994). The same antibody applied on the "typical" ependyma close to the choroid plexus did not reveal any significant reactivity. This result suggests that either the ZO-1 level is to low to produce a visualizable reaction or that ZO-1 molecules are absent. Probably, the "typical" ependymocytes do not express this protein, whereas actin revealed by rhodamine-phalloidine is present. But it is known that F-actin, a major component of the cytoskeleton, is bound to the plasma membranes via other intracellular proteins such as vinculin, α-actinin, ankyrin or cell adhesion molecules like cadherins, etc. (Gumbiner, 1992).

Recently, Balda et al. (1993) demonstrated on cultured MDCK cells that activation of protein kinase C (PKC) appears to be part of the assembly signaling cascade and that the PKC agonist diC8 induces movement of the

ZO-1 molecules from the cytosol to the membrane, rearrangements of F-actin, appearance of junctional fibrils and development of a epithelial transmonolayer barrier to mannitol flux. DiC8 has not been shown to induce the expression of TJ in cells that lack them. In our experiments carried out *ex vivo* on cerebral slices, diC8 did not induce TJ, probably because the various components bound to the junction are inadequately represented in the "typical" ependymocytes, especially ZO-1, a potential signaling protein located at initial cell-cell contacts. It is likely that the absence of TJ in the "typical" ependyma is independent of an inhibitory extrinsic signal coming from the supraependymal fibres. Rather it would be due to the deficiency in endogenous factors (adenylate cyclase, G proteins, PKC, phospholipase C, Ca^{++} ions and calmodulin) that modulate the assembly and sealing of TJ in epithelia.

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REFERENCES

Aghajanian, G.K. and Gallager, D.W. (1975) Raphe origin of serotonergic nerves terminating in the cerebral ventricles. Brain Res. 88, 221–231.

Arluison, M., Bouchaud, C., Derer, P. and Tramu, G. (1982) Etude immunocytochimique de l'arborisation des axones sérotoninergiques supraépendymaires du rat. C. R. Acad. Sc. (Paris) 294, 875–880.

Balda, M. S., Gonzalez-Mariscal, L., Matter, K., Cereijido, M. and Anderson, J. (1993) Assembly of the tight junction: the role of diacylglycerol. J. Cell Biol. 123, 293–302.

Balda, M. S. (1992) Intracellular signals in the assembly and sealing of tight junctions. In: Tight junctions (ed. Cereijido, M.), pp. 121–137. CRC Press, Boca Raton-Ann Arbor-London.

Balda, M. S., Gonzalez-Mariscal, L., Contreras, R. G., Macias-Silva, M., Torres-Marquez, M., Garcia-Sainz, J. A. and Cereijido, M (1991) Assembly and sealing of tight junctions: possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. J. Memb. Biol. 122, 193–202.

Baumgarten, H. G., Evetts, K. D., Holman, R. B., Iversen, L. L., Vogt, M. and Wilson, G. (1972) Effects of 5-6 dihydroxytryptamine on monoaminergic neurons in the central nervous system of the rat. J. Neurochem. 19, 1587–1597.

Baumgarten, H. G., Klemm, H. P., Sievers, J. and Schlossberger, H. G. (1982) Dihydroxytryptamines as tools to study the neurobiology of serotonin. Brain Res. Bull. 9, 131–150.

Bouchaud, C. and Arluison, M. (1977) Serotoninergic innervation of ependymal cells in the rat subcommisural organ. A fluorescence, electron microscopy and radioautographic study. Biol. Cell 30, 65–72.

Bouchaud, C. and Bosler, O. (1986) The circumventricular organs of the mammalian brain with special reference to monoaminergic innervation. Int. Rev. Cytol. 105, 283–327.

Brightman, M. W. and Palay, S. L. (1963) The fine structure of ependyma in the brain of the rat. J. Cell Biol. 19, 415–439.

Brightman, M. W., Prescott, L. and Reese, T. S. (1975) Intercellular junctions of special ependyma. In: Brain-endocrine Interaction (eds. Knigge, K. M., Scott, D. E., Kobayashi, H., Ishii, S.) Vol 2, pp. 199–137. Karger, Basel.

Brightman, M. W. and Reese, T. S. (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40, 648–677.

Brightman, M. W. and Tao-Cheng, J. H. (1993) Tight junctions of brain endothelium and epithelium. In: The blood-brain barrier (ed. Partridge, W. M.) pp. 107–125. Raven Press, New York.

Calas, A., Bosler, O., Arluison, M. and Bouchaud, C. (1977) Serotonin as a neurohormone in circumventricular organs and supraependymal fibers. In: Brain-endocrine Interaction (eds Scott, D. E., Kozlowski, Weindl) Vol. 3, pp. 238–250. Karger, Basel,

Cassio, D., Hamon-Benais, C., Guérin, M. and Lecoq, O. (1991) Hybrid cell lines constitute a potential reservoir of polarized cells: isolation and study of highly differentiated hepatoma-derived hybrid cells able to form functional bile caliculi *in vitro*. J. Cell Biol. 115, 1397–1408.

Chan-Palay, V. (1976) Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges-, their roles in local alterations of cerebrospinal fluid and vasomotor activity. Brain Res. 102, 103–120.

del Bigio, M. (1995) The ependyma: a protective barrier between brain and cerebrospinal fluid. Glia 14, 1–13.

Didier-Bazes, M., Aguera, M., Chouaf, L., Harandi, M., Calas, A., Meiniel, A. and Belin, M. F. (1989) Neuronal control of (^{3}H)-GABA uptake in the ependymocytes of the subcommissural organ: an *in vivo* model of neuron-glia interaction. Brain Res. 449, 137–145.

Didier-Bazes, M., Chouaf, L., Hardin, H., Aguera, M., Fèvre-Montange, M. and Belin, M. F. (1991) Developmental neuron-glia interaction: role of the serotonin innervation upon the onset of GABA uptake into the ependymocytes of the rat subcommissural organ. Dev. Brain Res. 63, 135–139.

Gonzalez-Mariscal, L., Chavez de Ramirez, B. and Cereijido, M. (1985) Tight junction formation in cultured epithelial cells (MDCK). J. Memb. Biol. 86, 113–125.

Gonzalez-Mariscal, L., Contreras, R. G., Bolivar, J. J., Ponce, A., Chavez de Ramirez, B. and Cereijido, M. (1990) Role of calcium in tight junction formation between epithelial cells. Am. J. Physiol. 259, C978–C986.

Gumbiner, B. (1992) Cell adhesion molecules in epithelia. In: Tight junctions. (ed. Cereijido, M.) pp. 91–104. CRC Press, Boca Raton-Ann Arbor-London.

Harandi, M., Didier, M., Aguera, M., Calas, A. and Belin, M. F. (1986) GABA and serotonin (5-HT) pattern in the supraependymal fibers of the rat epithalamus: combined radioautographic and immunocytochemical studies. Effect of 5-HT content on [^{3}H] GABA accumulation. Brain Res. 370, 241–249.

Hsu, S., Raine, L. and Fanger, H. (1981) Use of avidin-biotin-peroxidase complex (ABC) and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577–580.

Kimble, J. E., Sϕrensen, S. C. and Mϕllgård, K. (1973) Cell junctions in the subcommissural organ of the rabbit as revealed by use of ruthenium red. Z. Zellf. Mikr. Anat. 137, 375–386.

Lorez, H. P. and Richards, J. G. (1973) Distribution of indolealkylamine nerve terminals in the ventricles of the rat brain. Z. Zellf. Mikr. Anat. 144, 511–522.

Mack, A., Neuhaus, J. and Wolburg, H. (1987) Relationship between orthogonal arrays of particles and tight junctions as demonstrated in cells of the ventricular wall of the rat brain. Cell Tissue Res. 248, 619–625.

Madara, J. L. (1987) Intestinal absorptive cells tight junctions are linked to cytoskeleton. Am. J. Physiol. 253, C171–C175.

Madsen, J. K. and Møllgård, K. (1979) The tight epithelium of the mongolian gerbil subcommissural organ as revealed by freeze-fracturing. J. Neurocytol. 8, 481–491.

Matsuura, T., Takeuchi, Y., Kojima, M., Ueda, S., Yamada, H., Nojyo, Y., Ushijima, K. and Sano, Y. (1985) Immunohistochemical studies of the serotonergic supraependymal plexus in the mammalian ventricular system, with special reference to the characteristic reticular ramification. Acta Anat. 123, 207–219.

Monroe, B. G. and Holmes, E. M. (1982) The freeze-fractured median eminence. I. Development of intercellular junctions in the ependyma of the third ventricle of the rat. Cell Tissue Res. 222, 389–408.

Petrov, T., Howart, A. G., Krukoff, T. L. and Stevenson, B. R. (1994) Distribution of the tight junction-associated protein ZO-1 in circumventricular organs of the central nervous system. Mol. Brain Res. 21, 235–246.

Privat, A. (1977) The ependymal and subependymal layer of the young rat: a new contribution with freeze-fracture. Neuroscience 2, 447–457.

Reese, T. S. and Karnovsky, M. J. (1967) Fine structural localization of blood-brain barrier to exogenous peroxydase. J. Cell Biol. 34, 207–217.

Richards, J. G., Lorez, H. P. and Tranzer, J. P. (1973) Indolealkylamine nerve terminals in cerebral ventricles: identification by electron microscopy and fluorescence histochemistry. Brain Res. 57, 277–288.

Richards, J. G. and Tranzer, J. P. (1975) Localization of amine storage sites in the adrenergic cell body. J. Ultrastr. Res. 53, 204–216.

Risau, W. and Wolburg, H. (1990) Development of the blood-brain barrier. TINS 13, 174–178.

Smith, G. M. and Shine, H. D. (1992) Immunofluorescent labeling of tight junctions in the rat brain and spinal cord. Int. J. Dev. Neurosci. 10, 387–392.

Stagaard, M., Balslev, Y., Lundberg, J. J. and Mϕllgård, K. (1987) Microglia in the ependyma of the rat subcommissural organ following brain lesions with serotonin neurotoxin. J. Neurocytol. 16, 131–142.

Stevenson, B. R., Siciliano, J. D., Mooseker, M. S. and Goodenough, D. A. (1986) Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J. Cell Biol. 103, 755–766.

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Tramu, G., Pillez, A. and Leonardelli J. (1983) Serotonin axons of the ependyma and circumventricular organs in the forebrain of the guinea pig. Cell Tissue Res. 228, 297–311.

Vigh-Teichmann, I. and Vigh, B. (1989) The cerebrospinal fluid-contacting neuron: a peculiar cell type of the central nervous system. Immunocytochemical aspects. Arch. Histol. Cytol. 52, 195–207.

Voutsinos, B., Chouaf, L., Mertens, P., Ruiz-Flandes, P., Joubert, Y., Belin, M. F. and Didier-Bazes, M. (1994) Tropism of serotonergic neurons towards glial targets in the rat ependyma. Neuroscience 59, 663–672.

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Research report

PRESYNAPTIC MODIFICATION OF SYNAPTIC TRANSMISSION AT IDENTIFIED APLYSIA CENTRAL SYNAPSES, INDUCED BY CHANGES IN PROTEIN KINASE C ACTIVITY

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Summary: The effect of altered protein kinase C (PKC) activity level on the synaptic transmission in the central ganglia of Aplysia californica was studied. EPSPs or EPSCs evoked in the postsynaptic neuron by presynaptic nerve stimulation were recorded while the ganglion was in vitro treated with PKC activators (phorbol diacetate and SC-10) or PKC blockers (H-7, sphingosine, Cremophor-EL). It was found that the postsynaptic reaction was enhanced by increased and reduced by decreased PKC activity and that this modulatory influence of PKC was presynaptic.

Keywords: protein kinase C, Aplysia, postsynaptic potential and current, protein kinase C activators and blockers

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INTRODUCTION

The advances of neurophysiology in the last decades indicate clearly that functional plasticity of the nervous system is realized through changes in the synapses. Regulation of synaptic efficacy is an essential step in memory acquisition and recall (Alkon et al., 1991). Long-term potentiation or depression (LTP or LTD; Ben-Ari et al., 1992), considered crucial in memory function, also depend on changes in the synapses involved (Baranyi and Szente, 1987; Bramham et al., 1994). It has been shown in several cases that such plastic synaptic changes are bound to specific phosphorylating steps. Protein kinase C (PKC), a key enzyme of the intracellular signal transduction (Houslay, 1991), has been long supposed to have a role in synaptic modifications in various objects. In synaptosomes (Shearman et al., 1991) and hippocampal presynaptic endings (Saito et al., 1993) several PKC isoenzymes were found. In the mammalian hippocampus, Malenka et al. (1986) found that phorbol esters potentiate synaptic transmission. As phorbol esters had been known to activate PKC (Castagna et al., 1982), this pointed at a possible role of PKC in synaptic potentiation. This role was confirmed in cultured hippocampal neurons by Segal's (1989) observation that a phorbol ester enhanced the amplitude of the postsynaptic current. As the sensitivity of the postsynaptic receptors did not change, the effect was well due to increased transmitter release. Such an increase was described in the neuromuscular junction by Shapira et al. (1987). In intact mammalian brain, increase of EPSPs after phorbol ester injection into pyramidal neurons was demonstrated by Baranyi et al. (1988).

A connection between protein phosphorylation and LTP has also long been supposed. Direct relation between LTP and PKC-dependent phosphorylation was demonstrated by Lovinger et al. (1987) and Bachoo et al. (1992). It was also shown that activation of PKC can mimic (Malenka et al., 1987) while its inhibition can abolish (Cheng et al., 1994; Colley et al., 1990; Malinow et al., 1989) the induction or maintenance of LTP.

In Aplysia, the presence of PKC in the central nervous system has been well documented (DeRiemer et al., 1985a). DeRiemer et al. (1985b) also demonstrated that PKC activation increases the calcium inward current in the bag cells (Frazier et al., 1967). As these cells are neurosecretory, the enhanced calcium entry can enhance neuropeptide release. At an identified synapse in Aplysia buccal ganglion, Fossier et al. (1990) demonstrated that activity level of PKC influences stimulus-evoked acetylcholine (ACh) release

via the regulation of calcium influx where the calcium channels undergo a regulatory phosphorylation (Tauc et al., 1992). At another synapse, being involved in the Aplysia tail withdrawal reflex, simultaneous activation and translocation of PKC has been demonstrated (Sacktor and Schwartz, 1990), which lead to synaptic facilitation via enhanced transmitter release (Braha et al., 1990).

Based on the above, and on previous findings (Klee, 1990), our aim was to find out how alterations in PKC activity influenced synaptic transmission at identified synapses in Aplysia visceral ganglion and whether the changes were pre- or postsynaptic.

MATERIALS AND METHODS

Preparation

The experiments were carried out on neurons in isolated central ganglia of *Aplysia californica*. The identified neuron R15, and neurons in the indentified group RB, in the visceral ganglion were used preferentially as these neurons have a well-defined synaptic input through the right pleuroabdominal connective producing a large monophasic cholinergic EPSP (Fig. 1; Frazier et al., 1967). Prepared single ganglions were fixed to the Sylgard bottom of the recording chamber by needles. The left and right pleuroabdominal connectives (LC and RC, respectively) were laid over silver wire pairs for stimulation. The chamber was continuously perfused with artificial sea water (ASW). The composition of ASW was (in mM): NaCl, 480; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 20; $MgSO_4$, 15; HEPES, 5. The pH was set to 7.8; the chamber and the inflowing ASW were kept at a constant temperature of 16 °C.

Recording and stimulation

The cells were impaled with a single microelectrode of 2–6 MOhm resistance, filled with 3 M KCl or a special injecting solution (see below). An AXOCLAMP 2 bioamplifier/clamp device was used in all experiments. One of the connectives was stimulated using brief current pulses (2–5 μA; 4–5 msec) provided by a HIMED isolation unit driven by a HIMED pulse generator. The synaptic response was recorded in current or voltage clamp

mode. Action potentials and their first derivatives (dV/dt), observed in bridge mode, were visualized and plotted by a Philips digital storage scope and were used for monitoring the effects of the agents used (see Papp and Klee, 1995 for details). Postsynaptic potentials and currents were visualized and stored in a Nicolet digital scope having a floppy disk memory. Alternatively, voltage clamping, nerve stimulation and recording was performed by a computer using the CLAMPEX program.

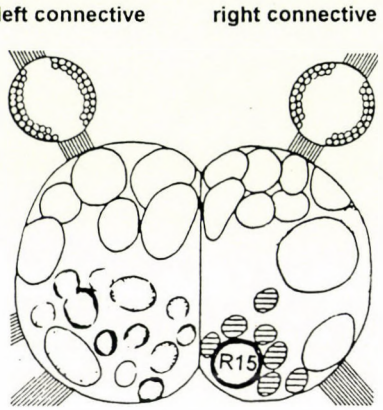


Fig. 1: Schematic drawing of the Aplysia visceral ganglion (taken from Frazier et al., 1967). The identified neuron R15 and the identified group RB (striped) are indicated (together with the outlines of other neurons)

Substances and pressure injections

Phorbol-12,13-diacetate (PDAc; Sigma) was made up to a 5.6 mM stock solution in 10% dimethyl sulfoxide (DMSO) - 90 % distilled water. This was added for perfusion to the ASW to give 2.5–10 µM final concentration. For intracellular injection, normal recording electrodes were filled with a solution containing PDAc, 0.5 mM; KCl, 2 M; Fast Green dye, 2 mg/ml. *N-n-heptyl-5-chloro-1-naphtalenesulfonamide (SC-10; Calbiochem)* was first dissolved in DMSO to a 20 mM stock solution. The amount of stock solution needed to yield the desired final concentration was dissolved in DMSO and this was filled up under ultrasonic treatment with ASW so that the final DMSO concentration was about 0.5%. *1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7; Sigma)* was dissolved in distilled water and was

applied either by the perfusion in 5–50 μM concentration or was injected from electrodes filled with a solution containing H-7, 2 mM; KCl, 2 M; Fast Green dye, 2 mg/ml. *D-Sphingosine (Sigma)* and *Cremophor EL (Sigma)* were dissolved in distilled water to a 100 μM stock solution and were so added to the perfunding ASW. The *PKC preparation* (kindly provided by Dr. W. Schiebler, Hoechst AG) was made up in a protective solution containing also 0.5 M KCl and 2 mg/ml Fast green dye and was kept on ice up to the moment of being used. All injections were done with ca. 200 kPa (~30 psi) pressure yielded by a pulse-controlled injection device (Neuro-Phore, Medical Systems Corp., USA). Before penetrating a neuron, the recording electrode was set under steady pressure and was pushed against the connective tissue around the ganglion until a fine trace of filling was forced out through the tip. The electrode now had about 2 MOhm resistance and was suitable both for recording and injection. The dyeing of the cytoplasm by Fast Green was used to determine the appropriate amount of injection.

RESULTS

Effects of PKC activation

Two PKC activators were used in our experiments. One of them, PDAc, is well known. It was applied in 5 μM concentration extracellularly or in form of intracellular injection. When PDAc was given extracellularly, a strong increase was observed in the amplitude of Aplysia EPSPs and EPSCs (recorded in current clamp or voltage clamp, respectively). This was a general effect, found both on the monosynaptic cholinergic inputs of identified neurons in the visceral ganglion (e.g. the RC-EPSP of neuron R15), and on less well defined synapses of pleural ganglion neurons. Beside the amplitude increment, a faster decay of EPSPs was sometimes observed, mostly on the RC-EPSP. Figure 1./1A illustrates the amplitude increment in neuron R15. Under voltage clamp, a clear increase of EPSC was seen. The averaged increment of EPSP/EPSC amplitude, which was statistically significant, is shown in Table 1.

Although the above effect was clear, it was not necessarily a PKC-mediated one. Therefore, we decided to use alternative PKC activators, chemically not related to phorbol esters. Several of them proved to be effective, including SC-10. The effectivity of this agent (or related compounds) has been shown in vitro (Ito et al., 1986) and in molluscan

nervous system (Kavaliers et al., 1991). SC-10 was used extracellularly in 100–200 μM concentration with 0.5% DMSO as a solvent (which, despite this concentration, produced no adverse effects).

The action of SC-10 was similar to that of PDAc. An increase of the EPSP/EPSC amplitude, being statistically significant, was found (see Table 1.). The increment of EPSC in a neuron in the left pleural ganglion, induced by SC-10, is shown in Fig. 1./1B. As seen in Fig. 1, the action of both PKC activators developed slowly. In case of PDAc, about 30 min was necessary for the effect to develop fully while in case of SC-10 it took even longer, up to 45 min. The effect of both substances was dose dependent and irreversible.

Table 1. Relative change (treated/control %) of the EPSP/EPSC peak amplitude on treatment with PKC activators and blockers

treatment	mean	s.d.	n	p<
PDAc; 1-10 μM	137.63	34.58	35	0.00005
PDAc; injected	97.25	28.61	27	n.s.
PKC injection	106.79	31.94	24	n.s.
SC-10; 100-200μM	114.93	34.16	32	0.01
H-7; 5-50 μM	63.10	18.64	22	0.00005
Sphingosine; 50-150μM	77.91	23.29	11	n.s.
Cremophor; 50-150 μM	81.56	23.70	16	0.005

Significance was tested by the one sample t-test (against 100% as null hypothesis)

Effect of PKC inhibition

The PKC blockers used in our experiments belong, according to their respective site of action, to several groups. H-7 exerts its action at the catalytic domain of the PKC molecule (Huang, 1989). H-7 was used in 5–50 μM concentration. The influence on the EPSP amplitude was opposite to that of PDAc and SC-10, i.e., a reduction, as shown in Fig. 2.A. The effect of H-7 was dose dependent and reversible, and it was significant.

Sphingosine and Cremophor EL are representatives of another group of PKC blockers: these drugs compete with the lipid PKC activators. Sphingosine, being a natural constituent of nervous tissue, is particularly

interesting. We applied both drugs in 50–150 µM concentration. Similar to the aforementioned blocker, these two substances reduced the EPSP amplitude, as seen in Fig. 2.B and C. The effect of both drugs was clear, although that of sphingosine was not statistically significant. The effect developed slowly and was irreversible.

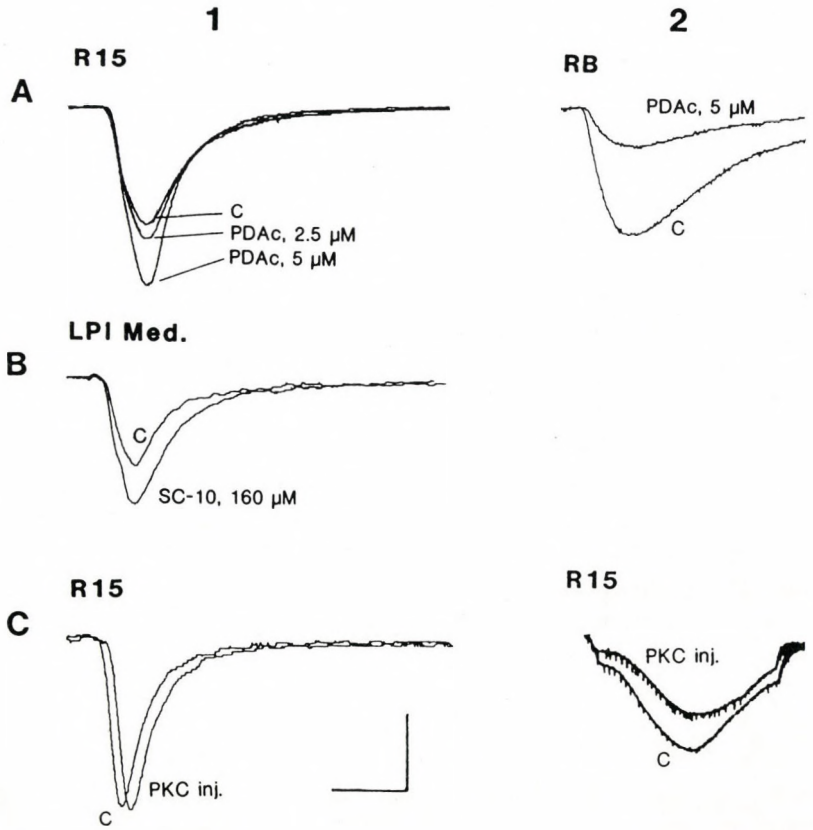


Fig. 2: Effect of elevated PKC activity on the amplitude of the synaptic current in Aplysia neurons (column 1) with comparison to its effect on the acetylcholine-evoked current (column 2). Records from identified neurons. Voltage clamp, $V_h = -90$ mV.
1A: The PKC activator PDAc, applied extracellularly in 2.5 and 5 µM concentration (calibration: 10 nA, 50 ms) induced a great increment in the EPSC peak amplitude. 2A: The ACh-induced current response, however, was diminished by PDAc (cal.: 6 nA, 5 s). 1B: A similar increment could be evoked also by 100 or 200 µM SC-10 (cal.: 4 nA, 50 ms). 1C: Injection of the purified enzyme PKC had no effect on the EPSC (cal.: 10 nA, 50 ms) but, 2C, reduced the ACh-induced current (cal.: 4 nA, 5 s)

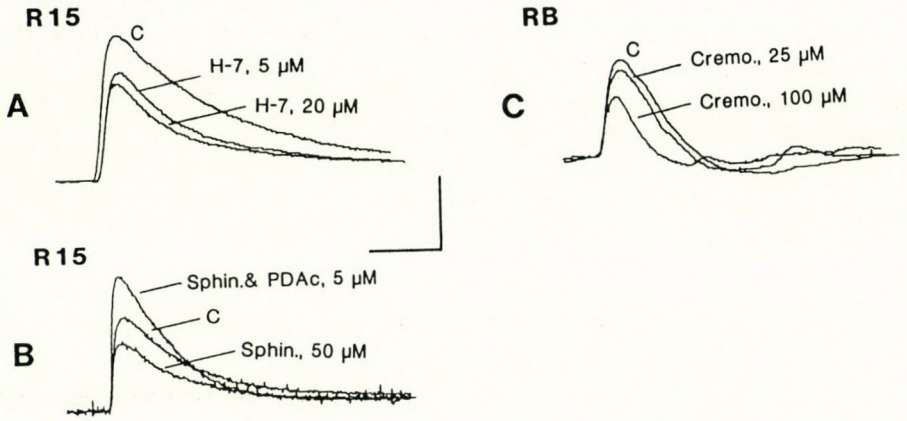


Fig. 3: Effects of different PKC blockers on the EPSP/EPSC amplitude in Aplysia neurons. Records from identified neurons. Current clamp at -90 mV.
A: Extracellular H-7 caused a rapidly onsetting (and dose dependent) reduction of the EPSP amplitude (calibration: 40 mV, 40 ms). B: The effect of sphingosine was similar. The records also show that the blocker's effect was reversed by PDAc (cal.: 20 mV, 100 ms). C: Cremophor EL also reduced the EPSP amplitude (cal.: 20 mV, 100 ms)

The last PKC blocker used in our experiments, melittin, affects PKC at the Ca/phospholipid binding site. It has been found very effective in several preparations (Takashima et al., 1991; Tomita et al., 1990). On EPSPs of Aplysia neurons, however, it exerted no effect. As shown by the Table, there was on the average no change in the postsynaptic phenomena. (Melittin was also of no effect on Aplysia action potentials.)

Effects of agents injected intracellularly

All effects described above were obtained with substances applied extracellularly. In order to get some information about the pre-or postsynaptic action of the substances used, we repeated certain experiments with intracellular drug injection. The substances were injected through the recording electrode so that the effect was confined to the postsynaptic cell. PDAc as a PKC activator and H-7 as a PKC blocker were injected. Prepared PKC (see Methods) was also injected to see if its action is similar to that of

PKC activators (Fig. 1./1C). We found that injected agents, even if they were active in extracellular application, had no influence on the EPSP/EPSC amplitude (see Table 1.).

A comparison of the synaptic modifications studied to PKC-dependent changes in the acetylcholine-induced membrane response revealed that the two processes are of opposite sign. As shown in Fig. 1./2A and C, extracellular PDAc application, or PKC injection, reduced the amplitude of the ACh response.

DISCUSSION

The results above indicate that the strength of transmission in the Aplysia central synapses studied is dependent on PKC activity. Potentiation of synaptic transmission by PKC-activating phorbol esters, similar to that found by us, has also been described in several nervous structures including hippocampus (Malenka et al., 1986) and neocortex (Baranyi et al., 1988). According to observations of Segal (1989), postsynaptic currents were enhanced on phorbol ester application to cultured hippocampal neurons with no change in the postsynaptic receptor sensitivity. Hence, a presynaptic alteration as explanation was probable. This alteration, augmented transmitter release, was found in frog neuromuscular junctions (Shapira et al., 1987) as well as in hippocampal slices (Malenka et al., 1987) and cortical synaptosomes (Herrero et al., 1992). The possible ultrastructural base of this PKC-dependent enhancement of transmitter release, accumulation of a PKC subspecies in the presynaptic area, was described by Arakawa et al. (1993). Two points of evidence indicate that the increase of EPSPs/EPSCs described here by us is of presynaptic origin as well. Firstly, when we applied a PKC activator extracellularly, i.e. the substance had access both to pre- and postsynaptic structures, an increment of EPSPs was present. When, however, the substance was injected into the postsynaptic neuron, no increase was ever seen. It would have been, of course, interesting to inject the activator presynaptically but our preparation did not permit that. The synapse itself was in the neuropil and was not to be visualized, and the presynaptic neuron was off the ganglion. The second point of evidence has been described in another paper of ours (Papp and Hoyer, 1996): the response of (somatic, i.e. extrasynaptic) ACh receptors on ACh pulse application was decreased at PKC activation. A rather similar phenomenon, increase of $GABA_A$ mediated IPSPs by presynaptic phorbol ester action in the hippocampus, was described

by (Pitler and Alger, 1994). Based on the increase of neuropeptide release from Aplysia bag cells on PKC activation (DeRiemer et al., 1985b), one can suppose that the increase of the postsynaptic reaction described here is in its mechanism common with those found in the bag cells, as well as Aplysia buccal ganglion synapses (Fossier et al., 1990) and various vertebrate preparations (Herrero et al., 1992; Malenka et al., 1986; Shapira et al., 1987). The similarity of the effect of PDAc and non-phorbol PKC activators as well as the opposedness of effects of PKC activators and blockers further support that the alterations observed by us were due to PKC activation.

A connection between protein phosphorylation and learning-like specific synaptic modifications has long been supposed. Increased PKC activation in rat hippocampus, as a consequence of associative learning, has been reported by Sunayashiki-Kusuzaki et al. (1993). Direct relation between LTP and PKC-dependent phosphorylation was demonstrated by Lovinger et al. (1987) and Bachoo (1992). According to Leahy (1993), presynaptically localized PKC is essential in LTP.

In invertebrates, including mollusks, no LTP can be induced but a shorter-term synaptic facilitation and some forms of associative learning are present. As described by Ghirardi et al. (1992) in Aplysia, PKC (activated through serotoninergic modulation) has a specific role in facilitation of depressed synapses. Translocation of PKC (being concomitant with its activation) in Aplysia sensory neurons, following sensitizing stimuli, was reported by Sacktor and Schwartz (1990). In the peptidergic modulation of the inhibitory synapses in Aplysia buccal ganglia, too, PKC is the enzyme of regulation (Fossier et al., 1990).

The data in the literature cited in the above paragraphs allow the conclusion that the PKC-dependent increase of the postsynaptic signals, as found by us, fits well in the trend of numerous publications. Our data give another evidence of the role played by PKC in presynaptic modifications.

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REFERENCES

Alkon, D.L., Amaral, D.G., Bear, M.F., Black, J., Carew, T.J., Cohen, N.J. et al. (1991) Learning and memory. Brain Res. Rev. 16, 193–220.

Arakawa, M., Mizoguchi, A., Masutani, M., Kawakita, N. and Ide, C. (1993) Ultrastructural localization of protein kinase C beta subspecies in the axon terminal of rat neuromuscular junction. Neurosci. Res. 16, 125–130.

Bachoo, M., Heppner, T., Fiekers, J. and Polosa, C. (1992) A role for protein kinase C in long-term potentiation of nicotinic transmission in the superior cervical ganglion of the rat. Brain Res. 585, 299–302.

Baranyi, A. and Szente, M.B. (1987) Long-lasting potentiation of synaptic transmission requires postsynaptic modifications in the neocortex. Brain Res. 423, 378–384.

Baranyi, A., Szente, M.B. and Woody, C.D. (1988) Activation of protein kinase C induces long-term changes of postsynaptic currents in neocortical neurons. Brain Res. 440, 341–347.

Ben-Ari, Y., Aniksztejn, L. and Bregestovski, P. (1992) Protein kinase C modulation of NMDA currents: an important link for LTP induction. TINS 15, 333–339.

Braha, O., Dale, N., Hochner, B., Klein, M., Abrams, T.W. and Kandel, E.R. (1990) Second messengers involved in the two processes of presynaptic facilitation that contribute to sensitization and dishabituation in Aplysia sensory neurons. P.N.A.S. 87, 2040–2044.

Bramham, C.R., Alkon, D.L. and Lester, D.S. (1994) Arachidonic acid and diacylglycerol act synergistically through protein kinase C to persistently enhance synaptic transmission in the hippocampus. Neuroscience 60, 737–743.

Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. 257, 7847–7851.

Cheng, G., Rong, X.W., Feng, T.P. (1994) Block of induction and maintenance of calcium-induced LTP by inhibition of protein kinase C in postsynaptic neuron in hippocampal CA1 region. Brain Res. 646, 230–234.

Colley, P.A., Sheu, F.-S. and Routtenberg, A. (1990) Inhibition of protein kinase C blocks two components of LTP persistence, leaving initial potentiation intact. J. Neurosci. 10, 3353–3360.

DeRiemer, S., Greengard, P. and Kaczmarek, L.K. (1985a) Calcium/phosphatidylserine/diacylglycerol-dependent protein phosphorylation in the Aplysia nervous system. J. Neurosci. 5, 2672–2676.

DeRiemer, S., Strong, J.A., Albert, K.A., Greengard, P. and Kaczmarek, L.K. (1985b) Enhancement of calcium current in Aplysia neurones by phorbol ester and protein kinase C. Nature 313, 313–316.

Fossier, P., Baux, G. and Tauc, L. (1990) Activation of protein kinase C by presynaptic FLRFamide receptors facilitates transmitter release at an Aplysia cholinergic synapse. Neuron 5, 479–486.

Frazier, W.T., Kandel, E.R., Kupfermann, I., Warizi, R. and Coggeshall, R.E. (1967) Morphological and functional properties of identified neurons in the abdominal ganglion of Aplysia californica. J. Neurophysiol. 30, 1288–1351.

Ghirardi, M., Braha, O., Hochner, B., Montarolo, P.G., Kandel, E.R. and Dale N. (1992) Role of PKA and PKC in facilitation of evoked and spontaneous transmitter release at depressed and nondepressed synapses in Aplysia sensory neurons. Neuron 9, 479–489.

Herrero, I., Míras-Portugal, M.T., Sánchez-Prieto, J. (1992) Activation of protein kinase C by phorbol esters and arachidonic acid required for the optimal potentiation of glutamate exocytosis. J. Neurochem. 59, 1574–1577.

Houslay, M.D. (1991) 'Crosstalk': a pivotal role for protein kinase C in modulating relationships between signal transduction pathways. Eur. J. Biochem. 195, 9–27.

Huang, K.P. (1989) The mechanism of protein kinase C activation. TINS 12, 425–432.

Ito, M., Tanaka, T., Inagaki, M., Nakanishi, K. and Hidaka, H. (1986) N-(6-phenylhexyl)-5-chloro-1-naphtalenesulfonamide, a novel activator of protein kinase C. Biochemistry 25, 4179–4184.

Kavaliers, M., Ossenkopp, K-P. and Tysdale, D.M. (1991) Evidence for the involvement of protein kinase C in the modulation of morphine-induced 'analgesia' and the inhibitory effects of exposure to 60-Hz magnetic field in the snail, Cepea nemoralis. Brain Res. 554, 65–71.

Klee, M.R. (1990) Different effects of a phorbol ester on membrane properties of Aplysia neurons. J. Bas. Clin. Physiol. Pharmacol. 1, 119–124.

Leahy, J.C. (1993) Demonstration of presynaptic protein kinase C activation following long-term potentiation in rat hippocampal slices. Neuroscience 52, 653–674.

Lovinger, D.M., Wong, K.L., Murakami, K. and Routtenberg, A. (1987) Protein kinase C inhibitors eliminate hippocampal long-term potentiation. Brain Res. 436, 177–183.

Malenka, R.C., Madison, D.V. and Nicoll, R.A. (1986) Potentiation of synaptic transmission in the hippocampus by phorbol esters. Nature 321, 175–177.

Malenka, R.C., Ayoub, G.S. and Nicoll, R.A. (1987) Phorbol esters enhance transmitter release in rat hippocampal slices. Brain Res. 403, 198–203.

Malinow, R., Schulman, H. and Tsien, R.W. (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245, 862–866.

Papp, A. and Hoyer, J. (1996) PKC-dependent reduction of the acetylcholine-evoked inward Na current in Aplysia D neurons. Effect of injected PKC and PKC activators. Brain Res. 708, 123–127.

Papp, A. and Klee, M.R. (1995) Alterations in the action potential of Aplysia neurons evoked by a phorbolester are mediated by protein kinase C. Brain Res. 684, 107–111.

Pitler, T.A. and Alger, B.E. (1994) Differences between presynaptic and postsynaptic $GABA_B$ mechanisms in rat hippocampal pyramidal cells. J. Neurophysiol. 72, 2317–2327.

Sacktor, T.C. and Schwartz, J.H. (1990) Sensitizing stimuli cause translocation of protein kinase C in Aplysia sensory neurons. P.N.A.S. 87, 2036–2039.

Saito, N., Itouji, A., Totani, Y., Osawa, T., Koide, H., Fujisawa, N., Ogita, K. and Tanaka, C. (1993) Cellular and intracellular localization of epsilon-subspecies of protein kinase C in the rat brain - Presynaptic localization of the epsilon-subspecies. Brain Res. 607, 241–248

Segal, M. (1989) Synaptic transmission between cultured rat hippocampal neurons is enhanced by activation of protein kinase C. Neurosci. Lett. 101, 169–174.

Shapira, R., Silberberg, S.D., Ginsburg, S. and Rahamimoff, R. (1987) Activation of protein kinase C augments evoked transmitter release. Nature 325, 58–60.

Shearman, M.S., Shinomura, T., Oda, T. and Niushizuka, Y. (1991) Protein kinase C subspecies in adult rat hippocampal synaptosomes. Activation by diacylglycerol and arachidonic acid. FEBS Letters 279, 261–264.

Sunayashiki-Kusuzaki, K., Lester, D.S., Schreurs, B.G. and Alkon D.L. (1993) Associative learning potentiates protein kinase C activation in synaptosomes of the hippocampus. P.N.A.S. 90, 4286–4289.

Takashima, A., Yokota, T., Maeda, Y. and Itoh, S. (1991) Pretreatment with caerulein protects against impairment induced by protein kinase C inhibitors in the rat. Peptides 12, 699–703.

Tauc, L., Fossier, P. and Baux, G. (1992) Regulation of transmitter release by presynaptic receptors at a cholinergic neuro-neuronal synapse. Acta Biol. Hung. 43, 49–58.

Tomita, H., Shibata, Y., Sakurai, T. and Okada, Y. (1990) Involvement of a protein kinase C-dependent process in long-term potentiation formation in guinea pig superior colliculus slices. Brain Res. 536, 146–152.

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Research report

SULFURTRANSFERASES ACTIVITY AND THE LEVEL OF LOW-MOLECULAR-WEIGHT THIOLS AND SULFANE SULFUR COMPOUNDS IN CORTEX AND BRAIN STEM OF MOUSE

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Summary: The level of 3-mercaptopyruvate sulfurtransferase (MPST) and rhodanese activity and the level of sulfane sulfur compounds, L-cysteine and non-protein sulfhydryl groups (NPSH) were compared in cortex and brain stem of mouse. The level of cysteine and sulfane sulfur compounds was higher in brain stem; 107% and 217% of the value determined in cortex, respectively. The activity of MPST and rhodanese showed also increased level in brain stem; 114% and 119% of the value determined in cortex, respectively. The level of NPSH in cortex was about 10% higher in comparison to brain stem. It seems that in brain stem metabolism of sulfane sulfur compounds and/or their accumulation may occur in a higher degree than in cortex.

Keywords: 3-mercaptopyruvate sulfurtransferase, rhodanese, cysteine, sulfhydryl groups, sulfane sulfur, mouse brain

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INTRODUCTION

Rhodanese (thiosulfate sulfurtransferase; EC 2.8.1.1) and 3-mercaptopyruvate sulfurtransferase (MPST) are involved in non-oxidative (desulfuration) pathway of L-cysteine metabolism. L-cysteine through the transaminative pathway is converted to 3-mercaptopyruvate; the substrate of 3-mercaptopyruvate sulfurtransferase (MPST; EC 2.8.1.2) (Meister et al., 1954; Cooper, 1983). MPST catalyzes the transfer of sulfur atom from 3-mercaptopyruvate to one of several acceptors including cyanide, thiols, sulfite, and sulfinates (Sörbo, 1957; Jarabak, 1981) or participates, among others, in the synthesis of iron-sulfur proteins (Taniguchi and Kimura, 1974).

Rhodanese carries a sulfane sulfur atom from a variety of sulfur donors e.g., thiosulfate, cystine trisulfide and persulfides (Wood, 1982) to various acceptors, for example to cyanide (Sörbo, 1975; Westley, 1980) and to proteins for Fe-S clusters formation (Finazzi-Agro et al., 1971; Taniguchi and Kimura, 1974) or to apoenzymes for their activity regulation. It is known that the sulfane sulfur atom have natural regulatory functions; it is effective in vitro at a very low concentration in regulating the activities of many enzymes (Toohey, 1989).

The desulfuration pathway of L-cysteine metabolism may be important as a source of metabolically active reduced sulfur (Stipanuk, 1986). The term, sulfane, designates sulfur atoms that are bonded covalently in chains only to other sulfur atoms. The importance of metabolic sulfane sulfur has been stressed in the context of cyanide detoxification (Westley, 1980) and the formation of iron sulfur centers (Finazzi-Agro et al., 1971; Taniguchi and Kimura, 1974). An operational definition of sulfane sulfur has been offered in terms of its susceptibility to cyanolysis (Wood, 1987).

The brains of mammals, particularly those of rodents, are very rich in sulfur compounds (Srebro, 1969; Srebro and Lach, 1972). Thiol rich glial granules have been found in astrocytes of the brain stem and surrounding the cerebral ventricles (Wisłocki and Leduc, 1954; Srebro, 1969).

The aim of the present study was to compare the level of sulfane sulfur compounds, L-cysteine, non-protein sulfhydryl groups (NPSH) and of rhodanese and MPST activity in cortex and brain stem of mouse.

MATERIALS AND METHODS

Two parts of Balb / c ♂ mouse brain were used in our experiment: cortex and brain stem. The removed parts were frozen in liquid nitrogen. After thawing tissues were washed with cold saline, blotted, weighted and homogenized using a Potter-Elvehjem homogenizer equipped with a Teflon pestle with 100 mM potassium phosphate buffer pH 7.50, in a proportion of 1 g of tissue to 3 ml.

NPSH was measured by the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to yield a compound that absorbs at 412 nm (Moron et al., 1979). Cysteine and cystine were determined according to Gaitonde (1967). The MPST activity was assayed according to the method of Valentine and Frankelfeld verified by Martensson and Sörbo (1978). The enzyme activity was expressed as μmoles of pyruvate produced per 1 g of fresh tissue during 15 min at 37 °C. Rhodanese was assayed according to Sörbo (1955). The enzyme activity was expressed as μmoles SCN^- formed during 5 min of incubation at 20 °C per 1 g of fresh tissue. Sulfane sulfur was determined by the method of Wood (1987) based on cold cyanolysis and colorimetric detection of ferric thiocyanate complex ion. Protein was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

The results were expressed as the average ±SD (standard deviation) of values determined for the tissue homogenate obtained from twenty mice. The statistical significance between the data was calculated by Student's t-test.

RESULTS AND DISCUSSION

The determined level of NPSH in cortex was about 10% higher in comparison to brain stem of mouse (Table 1). Differences between the brain regions indicate that the brain is not homogenous regarding glutathione (GSH) metabolism which is the most abundant non-protein thiol in the cell. This cellular protectant (Meister and Anderson, 1983) is widely distributed in the brain, however, its biological function in the central nervous system is not fully understood (Orlowsky and Karkowsky, 1976). It was noticed that the GSH content of the mouse brain showed considerable regional variation (Shivakumar and Ravindranath, 1992).

The level of L-cysteine seemed to be slightly (about 7%) lower in cortex than in brain stem of mouse. Similarly, the activity of MPST showed an increased level in brain stem; 114% of the value determined in cortex. L-cysteine is a source of 3-mercaptopyruvate; the substrate of MPST thus, higher concentration of L-cysteine may enhances the enzyme activity and the level of sulfane sulfur compounds (Toohey, 1989).

The level of sulfane-sulfur compounds was also significantly higher in brain stem; 217% of the value determined in cortex. Examples of sulfane sulfur compounds are the outer sulfur of thiosulfate and thiosulfonate ions, the internal chain sulfurs of organic and inorganic polysulfides, persulfides, polythionates, and elemental sulfur. Persulfide group are generated in the catalytic cycles of the enzymes rhodanese (Finazzi-Agro et al., 1972) and MPST (Meister et al., 1954). Other enzyme persulfides are found in a number of iron-sulfur proteins (Wood, 1982).

The rhodanese activity values determined in brain stem of mouse, in comparison with cortex, was 19% higher, what is in accordance with enhanced level of L-cysteine and sulfane sulfur compounds in this part of the

Table 1. The level of non-protein sulfhydryl groups, L-cysteine, sulfane-sulfur and the activity of rhodanese and MPST in the homogenate of cortex and brain stem of mouse

	Brain stem $\bar{x} \pm SD$	**Cortex** $\bar{x} \pm SD$
NPSH	1.28 ± 0.03	1.40 ± 0.05*
Cysteine	0.229 ± 0.016	0.213 ± 0.022
Sulfane sulfur	0.534 ± 0.029	0.246 ± 0.008*
Rhodanese	35.4 ± 0.58	29.8 ± 1.7*
MPST	1171 ± 152	1027 ± 49.6*

$\bar{x}$ — the average value from five to ten assays in the homogenate obtained from 20 mouse brains.

The level of NPSH and L-cysteine were expressed as μmoles per 1 g of fresh tissue, sulfane sulfur as μmoles of SCN^- per 1 g of fresh tissue.

The activity of rhodanese was expressed as μmoles of SCN^- formed during 5 min. of incubation in 25 °C per 1 g of fresh tissue.

The activity of MPST was expressed as μmoles of pyruvate formed during 15 min. of incubation in 37 °C per 1 g of fresh tissue.

* $p < 0.05$

brain. It seems that in this part of mice brain metabolism of sulfane sulfur compounds and/or their accumulation may occur in higher degree than in cortex. Thus, histochemical results (Srebro, 1969; Srebro and Lach, 1972; Wisłocki and Leduc, 1954) are in full agreement with the now disclosed activity of rhodanese and the sulfane sulfur compounds content. The latter are much more abundant in the brain stem than in cortex, this finding revealing a correlation of amount of the sulfur rich glia and the sulfane sulfur.

REFERENCES

Cooper, A.J.L. (1983) Biochemistry of sulfur-containing amino-acids. An. Rev. Biochem. 52, 187–222.

Finazzi-Agro, A., Cannella, C., Graziani, M.T., Cavallini, D. (1971) A possible role for rhodanese: the formation of "labile" sulfur from thiosulfate. FEBS Lett. 16, 172–174.

Gaitonde, M.K. (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acid. Biochem J. 104, 627–633.

Jarabak, R. (1981) 3-Mercaptopyruvate sulfurtransferase. In: Methods in Enzymology (eds. Colowick, S.P. and Kaplan, N.O.) Vol. 77, pp. 291–294, Academic Press, New York

Lowry, O., Rosebrough, N.J., Farr, A.L., Randal, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Martensson, J., Sörbo, B. (1978) Human, β-mercaptopyruvate sulfurtransferase: Distribution in cellular compartments of blood and activity in erythrocytes from patients with hematological disorders. Clin. Chim. Acta 87, 11–15.

Meister, A., Anderson, M.E. (1983) Glutathione. An. Rev. Biochem. 52, 711–760.

Meister, A., Fraser, P.E., Tice, S.V. (1954) Enzymatic desulfuration of ,β-mercaptopyruvate to pyruvate. J. Biol. Chem. 206, 561–575.

Moron, M.S., Depierre, J.W., Mannervik, B. (1979) Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582, 67–78.

Orlowsky, M., Karkowsky, A. (1976) Glutathione metabolism and some possible functions of glutathione in the nervous system. Int. Rev. Neurobiol. 19, 75–121.

Shivakumar, B. R., Ravindranath, V. (1992) Selective modulation in mouse brain regions and its effect on acrylamide-induced neurotoxicity. Biochem. Pharmacol. 43, 263–269.

Sörbo, B. (1955) Rhodanese. In: Methods in Enzymology (eds. Colowick, S.P. and Kaplan, N.O.). Vol. 2, pp. 334–337. Academic Press, New York

Sörbo, B. (1957) Enzymatic transfer of sulfur from mercaptopyruvate to sulfite or sulfinates. Biochim. Biophys. Acta 24, 324–329.

Sörbo, B. (1975) Thiosulfate sulfurtransferase and mercaptopyruvate sulfurtransferase. In: Metabolic Pathways. Vol. 7, pp. 433–456, Academic Press, New York.

Srebro, Z. (1969) A comparative and experimental study of the Gömöri - positive glia. Folia Biol. /Krakow/ 17, 177–192.

Srebro, Z., Lach, H. (1972) X-ray- and UV-induced increase in number of cysteine-rich periventricular glial cells in the brains of rats and mice. Acta Biol. Acad. Sci. Hung. 23, 145–151.

Stipanuk M. H. (1986) Metabolism of sulfur-containing amino acids. Annu. Rev. Nutr. 6, 170–209.

Taniguchi, T., Kimura, T. (1974) Role of 3-mercaptopyruvate sulfurtransferase in the formation of the iron-sulfur chromophore of adrenal ferredoxin. Biochim. Biophys. Acta 364, 284–295.

Toohey, J.L. (1989) Sulphane sulphur in biological systems: a possible regulatory role. Biochem. J. 264, 625–632.

Westley, J. (1980) Rhodanese and the sulfane pool. In: Enzymatic Basis of Detoxication. Vol. 2, pp. 245-262, Academic Press, New York.

Wisłocki, G.B., Leduc, E.H. (1954) The cytology of the subcommissural organ, Reissner's fiber, periventricular glial cells and posterior collicular recess of the rat's brain. J. Comp. Neurol. 101, 283-309.

Wood JL (1982) Biochemical functions of persulfides. Adv. Exp. Med. Biol. 148, 327-342.

Wood L. (1987) Sulfane sulfur. In: Methods in Enzymology (eds. Jakoby, W.B., Griffith, O.W.). Vol. 143, pp. 25–29, Academic Press, San Diego.

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Preliminary note

ROLE OF NITRIC OXIDE (NO) IN EXPLORATORY ACTIVITY INDUCED BY αCGRP(8–37) IN RATS

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Previously we have shown that calcitonin gene-related peptide (CGRP) has a role in learning, consolidation and retrieval of memory (1). The peptide delayed the extinction in active avoidance behavior (2) prevented the ECS-induced retrograde amnesia (3). In open field it decreased the exploratory activity, increased the rearing and grooming activity of the rats (4). I.c.v. administration of the receptor antagonist substance of peptide, αCGRP(8–37) dose dependently decreased the entering latency in passive and facilitated the extinction in active avoidance experiments, suggesting the role of endogenous CGRP in these behavioral paradigms (5).

Nitric oxide is involved in many neuronal processes such as fever (6), nociception (7, 8), memory (9,10). NO is a putative retrograde messenger at synapses involved in processes of learning and memory formation (11). The nitric oxide synthase (NOS) can be inhibited by i.c.v. administration of N-nitro-L-arginine (L-NA) and its methyl ester (LNAME) (12). Synaptic plasticity and memory formation was altered in NOS inhibitor-treated rats (13).

In the present studies we examined the action of αCGRP(8–37) an antagonist of CGRP on exploratory activity of the rats in open field, furthermore we were interested to reveal the possible role of NO in mediation of behavioral action of endogenous CGRP.

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The experiments have been done on adult male rats. A stainless steel cannula was implanted into the right lateral brain vetricle under pentobarbital-Na anaesthesia one week prior to experiments. Different doses (250 ng, 500 ng, 1 μg) of αCGRP(8–37) were injected i.c.v. and open field activity of animals were tested at 30 min and 1 hour.

In further experiments we pretreated the animals i.c.v. 5 μg L-nitro arginine (LNA) to inhibit the NOS before the i.c.v. administration of 1 mg of αCGRP(8–37). The 5 μg dose of L-NA was chosen in accordance with previous experience. This dose of L-NA inhibited the nitric oxide synthesis, but it has not influenced the behavioral paradigm. Open field activity was tested 30 min after the αCGRP(8–37) treatment.

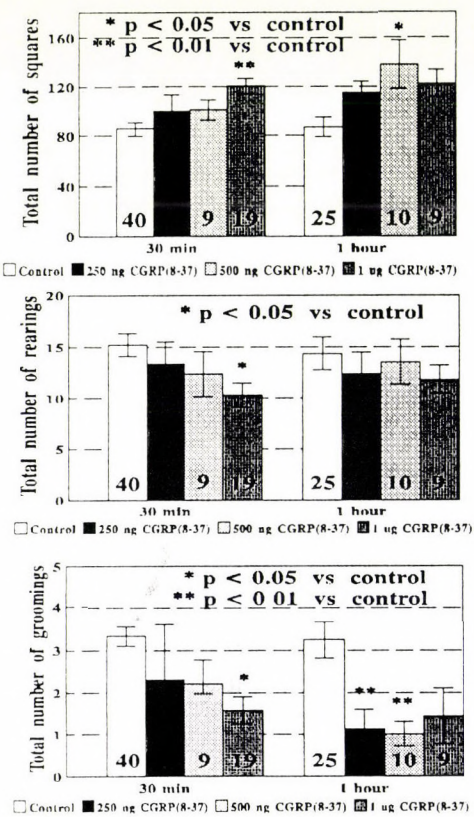


Fig. 1

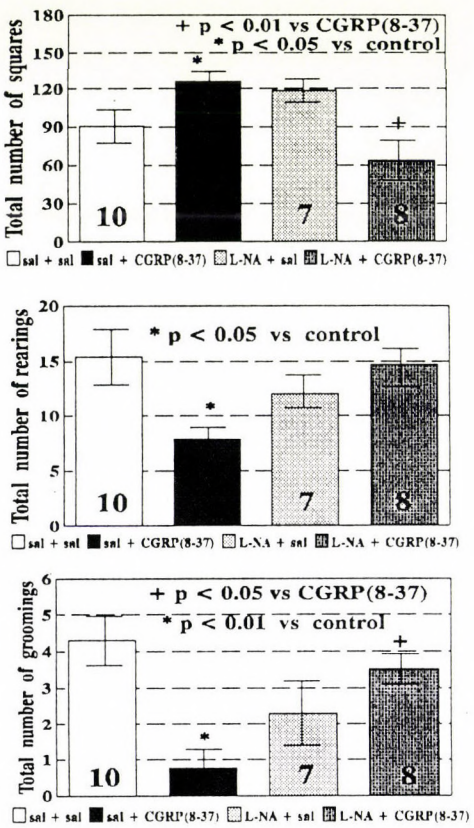


Fig. 2

I.c.v. administration of αCGRP(8–37) dose dependently increased the exploratory activity 30 min after the treatment. In 1 hour test only 500 ng dose of peptide was effective. 1 mg of αCGRP(8–37) decreased the rearing activity 30 min after the administration, in 1 hour test it had not effect. Numbers of groomings were decreased at both test times, more significantly in the 1 h test (Fig. l).

L-NA alone did not change the behavior of rats. In combined treatment L-NA prevented the increasing effect of αCGRP(8–37) in exploratory activity. L-NA also prevented the decreasing effect of the CGRP antagonist in grooming activity. Rearing activity was not influenced by L-NA (Fig. 2).

The results show that the endogenous CGRP is involved in the three parameters of open field activities (exploratory, rearing and grooming). Since the action of αCGRP(8–37) can be inhibited by nitric oxide synthase enzyme inhibition suggests that NO is involved in the mediation αCGRP(8–37) on open field behavior.

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REFERENCES

(1) Kovács, A., Telegdy, G.: Neuropeptides 23: 51–54 1992.
(2) Kovács, A., Telegdy, G.: Physiol. Behav. 58: 429–435 1995.
(3) Kovács, A., Telegdy, G.: Pharmacol. Biochem. Behav. 47: 121–125 1994.
(4) Kovács, A., Telegdy, G.: Pharmacol. Biochem. Behav. (Submitted).
(5) Kovács, A., Telegdy, G.: J. Neuroendocrinology 60: 96, 1994.
(6) Reimers, J.I., Bjerre, U., Mandrup-Poulsen, 1:, Nerup. J: Cytokine 6: 512–520 1994.
(7) Hara, S., Gagnon, M.J, Quock, R.M., Shibuya, T.: Pharmacol. Biochem. Behav. 48: 699–0702 1994.
(8) Meller, S.:, Gebhart, G.F.: Pain 52: 127–136 1993.
(9) Estall, L.B., Grant, S.J, Cicala, G.A.: Pharmacol. Biochem. Behav. 46: 959–962 1993.

(10) Holscher, C., Rose, S.P.: Neurosci. Lett. 145: 165–167 1992.
(11) Barinaga, M.: Science 263: 466 1994.
(12) Salter, M., Duffy, C., Garthwaite, J. Strijbos, P. J.L.M.: Neuropharmacology 34: 639–649 1995.
(13) Böhme, G.A., Bon, C., Lemaire, M., Reibaud, M., Piot, O., Stutzmann, J-M., Doble, A., Blanchard, J-C.: Proc. Natl. Acad. Sci. USA 90: 9191–9194 1993.

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Preliminary note

LONG-LASTING CYCLES IN FORMAL NEURAL NETS

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Recurrent activities are very frequent phenomena both in excitable and in the so-called non-exitable organs. They are recorded under various names (periodicity, oscillations, rhythmicity). The notion of reverberation is attributed to Lashley (1942) who regarded it as maintaining past activity for storage. McCulloch and Piffs (MCP, 1943) have already simulated reverberations. But, in order to simulate periodic locomotory actions it was most probably used first in works of Székely (1962-65, and later with coauthors Kling, Adam). Until that time, for locomotions detailed descriptions can be found without generating mechanisms. Interestingly, already Aristotle raised the problem of generation of motion in his *De motu animalium.*

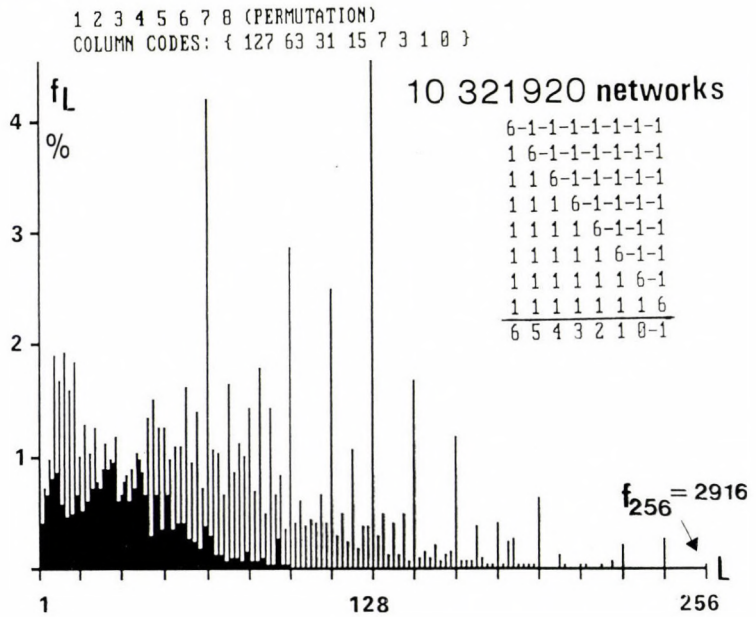
On the instruments of mathematical simulations. - Let us concentrate to a question arised investigating threshold logical networks. How to generate very long-lasting complicated cycles? The cycle - and other dynamical simulations may belong to the following categories. (1) *Ad hoc* reproductions one or multi-channel recurrent behaviour. These are good for interpretations of any singular functional pattern record of activities, but only vaguely reproducible if the interpreted records are also roughly repeatable. (2) Functional explanations of dynamics (e.g. oligocyclic) organized at a well reproducible macro-level. Perceptual or motoric performances as well as their relations are included here. (3) General studies of connections between temporal structure, cycle lengths, dynamics in general and the wiring (or component interactions which is usually called structure). All of these can be done with a great variety of units and nets. By units falling in

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the range from bistable formal neurons to HH-models (ion-channel based systems).

By network interactions being either matrix represented (MS), matrix system representable (MSR) or interactions not or only partially separable (NS, PS) into matrix entries. The controls are exerted either on variables or parameters.

The long cycle (LC) problem (LCP) for threshold gate neural networks. When synthetizing neural nets, soon we face the following problem. How can we reproduce one single activity pattern or a bundle of activity patterns with a network of the smallest required number of neurons? How we can reach the longest cycle of simple or of complicated inner structure with a network of a given size? The question is a version of "maximal exploitation of a net" problem. This or related questions were addressed perhaps first by Arimoto, the Caianiello-school, Moreno-Diaz, Da Fonseca, Sato, Lábos, Sette and in the last years Nützel and Matamala. Different network dynamical problems were studied by Aman and his followers. This short list of authors who dealt with LCP obviously does not exhaust the presentations of "cycle problem" discussed for formal neuronal nets. It will be surveyed more in details later (Lábos).



The approaches and problems emerged are either constructive or statistical. The LCP is a prototype of network analytical (NA) and synthesis (NS) problems. Most often both NA and NS can be formulated as computations, but intuitive methods are still profitable at the beginning and will play their further fertilizing role. The animal brains are limit cases of long cycle generation. Their genuine aperiodicity requires completely different theoretical apparatus.

A cycle in the activity of a net is called long if its length cannot be reproduced with other nets consisting of fewer neurons. Such cycles are rare. Here, we display the result of a computer search of 10 millions of 8-neuronal-network-dynamics when only 3000 cycles (0.0283%, 1/3539) proved to be maximal, not only long. The figure is a length spectrum. To reach this result an extensive theoretical preparation was unavoidable.

In a textual example several procedures were used and long cycles was generated with sparse wiring until the case of n=75 units, as displayed below. The few1 locally dense disjoint subnetworks are as follows (number of neurons in 11 subnets and the generated subnet-cycle (subcycle) lengths are written): Sum of Subnet Sizes: 9+7+7+7+7+7+7+7+6+6+5 (Sum = 75) Lengths: 512,127,125,123,121,119,113,109,61,59,31 (Product larger than 2^{74}).

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Preliminary note

REINNERVATION OF DENERVATED HINDLIMB MUSCLES BY AXONS OF GRAFTED MOTONEURONS VIA THE REIMPLANTED L4 VENTRAL ROOT

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Embryonic spinal cord grafts placed into the lumbar region of an adult spinal cord survive, differentiate but neurones from such grafts are not able to extend their axons into denervated ventral roots. However, they can be made to reinnervate a skeletal muscles via a peripheral nerve bridge. We attempted to guide out axons of grafted motoneurones via the reimplanted L4 ventral root in order to reinnervate denervated hindlimb muscles.

Table 1. Numbers of retrogradely labeled cells and those examined for the presence of BrdU labeling from the spinal cords of 4 animals processed for double-labeling (FB/DY + BrdU) are shown.

	Total number of retrogradely labeled cells	Number of cells investigated	Number of double labeled neurones	%
	142	81	16	20
	70	19	2	10
	133	41	9	22
	121	60	5	8.3
Total	466	201	32	15 ± 2.9
Mean	116 ± 16	50 ± 13	8 ± 3	

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Solid pieces of embryonic spinal cords prelabeled with 5-bromo-deoxyuridine were grafted into the L5 spinal segment, the L4 ventral root was pulled out and reimplanted close to the graft. Three to six months later fluorescent dyes were applied both to the L4 ventral ramus and hindlimb muscles and retrogradely labeled neurones were traced.

A considerable number of labeled neurones had grown axons into the ventral root and 75% of these reached the hindlimb muscles originally supplied by L4 ventral root axons. Many of the retrogradely labeled neurones were of embryonic origin and also calcitonin gene-related protein immunoreactive. Intracellular labeling of these retrogradely labeled cells with biocytin showed that approximately 30% of them exhibited the features of differentiated motoneurone-like cells with an extensive dendritic tree. Stimulation of the L4 ventral ramus elicited a strong contractions in the reinnervated Tibialis Anterior and Extensor Digitorum muscles (37% op/ctrl). In the absence of embryonic grafts the reimplantation of the L4 ventral root resulted in poor reinnervation. These results show that grafted embryonic motoneurones are able to reinnervate remote denervated skeletal muscles via reimplanted ventral roots.

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Preliminary note

NEUROTRANSMITTER BACKGROUND OF THE ANTI-INFLAMMATORY EFFECT EVOKED BY ACTIVATION OF SENSORY NERVE FIBERS

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Neuropeptides released by electrical or chemical stimulation from peripheral endings of the capsaicin-sensitive primary afferent neurons evoke local neurogenic inflammation in the innervated skin and mucosal areas. It has been established by previous studies that a local neurogenic inflammation (primary reaction) induced by antidromic as well as orthodromic stimulation prevents the development of a subsequent neurogenic or non-neurogenic inflammation on a distant part of the body (secondary reaction). A pure non-neurogenic inflammation evoked by dextran had no anti-inflammatory activity.

The aim of the present study was to determine the neurotransmitter background of this phenomenon. Following the primary reaction induced by 1% mustard oil application on acutely denervated leg skin of the rats, the secondary plasma protein extravasation on the contralateral leg evoked also by mustard oil was inhibited by 50 percent. Pretreatment with an opiate antagonist naloxone (1 mg/kg), almost totally abolished this anti-inflammatory effect of the primary reaction (Fig. 1).

Neurogenic inflammation produced by 1% mustard oil smearing in the skin of acutely denervated leg (primary reaction) significantly decreased the oedema induced by subcutaneously injected dextran in the contralateral leg after chronic denervation (secondary reaction). Although naloxone pretreatment diminished the anti-inflammatory effect, the oedema developed in the mustard oil treated animals was significantly smaller than in the paraffin oil treated animals (Fig. 2).

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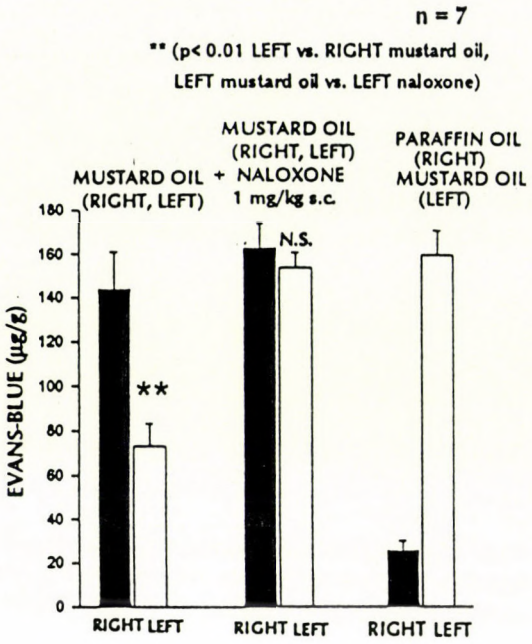


Fig. 1. Effect of neurogenic inflammation evoked by mustard oil on a contralateral neurogenic inflammation induced by mustard oil also, in the skin of hind legs in naloxone pretreated and untreated rats

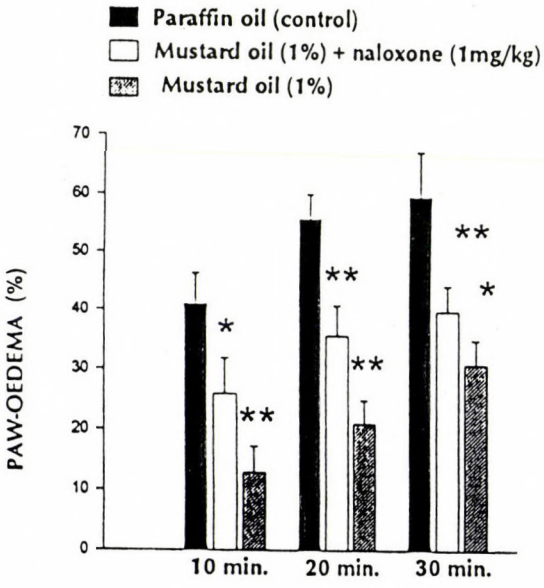


Fig. 2. Effect of neurogenic inflammation evoked by mustard oil on dextran-oedema on the contralateral leg, in naloxone pretreated and untreated animals. (** $p<0.01$, * $p<0.05$)

CONCLUSIONS

A primary local neurogenic inflammation prevents the development of a secondary inflammatory reaction. If the secondary reaction has a neurogenic component, the enkephalinergic transmission plays key-role in the mediation of the anti-inflammatory effect. If the secondary reaction is a non-neurogenic inflammation participation of other anti-inflammatory mediators (somatostatin, galanin?) are also important besides enkephalins.

REFERENCES

Pintér, E. and Szolcsányi, J. (1988) Agents and Actions 25, 240–242.

Lembeck, F., Donnerer, J. and Barthó, L. (1982) Eur. J. Pharmacol. 85, 171–176.

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Preliminary note

THE ROLE OF HUMAN GALANIN (hGAL1-30), hGAL1-19 AND hGAL17-30 IN THE RELEASE OF ACETYLCHOLINE IN THE STRIATUM: A NEUROPHARMACOLOGICAL STUDY

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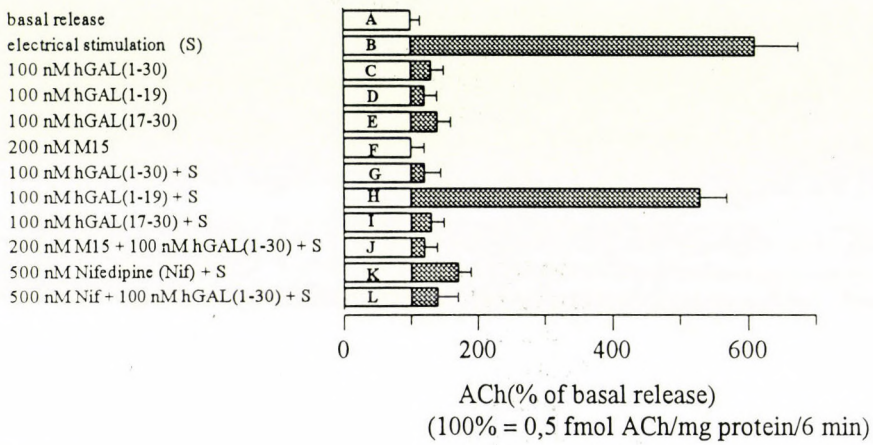
Galanin (GAL) is a neuropeptide that was discovered by Tatemoto in 1983 (4). Electrophysiological experiments have revealed that its application both *in vivo* and *in vitro* inhibits the evoked release of acetylcholine (ACh). In morphological studies, Chan-Palay (1) concluded that in Alzheimer's disease (AD), when the cholinergic neurons degenerate in the basal forebrain, the GAL-contaning fibers become hypertrophic. If this is the case, the hyperinnervated cholinergic neurons in the basal forebrain will be less effective and the hypocholinergic activity of these neurons will lead to reductions in memory and the cognitive function. Ögren and Pramanik (3), however, demonstrated that GAL causes a facilitatory modulation of the neurotransmission in the brain since porcine GAL (pGAL1-29) enhances ACh release *in vivo* in the rat striatum. In earlier experiments, we obtained neuropharmacological evidence of the divergent effects of synthetic human GAL (hGAL1-30) and pGAL129 on the ACh release from the striatum of rat. In *in vitro* experiments on the striatum, neither hGAL1-30 nor hGAL1-19 stimulated the basal release of ACh, whereas pGAL1-29 enhanced it. On the other hand, hGAL1-30 and hGAL17-30 inhibited the K^+-evoked transmitter release in the striatum, whereas p-GAL1-29 and hGAL1-19 had no such effect. Since the N-terminal aminoacid sequence of hGAL1-15 and

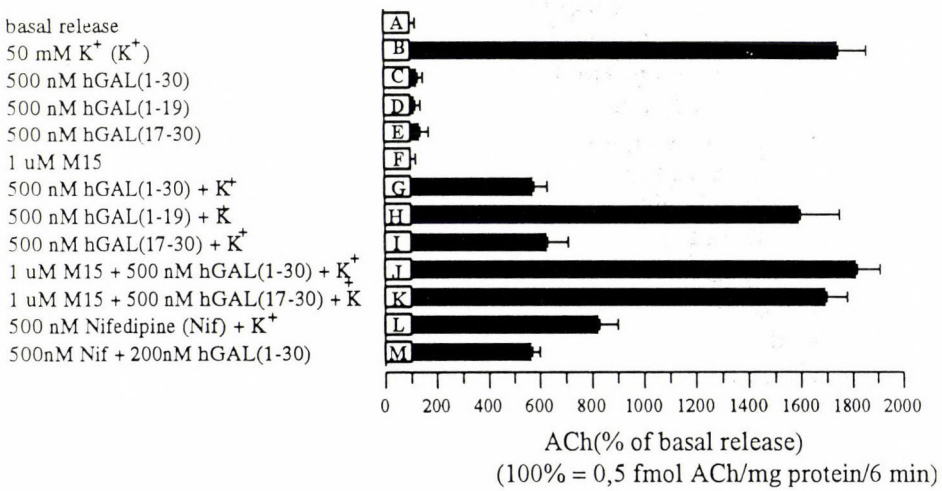
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pGAL1-15 are identical, we suggested that the C-terminal fragment of hGAL (hGAL17-30) may have an important role in the modulation of ACh release.

Our main goal in the present experiment is to demonstrate the roles of the N and C-terminals in the release of ACh, and the effects of hGAL1-30, hGAL1-19 and hGAL17-30 on the release of ACh evoked by K^+ or electrical stimulation.

To study the effects of GALs and combinations of GALs either with galantide (M15, a GAL receptor antagonist) or with nifedipine (a calcium channel blocker), the striatum of rat was used as described earlier (2).





The results of the experiments are summarized in Figs 1 and 2. It is to be observed that hGAL1-30, hGAL1-19, and hGAL17-30 had no stimulatory effect on the basal release of ACh. However, hGAL1-30 and hGAL17-30 diminished both K^+ and electrically evoked ACh release. Somewhat surprisingly, whereas M15 can antagonize the inhibitory effects of hGAL1-30 and hGAL17-30 on K^+-evoked ACh release, no such effect of M15 was to be observed during electrical stimulation. The combination of nifedipine with hGAL1-30 dramatically reduced electrically induced ACh release, but nifedipine had no such effect on K^+-evoked ACh release.

CONCULSION

The inhibitory effect of hGAL on electrically evoked ACh release is more pronounced than on that evoked by K^+. The C-terminal fragment of hGAL (hGAL17-30) is more effective in inhibiting the release of ACh than the N-terminal part (hGAL1-19).

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REFERENCES

1. Chan-Palay, V.L., J. Comp. Neurol., 273, 543-557 (1988)
2. Kása, P. et al., Neuroscience (in press 1996)
3. Ögren, S.O., Pramanik, A., Neurosci. Lett., 128, 253-256 (1991)
4. Tatemoto, K. et al., FEBS Lett., 164, 124-128 (1983)

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Preliminary note

NEW POLYCLONAL ANTISERUM AGAINST MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2); PREPARATION AND PRELIMINARY CHARACTERIZATION

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Microtubule-associated proteins (MAPs), a diverse set of tubulin-binding proteins take part in regulating assembly, stability and interactions of microtubules. Of special importance is the family of MAP2 proteins, whose most important members are the high molecular weight forms, MAP2a and MAP2b, and the low molecular weight form, MAP2c, expressed predominantly in the embryonic tissue (Riederer and Matus, 1985). MAP2 proteins are thought to influence neuronal morphogenesis, plasticity and regeneration (Johnson and Jope, 1992; Matus 1988) most probably by taking part, along with other filamentous MAPs and motor proteins, in the formation of crossbridges interconnecting microtubule filaments and other subcellular organelles (Hirokawa et al., 1988; Jung et al., 1993). Our

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motivation for raising new antibodies against MAP2 came from the interest of gaining insight into the above phenomena.

On the molecular level, MAP2 proteins are highly asymmetric, flexible molecules, consisting of two principal domains: the microtubule-binding and the projection domain. The microtubule-binding domain corresponds to the C terminal region of the protein. The second, rod-like domain is thought to form projections emerging from the surface of the microtubules. These two domains can be separated following proteolytic digestion of purified MAP2 (cf. Jancsik et al., 1989). We used purified proteolytic fragments of the projection domain of MAP2 to raise antiserum in rabbits. IgG fraction was prepared from the crude antiserum by ion exchange chromatography. Both crude serum and IgG fractions could be stored at -20 ^{o}C for months without loss of activity.

Among electrophoretically separated (Laemmli et al., 1970) protein components of total brain homogenate or purified microtubules, electroblotted onto nitrocellulose membrane (Towbin et al., 1979), the purified IgG fraction specifically recognized a protein doublet of approximately 280-300 kDa (result not shown).

Testing of the antiserum on the immunohistochemical level gave the results shown below.

Adult rat brain was fixed by transcardial perfusion with Zamboni's fixative, then 60 μm thick saggital vibratome sections were cut. Free floating sections were incubated with anti-MAP2 IgG (dilution 1:10000) for 48 hr at + 5 ^{o}C and were further processed by using the ABC-Immunoperoxidase Kit (Vectastain®, Vector Laboratories, USA). For visualization, 3,3′-diaminobenzidine tetra hydrochloride (DAB) was used as chromogen.

The technique described in Fig. 1 has been applied for sections from the cervical part of the spinal cord.

The most prominent feature in the cerebellum (Fig. 1) is the intensive staining of the Purkinje cell perikaya and dendrites. The IR of dendrites is more pronounced than that of the perykaria. It is worth noting that in the distal part of the dendrites IR is usually higher than near the perikarya. In the ventral horn of the medulla oblongata (Fig. 2), perikarya of the great motor neurons are moderately/strongly stained; dendrites are strongly stained.

The above findings are in perfect agreement with the strict compartmentalization of MAP2 in the rat nervous system (De Camilli et al., 1984): the protein was confined to the perikarya and dendrites. Within the cerebellum, the most intensive staining was the one of dendritic branching

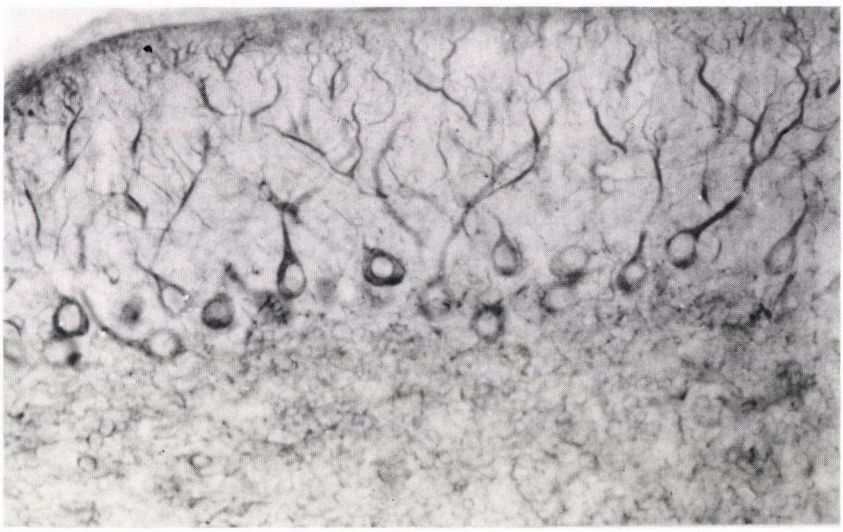


Fig. 1. MAP2 immunoreactivity (IR) in adult rat cerebellum

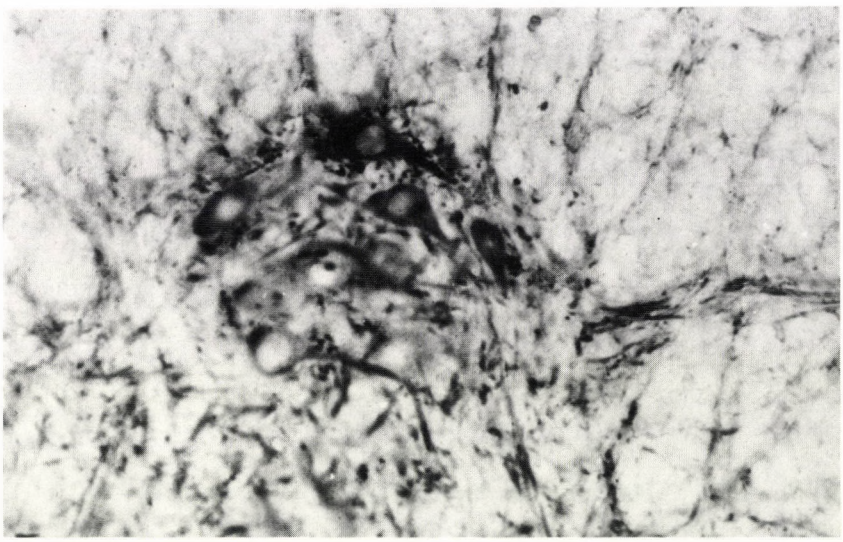


Fig. 2. MAP2 IR in adult rat medulla oblongata

of the Purkinje cells. This intracellular compartmentalization of MAP2 has been further confirmed by several authors (e.g. in a comparative study on three classes of vertebrates: Viereck et al., 1988).

Further characterization of this antiserum on the biochemical and immunohistochemical levels is underway in our laboratories.

The observations shown in the present work strongly speak for the specificity of the antiserum described.

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REFERENCES

De Camilli, P., Miller, P.E., Navone, F., Theurkauf, W.E., Vallee, R.B. (1984) Distribution of Microtubule-Associated Protein 2 in the Nervous System of the Rat Studied by Immunofluorescence. Neuroscience 11, 819–846.

Hirokawa, N., Hisanaga, S.I., Shiomura, Y. (1988) MAP2 is a Component of Crossbridges between Microtubules and Neurofilaments in the Neuronal Cytoskeleton: Quick-Freeze, Deep-Etch Immunoelectron Microscopy and Reconstitution Studies. J. Neurosci. 8, 2769–2779.

Jancsik, V., Filliol, D., Felter, S., Rendon, A. (1989) Binding of Microtubule-Associated Proteins (MAPs) to Rat Brain Mitochondria: A Comparative Study of the Binding of MAP2, its Microtubule-Binding and Projection Domains, and Tau Proteins. Cell Motil. Cytoskel. 14, 372–381.

Johnson, G.V.W., Jope, R.S. (1992) The Role of Microtubule-Associated Protein 2 (MAP-2) in Neuronal Growth, Plasticity, and Degeneration. J. Neurosci. Res. 33, 505–512.

Jung, D., Filliol, D., Miehe, M., Rendon, A. (1993) Interaction of brain microtubules from rat brain tubulin and MAP2 or TAU. Cell Motil. Cytoskel. 24, 245–255.

Laemmli, U.K. (1970) Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 277, 680–685.

Matus, A. (1988) Microtubule-Associated Proteins. Their Potential Role in Determining Neuronal Morphology. Ann. Rev. Neurosci. 11, 29–44.

Riederer, B.M., Matus, A. (1985) Differential Expression of Distinct Microtubule-Associated Proteins during Brain Development. Proc. Natl. Acad. Sci. USA 82, 6006–6009.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.

Viereck, C., Tucker, R.P., Binder, L.I., Matus, A. (1988) Phylogenetic Conservation of Brain Microtubule-Associated Proteins MAP2 and Tau. Neuroscience 26, 893–904.

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Preliminary note

MULTICOMPARTMENTAL MODELING OF HIPPOCAMPAL PYRAMIDAL CELLS AND INTERNEURONS WITH THE GENESIS SOFTWARE TOOL

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Three classes of problems related to multicompartmental modeling of hippocampal cells are studied by using the GENESIS software tool (Bower and Beeman 1994).

(1) The contribution of the individual conductances to the overall cell function is analyzed. (2) A preliminary model of the basket cell is specified and its elementary properties are demonstrated. (3) The pyramidal cell soma itself is considered as a multicompartmental unit.

1. Hippocampal pyramidal cells were the subject of multicompartmental modeling studies (Traub et al. 1991, Traub et al. 1994, Migliore et al. 1995). The maximal model takes into account a single Na^+ channel, three Ca^{2+} channels (Ca_N, Ca_L and Ca_T), three Ca^{2+} independent K^+ channels (K_{DR}, K_A, K_M), two Ca^{2+}-dependent K^+ channels (K_{AHP} K_{Ca}). Intracellular Ca^{2+}-related processes, such as buffering, pumping, and radial diffusion may also be involved.

The role of the individual channels in establishing both the bursting and non-bursting firing modes are studied here. The relationship between the firing frequency and the strength of the applied current is also calculated. The intention of our work is to have a realistic minimal single cell model that should become the building block of a medium-scale network model.

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Since the role of the densities and the (nonuniform) distribution of ionic channels are not (well) known, some sensitivity analysis for these parameters has also been done.

2. A preliminary model for the basket cell has been set up and studied. To construct a realistic model, we took into account a set of anatomical and physiological properties (Sik et al. 1995):

(i) the cells are electrotonically much more compact than the pyramidal cells;

(ii) basket cells have smaller amplitude after hyperpolerization (AHP) than other interneurons;

(iii) the threshold for evoking action potential in basket cells is lower than in pyramidal cells;

(iv) since firing adaptation is small, it is assumed that K_{AHP} is very small;

(v) because of the very short, non-overshooting nature of the action potential and the very fast AHP, it is assumed that the repolarizing K^+ currents are very strong;

(vi) the transition from depolarization is very rapid: the kinetics of the Na^+ channel should be different from that of pyramidal cells to describe the rapid spike initiation.

3. Multicompartmental models consider the cell body as a single compartment which is connected to the axon initial segment and the final dendritic compartments. It seems important, however, to have a more detailed picture of the function of the soma itself. In the CA3 region of the hippocampus (as well as in many other cortical regions), basket cell inhibitory terminals give connections to the somata (and to the perisomatic regions) of pyramidal cells.

To learn more about the effect of these inhibitory connections, a multicompartmental model of the pyramidal cell soma has been developed. The soma is considered as a sphere, and its surface is divided into compartments corresponding to the spherical coordinate system of "latitudes" and "longitudes". Cable theory has been transformed to describe electric conduction of such compartments, and the (usually quite large) nucleus has also been taken into account. The simulation package GENESIS has been extended to treat this multicompartmental soma model. The somatic integration of different synaptic inputs is studied by the new model. Random inputs arriving through the dendrites as well as inhibitory inputs coming from the basket cells at different somatic sites can be studied and compared to traditional compartmental models.

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REFERENCES

Bower JM, Beeman D (1994): The book of GENESIS. Exploring realistic neural models with the GEneral NEural SImulation System, TELOS, Springer-Verlag, New York.

Traub RD, Wong RKS, Miles R, Michelson H (1991): A model of a CA3 hippocampal neuron incorporating voltage-clamp data on intrinsic conductances, J. Neurophysiol., 66, (635-650).

Traub RD, Jefferys, Miles R, Whittington, Toth K (1994): A branching dendritic model of a rodent CA3pyramidal neurone. J. Physiol., 481, (79-95).

Migliore M, Cook EP, Jaffe DB, Turner DA, Johnston D (1995): Computer simulation of morphologically reconstructed CA3 Hippocampal Neuron. J. Neurophysiol., 73, (11571 168).

Sik A, Penttonen M, Ylinen A, Buzsáki G (1995): Hippocampal CA1 Interneurons: An in vivo labeling study. J. Neuroscience, 15, (6651-6665) .

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Preliminary note

SIMULATION OF THE WHOLE OLFACTORY BULB BASED ON DETAILED SINGLE CELL MODELS

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Data of intracellular studies on the kinetics of voltage- and calcium-dependent ion channels have been started to be available for different cells. Consequently, multi-compartmental models supplemented with kinetic equations for the intrinsic membrane currents may be the building blocks of (moderately large) network models. At first, the generation and propagation of action potentials in the two major cell types of the olfactory bulb, i.e. in the mitral and granule cells are simulated by applying multi-compartmental modeling technique. The effects of the individual currents and their role in the generation and supression of action potentials, and in the control of firing frequencies are particularly detected previously (Aradi and Érdi 1996, in press).

Simulation of detailed models of single mitral and granule cells (i.e. the main cell types of the olfactory bulb have been done using morphological and electrophysiological data (see also Aradi and Érdi 1996, in press). Passive and active membrane data were either taken from Bhalla and Bower (1993), who have already studied many aspects of the dynamics of the mitral and granule cells. Signal propagation through the compartments of both the excitatory and inhibitory cells of the OB have been simulated. The effect of both orthodromic and antidromic stimulation of the mitral has been demonstrated. Further simulations on granule cells have been done to find

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proper parameters matching to the experimental data (Wellis and Scott, 1990).

Elementary synaptic interactions and dynamic behaviour of small networks of mitral and granule were studied by a series of simulations. In more complicated network models, the effect of other two cell types, namely the periglomerular and deep short axon cells, have been taken into account, and the structure of these networks were based on anatomical findings (Halász, 1990). Suppression by partial blockade (Duchamp et al., 1993, Nicoll et al., 1982) of the GABAergic inhibitory effect of either the periglomerular cells or of the granule cells has been shown.

The NEURON software (Hines 1993) has been used.

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REFERENCES

I. Aradi and P. Érdi, Signal Generation and Propagation in the Olfactory Bulb: Multicompartmental Modeling. Computers & Mathematics with Application (1996) (in press).

U. S. Bhalla and J. M. Bower, Exploring Parameter Space in Detailed Single Neuron Models: Simulations of the Mitral and Granule Cells of the Olfactory Bulb. J. Neurophysiology 69, 1948–1963 (1993).

N. Halász, Morphological basis of information processing in the olfactory bulb.NATO ASI Series, Vol. H 39 Chemosensory Information Processing ed.: D. Schild, Springer-Verlag Berlin Heidelberg (1990).

M. Hines, The NEURON simulation program. In: Neural network simulation environments Skrzypek (ed.), Kluwer Acad. Publ., Norwell, Ma (1993).

P. Duchamp-Viret and A. Duchamp, GABAergic Control of Odour-induced Activity in the Frog Olfactory Bulb: Possible GABAergic Modulation of Granule Cell Inhibitory Action. Neuroscience 56, 905-914 (1993)

R. Nicoll and C. E. Jahr, Self-excitation of Olfactory Bulb Neurones. Nature 296, 441–444 (1982).

Wellis D. P. and Scott J. W. Intracellular Responses of Identified Rat Olfactory Bulb Interneurons to Electrical and Odor Stimulation. J. of Neurophysiology 64, 932–947 (1990).

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Preliminary note

MCPP-INDUCED ANXIETY — A POTENTIAL NEW METHOD FOR SCREENING ANXIOLYTIC ACTIVITY

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The majority of tests for screening anxiolytic activity of drugs are based on the measurement of drug-induced changes in the normal reaction to an aversive stimulus. However, anxiolytic drugs have to decrease pathological reaction to some kind of aversive (or pathologically considered as aversive) stimulus. Animals treated with anxiogenic compounds react inordinately to stress which is only mildly aversive for normal, untreated animals. m-chlorophenylpiperazine (mCPP), a major metabolite of the atypical antidepressant drug trazodone has been found to exert significant activity in the serotonergic systems (Murphy et al. 1991). It has particularly high affinity to the 5-HT_{2C} and 5-HT_{2A} receptors, with K_d values of 20 and 200 nm, respectively (Fiorella et al. 1995). mCPP produces anxiety in humans (Charney et al. 1987) and in rodents. In light-dark test mCPP reduces the activity of the animals in the light compartment indicating its anxiogenic effect. The aim of our study was to investigate the conditions under which mCPP produces a measurable and reproducible anxiogenic reaction and the effect of anxiolytic drugs with various mode of action on mCPP-induced anxiety. Male Wistar rats (Charles River) were treated subcutaneously with saline or mCPP and 20 minutes later behavioral test was conducted in six, 2-compartment automated test chambers. Access between the lit and dark areas was provided by a 8x8 cm passageway. A 40 W red tungsten bulb located 30 cm above the floor of the box was the source of light in the lit compartment in each box. Interruptions of the infrared beams (16 at 2 cm height) in the chamber were automatically recorded by the digiscan analyzer and than transmitted to an IBM compatible computer. Movement time in the

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light compartment was analyzed by one-way ANOVA followed by Duncan test.

In order to investigate the adequate conditions of the method, the influence of lighting, the length of pretreatment time, duration of the effect of mCPP and dose-response relation were studied. It was pointed out, that rats are very sensitive to light intensity, relevant control activity was measured only in dim red light. Pretreatment time failed to influence significantly the effect of mCPP, however, the difference between the control and mCPP treated groups proved to be significant according to Duncan test when the pretreatment time was 0 or 20 minutes. mCPP significantly decreased the activity of the animals in the light compartment from the second minute. The difference between the control and treated groups increased during the first ten minutes, and it remained nearly constant until the end of the experiment (20 minutes). The drug significantly decreased the motor activity of the animals in the lit compartment in the dose of 0.5; 1.0 and 2.0 mg/kg, in higher doses strong decrease in the general motor activity was detected.

Studying the effect of anxiolytics in this model eight drugs were studied with four different mechanisms of action. 1,4-benzodiazepines (chlordiazepoxide and diazepam) were very active, the amplitude of their effect was high. Their activity decreased in higher doses, where the sedative effect of the drugs influenced the activity of the animals. Similar decrease in activity in high doses was observed in the case of 2,3-benzodiazepines (Tofisopam and Girisopam). Tofisopam failed to increase significantly the activity of mCPP-treated animals, but its effect was dose-dependent and its activity was considerable. 5-HT_{2C} antagonists (Deramcyclane and Ritanserin) were also active in a relatively wide dose-range, but the intensity of their effect was lower compared to 1,4-benzodiazepines, and it was not possible to observe any doseresponse relation in its active range. In the case of 5-HT_{1A} agonists, buspiron was active, although its dose-range was narrow, and the amplitude was not very high. It was sedative in the dose of 1.0 mg/kg. 8-(OH)DPAT was the only drug studied that clearly lacked any activity in this model. Although 8-(OH)DPAT has a relatively strong activity on 5-HT_{1A} receptor but it has very inconsistent effect in anxiolytic tests, even its anxiolytic activity is debated.

As a conclusion, mCPP-induced anxiety model is a potent and useful test for screening anxiolytic activity of drugs with various modes of action.

REFERENCES

Charney, D.S., S.W. Woods, W.K. Goodman and G.R. Heninger: Serotonin function in anxiety II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. Psychopharmacology, g2 p. 14 (1987)

Fiorella, D., R.A. Rabin and J.C. Winter: The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of m-chlorophenylpiperazine. Psychopharmacology, 119, p. 222 (1995)

Murphy, D.L., K.P. Lesch, C.S. Aulakh, and T.A. Pigo, K: Serotonin-selective arylpiperazines with neuroendocrine, temperature and cardiovascular effects in humans. Pharmacol. Rev., 43, p. 527 (1991)

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Preliminary note

EFFECT OF N-CYCLOPROPYLMETHYL-NORAZIDODIHYDROISOMORPHINE (CAM) ON PARTNER-SEEKING BEHAVIOR AND COPULATION IN MALE RATS

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N-cyclopropylmethyl-norazidoisomorphine (CAM) is an azidomorphine derivative which exerts both opiate agonist and opiate antagonist properties (1). It was tested in two sorts of manifestation of male rat's sexuality: partner seeking and copulation. The watching of the partner-seeking behavior was performed in the following way (2): The males (Wistar strain, body weight 200-250 g) were placed individually in an ovoid-shaped box. After five minutes' adaptation period the females were put to the male one by one. When the male has displayed three mountings or one intromission, he received a new female. This procedure lasted for 30 minutes and was repeated at least on fourteen successive days on a group of fifteen males. After the completion of this period the males were tested for female-seeking behavior. The male was put on a table at 1,5 meter distance from the receptive female who was in the test box having a small gate. We recorded the time when the male entered the box of the female (seeking time) and the copulatory patterns, mounting, intromission until one ejaculation. (After the ejaculation the female-seeking could be elicited for several times.) Eight males proved to meet this criterion of female-seekers. Males which were left to copulate with receptive females for 30 minutes never displayed this female seeking behavior.

Before the administration of CAM to the males, they were tested for three weeks in order to check their partner seeking time (in seconds) and their copulatory activity. Each male served as his own control.

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10 μg/kg CAM subcutaneously one hour after the treatment has not altered the seeking time and copulatory activity of the males in comparison to the control tests. Seeking time was 5-30 seconds and all males displayed mounting, intromission and ejaculation.

In contrast to the 10 μg/lcg CAM, 50 μg/lcg completely inhibits the copulatory activity of the males but the seeking time remains on the control range (5-30 sec.)

200 μg/kg naloxone completely antagonized the inhibitory effect of 50 μg/kg CAM on the copulation. According to our experiments female seeking and copulation were sharply dissociated by CAM treatment. These data could be interpreted in the light of the dual concept of copulatory behavior (3). According to this theory males' sexual behavior consists of two different parts: desire and ability (3) (in our experiments female-seeking and copulation).

50 μg/kg CAM inhibits copulation but this dose is ineffective on the female-seeking behavior. Receptor systems which mediated this effect need further investigations.

REFERENCES

Fürst, S., Friedmann, T., Knoll, J.: Regulatory role of Opioid Peptides (Eds. P. Illes and C. Farsang 1988. pp. 358–377)

Dalló, J.: Activ. Nerv. Sup. 19, 239–240 (1977.)

Clark, T.J.: Methods in Neurosciences 14, 32–53 (1993.)

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Preliminary note

THE DISTRIBUTION OF NITRERG NEURONS IN THE BRAIN OF CARP AND THE EFFECT OF NOS SPECIFIC INHIBITORS ON THEIR NADPH DIAPHORASE ACTIVITY

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Up to now only a few investigations have dealt with the localization of NADPH-d/NOS containing neurons in fish (Holmqvist et al. 1994, Schober et al. 1993). These experiments were mostly performed with NADPH-d histochemistry although the specificity of NADPH-d as a histochemical marker for NOS activity has been compromised by the coexistence of other members of the dehydrogenase/electron transferase family of enzymes.

The present experiments have been undertaken to determine whether neurons and fibers that exhibit NADPH-d activity in the carp brain can also exhibit NOS immunoreactivity (NOS-IR), and whether NOS specific inhibitors like L-nitroarginine (LNNA) and arginase influence the NADPH-d histochemistry.

NADPH-d histochemistry was performed according to Scherer-Singler (1983). For the inhibition experiments L-NNA or arginase was applied before the histochemical reaction (Blottner et al. 1995). For immunocytochemistry a mouse monoclonal anti-bNOS primary antibody was used (Hai et al. 1995).

The combination of NADPH-d histochemistry and NOS immunocytochemistry revealed only a limited coincidence of NOS-IR and NADPH-d.

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Based on the inhibition experiments with L-NNA and arginase three populations of NADPH-d positive neurons were distinguished:

1./ NADPH-d staining was not altered

2/ NADPH-d staining was diminished to various extent

3/ NADPH-d staining was totally abolished after treatment.

The results of the present experiments indicate that bNOS immunoreactive neurons and NADPH-d neurons are not absolutely overlapping in the brain of carp, consequently the NADPH-d activity is not always a specific histochemical marker for NO containing neurons. Inhibition experiments with L-NNA and arginase support the existence of a subpopulation of NADPH-d positive neurons in the carp brain whose diaphorase activity is dependent on arginin.

REFERENCES

Holmqvist et al. (1994) Neurosci Lett 171, 205–208.
Schober et al. (1993) Neurosci Lett 151, 67–70.
Blottner et al. (1995) Histochemistry 103, 379–385.
Scherer-Singler et al. (1983) JNeurosci Methods 9, 229–234.
Hai et al. (1995) 2nd Int Congr Hung Biochem Soc Szeged, Hungary.

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Preliminary note

ALTERNATIVE MODELING STRATEGIES AND THEIR APPLICATIONS IN THE OLFACTORY SYSTEM AND THE HIPPOCAMPUS

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Bottom-up modeling relies on anatomical data, while top-down approaches use behavioral data first. By applying a bottom-up neural modeling strategy, the emphasis tends to be on single neurons, synaptic transmission and neural networks built from these elements. Multi-compartmental modeling in the spirit of Hodgkin and Huxley (1952) and population modeling techniques seem to be complementary. (For the technical frameworks of neurodynamic modeling see Szentágothai, Arbib, Érdi, in press).

Given a set of neurons, interconnected by excitatory and inhibitory synapses, theory can answer the question how the network is able to implement a given function. The general method by applying the dynamic approach is to build network models to describe the spatiotemporal activity patterns and the change of synaptic modifiability for explaining developmental, plastic and functional behaviors. The refinement of the anatomical tracing techniques combined with new physiological methods, such as dual intercellular recording, give the possibility to build and test very detailed single cell models in the spirit of Hodgkin and Huxley (1952), incorporating biophysical, physiological, and pharmacological properties of cells, the types and kinetics of channels, the connectivity patterns and synaptic distributions between cells, etc. (A masterpiece prepared in this spirit is the work of Traub and Miles 1991). For our own use of this technique to model the olfacory bulb, see Aradi and Érdi (in press). The results of this realistic modeling philosophy promise that even from this

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rather extremely reductionist approach some behavioral phenomena can be explained. Despite of its successes, alternative (such as lumped or statistical) strategies to this realistic-reductionist approach may also have relevance. The incorporation of results of single cell modeling into statistical population models may increase the efficiency of modeling.

The behavior of large networks of neurons may be studied by population theories. Just as collective phenomena emerging in physical systems made from large numbers of elementary components (spins, molecules, etc.) are treated by statistical mechanics, analogously, statistical dynamic theories of neural populations have been established. (A version of this theory is the kinetic theory (Ventriglia 1974, Ventriglia 1994.) Statistical theories work with distribution functions, and averaged quantities. The future of population theories depends on how knowledge about single neurons can be incorporated into their frameworks.

A step into this direction has been made by Grőbler and Barna (1996) who started from Ventriglia's kinetic theory. In this theory neural population activities are characterized in terms of the probability functions (p.d.f's) for (i) the neurons and (ii) spikes travelling between the neurons. The neural continuum has the following properties: The different excitatory and inhibitory neural populations have their own neural fields, and they can interact through the emission and absorption of spikes. The neurons are fixed in space while spikes can freely travel among them. The change of subthreshold potentials caused by absorbed spikes. Grőbler and Barna incorporated the dependence on the different ionic currents into the model, too. They defined a two-dimensional stat-space (both for neurons and spikes) which consists of a subthreshold membrane potential coordinate and an intracellular calcium concentration. Instead of having a mechanism based on channel kinetics, a dynamical threshold mechanism related to Na^+-kinetics has been given, (as a compromise between detailed description and the complete neglection of biophysical details) which determines the proportion of neurons that will fire. The p.d.f. of neurons gives the probability of neurons in a fixed point and at a given time in an infinitesimal membrane potential and calcium concentration interval. The p.d.f of spikes denotes the probable number of spikes in a given environment of a fixed point. Evolution equations for the p.d.f.s are basically diffusion equations which under the present conditions can be solved only by simulations. An elementary application of this model for the dynamics of the hippocampal CA3 region was mentioned in Grőbler and Barna (1996).

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REFERENCES

Aradi I and Érdi P: Signal generation and propagation in the olfactory bulb: multicompartmental modeling. Computers and Mathematics (in press).

Grőbler T and Barna G: A statistical model of the CA3 region of the hippocampus. In: Cybernetics and Systems' 96, Trappl R (ed.) (in press).

Hodgkin AL and Huxley AF: A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.) 117 (500–544), 1952.

Szentágothai J, Arbib MA, Érdi P: Neural Organization: Structure, Function, Dynamics. MIT Press, Cambridge, Mass. (in press).

Traub RD and Miles R: Neuronal Networks of the Hippocampus. Cambridge Univ. Press, 1991.

Ventriglia F: Kinetic theory of neural systems. I. Bull. Math. Biol. 36 (534–544),1974.

Ventriglia F: Toward a kinetic theory of cortical-like neural fields. In: Neural modeling and neural networks (pp. 217–249), Ventriglia F (ed.), Pergamon Press, Oxford, 1994.

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Preliminary note

NORMAL AND EPILEPTIC ACTIVITIES IN A POPULATION MODEL OF THE HIPPOCAMPAL CA3 REGION

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Modeling the function of large neural populations is becoming extremely difficult as more and more anatomical, biochemical, biophysical, and electrophysiological data are available about the behavior of individual neurons and their synaptic connections. Current mathematical models of neural networks either lack the biological details of these mechanisms or are unable to treat more than a few hundreds of neurons (Traub and Miles, 1991).

Both experiments and theoretical studies suggest the existence of a universal synchronization mechanism in the hippocampal CA3 region. Synaptic inhibition regulates the spread of firing of pyramidal neurons. Inhibition may be reduced by applying drugs to block (mostly) $GABA_A$ receptors.

Collective network properties *in vitro* have been studied successfully by Traub and Miles (1991): they found a few different epileptic phenomena such as synchronized bursts, synchronized multiple bursts and seizure-like events. At increasing levels of inhibition, different phenomena have been obtained, ranging even to normal, non-epileptic activity such as synchronized synaptic potentials (SSP). Synchronized bursts last 50-100 ms, and interburst intervals are generally longer than 1s. They are analogous to interictal events found *in vivo*, and can be elicited by applying a localized $GABA_A$-blocking agent (e.g., picrotoxin, bicucullin, penicillin). A burst consists of a series of spikes at intervals of 5-10 ms, terminating in a slow, Ca-spike.

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In our study, a statistical model, based on the kinetic population model by Ventriglia (1988), is given to describe the above phenomena of the hippocampal CA3 region. The population model incorporates basic electrophysiological properties of hippocampal pyramidal and inhibitory neurons. Population activities as well as underlying single cell voltages are simulated during normal and epileptiform activities in the CA3 region of the hippocampus.

The size, connection patterns, and other anatomical details of the model are taken from available data on rat hippocampus. Three basic neuron types are taken into account: (i) excitatory pyramidal cells, (ii) inhibitory cells initiating fast ($GABA_A$ mediated) and (iii) slow ($GABA_g$ mediated) IPSP's. The membrane potential distribution of a given neuron population changes according to the corresponding postsynaptic potential.

Electrophysiological properties of the cells are also incorporated, thus the model has been extended to describe characteristic ionic currents and bursting properties of hippocampal pyramidal cells (Grőbler and Barna 1996). Activities of typical individual cells receiving average input of a subpopulation can also be observed during the simulations.

Computer simulations were run to model synchronized population bursts in the CA3 region of the disinhibited hippocampal slice. It is demonstrated that our model can reproduce the electrophysiological phenomena characteristic to both single cell and population activity.

First, the response of individual pyramidal cells to injected currents, namely intrinsic burst discharges, were reproduced. Some characteristic features of the physiological responses to be reproduced were (i) summation of spike after depolarization due to the increase of intracellular Ca-concentration; (ii) Ca-spike; (iii) intrinsic burst followed by a long (1000 msec) after-hyperpolarization (AHP); (iv) the ability to prevent full burst generation by properly timed hyperpolarizing input.

Second, fully synchronized population bursts in the CA3 slice were reproduced by blocking the GABA synapses. The total suppression of inhibition lead to synchronized population activity in consequence of the excitatory interactions between pyramidal cells. Locally synchronized bursts spread over the slice more slowly than the axonal conduction velocity, as other comparative experiments and simulations with networks have also demonstrated (cf. Traub and Miles, 1991).

Third, at different levels of inhibition, a wide range of normal and epileptiform population activities were obtained, ranging from synchronized synaptic potentials to sustained multiple population bursts.

We can conclude that the statistical model presented here is capable of reproducing population activities of the CA3 region of the hippocampus while it is also possible to monitor typical single cell activity underlying population behaviour. The single cell model included in the population model is detailed enough to account for the intrinsic bursting property of pyramidal cells. The population model, however, allows for any other extension of the single cell model which might be necessary to simulate more complex phenomena.

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REFERENCES

Grőbler T, Barna G (1996): A statistical model of the CA3 region of the hippocampus. In: R. Trappl (ed.) Cybernetics and Systems Research '96, World Scientific (to appear).

Traub RD and Miles R (1991): Neuronal Networks of the Hippocampus, Cambridge University Press, New York.

Ventriglia F (1988): Computational simulation of activity of cortical-like neural systems. Bull. Math. Biol. 50 (143–185).

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Preliminary note

SITES OF SYNAPTIC JUNCTIONS ESTABLISHED BY A GABAERGIC BASKET CELL ON AN INTERNEURON IN THE CA1 AREA OF THE RAT HIPPOCAMPUS

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GABAergic cortical interneurons have been reported to receive GABAergic input from at least 3 different sources: i. extrinsic innervation from subcortical areas (Freund and Antal, 1988); ii. intrinsic innervation from neurons highly specialized to innervate other GABAergic cells (Acsády et al., 1995); iii. via self-innervation (Tamás et al., 1995).

In the course of studying the postsynaptic effect of identified GABAergic basket cells, we encountered a fourth type of connection. Dual intracellular recordings followed by biocytin filling in vitro revealed that a GABAergic cell which mainly innervates pyramidal cells also provides input to another interneuron in the pyramidal layer of the rat hippocampal CAl area. The presynaptic cell was identified as a basket cell, as 50 % of its boutons terminated on somata and the remaining 50 % on dendritic shafts of pyramidal cells. The identified postsynaptic cell was also a fast spiking interneuron, but its partially filled axonal arbor was not sufficient to define the cell. The physiological effect of the basket cell on the other interneuron was a short latency, fast rising IPSP resembling those mediated by GABAA receptors. This effect was mediated via 10 synaptic boutons. Five boutons established type 2 symmetrical synapses on the somatic membrane with 6 release sites. The remaining 5 boutons terminated on the proximal dendritic shafts of the identfied postsynaptic neuron. This termination pattern (55 % / 45 %) is in good agreement with the proportion of somatic and dendritic

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targets in the random sample on pyramidal cells. Boutons of the same axonal branches were found to innervate the interneuron and adjacent pyramidal cells.

Our results show that GABAergic innervation of interneurons also involves presynaptic neurons such as the basket cells, which mainly innervate principal cells.

REFERENCES

Acsády et al. (1995) Neuroscience, in press.
Freund and Antal (1988) Nature 336, 170-173
Tamás et al. (1995) J. Physiol. (Lond.) in press

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Preliminary note

SPONTANEOUS ACTIVITY OF VARIOUS POSTGANGLIONIC SYMPATHETIC NERVES IN ANAESTHETIZED CATS

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In anaesthetized animals, postganglionic sympathetic nerves are able to show a great variety of background activity patterns. Usually, the volleys are heart-rate-related. The discharges running in cardiac rhythm can display respiration-locked (in immobilized animals ventilation-locked) modulations. According to the degree of the latter, the activity can be continuous, continuous undulatory or intermittent, i.e., its continuity can be periodically broken by totally silent periods. Moreover, the volleys of mass discharges can be synchronized or desynchronized. Alternating synchronization/ desynchronization can occur in fixed phases of activity. The conditions defining the appearance of the individual patterns have not been identified so far.

As to the similarity or dissimilarity of spontaneous activities in sympathetic outputs of various destinations, similarity has long been thought to be the case (reviewed by Koizumi and Brooks, 1972), although contradicting reports (e.g. Ninomiya et al. 1973, Numao et al. 1987) have also been published. The issue still waits for further research.

In order to contribute to the latter, experiments were performed on cats anaesthetized with chloralose-urethane (33-50 and 133-200 mg/kg, respectively) and immobilized with pipecuronium bromide (60 μg/kg). Artificial ventilation was adjusted to maintain end-tidal CO_2 at about 4%. Monophasic compound achon potentials of renal and vertebral postganglionic efferent nerve branches (innervating vessels of a visceral

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organ and skeletal muscles, respectively), ECG, arterial blood pressure, ventilation, and capnogram were continuously recorded.

The results have shown that in the simultaneously recorded visceral and muscular sympathetic outputs similar as well as dissimilar background activity patterns can be observed. In the majority of cases, however, the mass discharges used to display partially or totally differential patterns. In our records, the following differential patterns were found: 1) intermittent synchronized activity of muscular output consisting of ventilation-locked groupings of pulse-synchronous volleys accompanied by a continuous visceral output of pulse-related volleys showing weak ventillatory modulation; 2) the opposite of 1), i.e. continuous muscular and intermittent visceral outflows, respectively; 3) ventilation-modulated intermittent activity in both outputs with synchronized pulse-related volleys in the muscular sympathetic outflow and desynchronized pulse-related volleys in the visceral one; 4) Ventilation-modulated desynchronized intermittent activity in both outputs and 5) continuous desynchronized activity in both outputs with weak ventilation-related modulation.

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REFERENCES

Koizumi, K., Brooks, C. McC.: The integration of autonomic system reactions: A discussion of autonomic reflexes, their control and their association with somatic reactions. Rev. Physiol. 67: 1-68 (1972).

Ninomiya, I., Irisawa, A., Nisimaru, N.: Nonuniformity of sympathetic nerve activity to the skin and kidney. Amer. J. Physiol. 224: 256–264 (1973).

Numao, Y., Koshiya, N., Gilbey, M.P., Spyer, K.M.: Central respiratory drive-related activity in sympathetic nerves of the rat: the regional differences. Neurosci. Lett. 81: 279–284 (1987).

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Preliminary note

ROLE OF LEU(34)-MET(35) IN NEUROTOXICITY CAUSED BY HUMAN β-AMYLOID (1-42) PEPTIDE IN VITRO

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β-Amyloid peptide (βAP), the principle component of cerebral amyloid in Alzheime's disease (AD), has been found toxic to neuronal cells in both *in vivo* (Kowall et al. 1992) and *in vitro* (Yankner et al. 1990) experiments. We recently showed that βAP 1 - 42 damaged the GABAergic and AChE-positive neurons in rat basal forebrain cultures (Kása et al. 1993). It has been suggested that the biologically active domain of βAP is a discrete 11-amino acid internal sequence corresponding to amino acids 25-35 (βAP 25-35) (Yankner et al. 1990), but little is known about the toxicity of shorter amyloid peptides. To address this question, we have investigated the bioactivity of full-length βAP 1-42 and some of its fragments (βAP 31-35, βAP 33-35 and βAP 34-39).

In the present study, dissociated cells from E16 rat basal forebrain were cultured for 4 days in vitro (DIV). The cultures were then grown in the presence of 20 μmol βAP 1-42, βAP 31-35, βAP 33-35 or βAP 34-39. Protein gene product 9.5 (PGP 9.5) and GABA immunohistochemistIy and AChE histochemistry were used to analyze the neurotoxicity of these amyloid peptides.

Addition of 20 μmol βAP 1-42, βAP 31-35 or βAP 34-39 to the culture medium for 1-4 DIV induced similar neuronal damage. On DIV 4+1, the neurotoxicity included varicosities particularly localized to the processes

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of small, bipolar neurons, as revealed by PGP 9. 5 immunohistochemistry. By DIV 4+3 and DIV 4+4, the neuronal cultures exhibited more serious signs of neuronal damage, including decreases in neurite length and cell number. GABA immunostaining and AChE enzyme histochemistry also revealed morphological indications of the degeneration process, i.e. pruning and fragmentation. The only amyloid peptide which did not alter the morphology of cells labelled by PGP 9.5, GABA or AChE was βAP 33-35.

It is interesting to note that Leu and Met at positions 34 and 35 of the amino acid sequence are common in both amyloid pentapeptides and the tripeptide we used. Since there is a correlation between βAP aggregation and neurotoxicity (Pike et al. 1991), the differences in the biological actions elicited by these two amyloid pentapeptides and the tripeptide may be attributed to their different aggregation states.

From these data, we concluded that Leu 34 and Met 35 may play a critical role in the biochemical mechanism of βAP neurotoxicity. Our results also suggest that Leu 34 and Met 35 may induce a neurotoxic effect in that form of amyloid peptide which contains at least five amino acids and which is long enough to be in a stable aggregation state.

REFERENCES

Kása, P., Pákáski, M. and Penke, B. (1993) Synthetic human β amyloid has selective vulnerable effects on different types of neurons. In: Alzheimer's Disease and Related Disorders. Communications. (Eds. Zatta, P.F. and Nicolini, M.) 311–312.

Kowall, N.W., McKee, A.C., Yankner, B.A. and Beal, M.F. (1992) In vivo neurotoxicity of beta-amyloid β(1-40) and the β(25-35) fragment. Neurobiol. Aging 13, 537–542.

Pike, C.J., Walencewicz, A.J., Glabe, C.G. and Cotman, C.W. (1991) In vitro aging of β amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 563, 311–314.

Yankner, B.A., Duffy, L.K. and Kirschner, D.A. (1990) Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. Science 250, 279–282.

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Preliminary note

CHARACTERIZATION OF THE HYPERPOLARIZATION ACTIVATED NONSPECIFIC CATION CURRENT (I_h) OF BUSHY NEURONES FROM THE RAT ANTEROVENTRAL COCHLEAR NUCLEUS STUDIED IN A THIN BRAIN SLICE PREPARATION

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The ventral cochlear nucleus (VCN) plays a central role in the processing of incoming auditory information. It contains several cell types which exhibit distinct morphology, membrane currents, firing patterns and function. One of these cell types is the globular bushy cell, which relays the auditory stimuli to the contralateral medial nucleus of the trapezoid body (MNTB) through a giant somatic synapse called the calyx of Held. This unusual morphological arrangement provided us with an opportunity to study currents at two different locations of the bushy neurone: on their soma and presynaptic terminals. Our data concern the hyperpolarization activated nonspecific cation current (I_h) at these two locations.

Two brain slice preparations were utilised from Lister hooded rats aged 5-14 days. Sagittal slices (200 μm thick) were used for bushy cell body recordings in the VCN or transverse slices (150 μm thick) were cut from the brain stem at the level of the 7th nerve in order to record from the synaptic terminals of the bushy neurones located in the MNTB. The whole-cell configuration of the patch-clamp technique was employed under visual control. Identification of the cells and terminals was achieved by Nomarski optics and fluorescent labelling by including Lucifer Yellow (0.5-2 mgml^{-1}) in the pipette solution.

The average whole-cell capacity and input resistance of the cell body were 32 ± 8 pF (n=39) and 212 ± 97 MΩ (n=24), respectively (mean±SD).

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On hyperpolarizing voltage steps both somas and presynaptic terminals produced a slowly activating inward current with no apparent inactivation. The activation time constants were determined by fitting the first 300 ms of the current traces by a single exponential function. τ_{act} at -140 mV was 70 ± 10 ms (n=17) and 82 ± 4 ms (n=3) in the cases of the somata and terminals, respectively. The activation time constant was voltage dependent; increasing as less negative test pulses were applied. This type of current could be well blocked by 1 mM CsCl at both locations (at -140 mV the blocking efficiency was more than 93%). Application of 1 mM $BaCl_2$, however, resulted in a much less effective block: 31 ± 8%; n=4 at -140 mV (this experiment was not performed on presynaptic terminals).

In both locations the reversal potential of the current was determined by extrapolation; resulting in values of -33 ± 2 mV (n=5) on the somas and -40 ± 6 mV (n=3) on the presynaptic terminals. The steady-state activation of the current was calculated by using a tail-current protocol. The half activation voltage appeared to be -94 ± t6 mV with a slope factor of 13 ± 2 mV (n=15) on the somas. Similar values were obtained from the presynaptic terminals.

In addition, in the cell bodies we have observed a strong correlation between the age of the animals and the current density of I_h. In animals older than 10 postnatal days the current densities were significantly higher than in those under 10 days, suggesting that I_h is developmentally regulated.

Our data indicate that bushy neurones of the VCN possess Ih with similar properties at both the cell body and their synaptic terminals in the MNTB. A similar current has been described in the principal neurones of the MNTB (Banks et al, 1993). We conclude that the expression of I_h in the auditory pathway may be a factor in the maturation of hearing during development.

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REFERENCE

Banks, M.I., Pearce, R.A., Smith, P.H.: Hyperpolarization-activated cation current (Ih) in neurons of the medial nucleus of the trapezoid body: Voltage clamp analysis and enhancement by norepinephrine and cAMP suggest a modulatory mechanism in the auditory brainstem. J. Neurophys. 70, 1420–1432, 1993.

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Preliminary note

TOXIC HEAVY METALS POTENTIATE LIGAND-GATED C1-CURRENTS IN GASTROPODA NEURONS

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Studying the effect of toxic heavy metals it has been shown, that they may cause both enhancement and depression of neuronal activity through modulating membrane conductance to different ions as well as the permeability of voltage and ligand gated channels (S.-Rózsa and Salánki, 1987, 1991, Arakawa et al., 1991).

Ligand activated chlorid channels play an important role in postsynaptic inhibitory processes both in vertebrates and invertebrates. Four main agonists have been described as specific transmitters at interneuronal synapses capable of activating Cl-channels: ACh, GABA, glutamate and glycin. In the present report we summarize our results obtained under the effect of mercury and lead on transmitter activated chloride channels of identified molluscan neurons.The results suggest, that potentiation of ligand gated Cl-currents by toxic heavy metals is a rather general phenomenon in various molluscan species.

Experiments were performed on *Aplysia, Helix* and *Lymnnea* neurons, on transmitter activated Cl-channels. Chlorid channels were activated in *Aplysia* by ACh and glutamate, in *Helix* by glutamate and in *Lymnaea* by GABA. Of toxic metals mercury and lead were used in 0.1-10 μM concentrations, added into the external solution. On *Aplysia* neurons conventional two microelectrode voltage clamp techniques, while on isolated and internally perfused *Helix* and *Lymnaea* neurons whole cell clamp technique was applied.

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In *Aplysia* medial neurons the ACh and carbachol activated Cl-current was increased by two-three times in the presence of mercury ions. Glutamate induced Cl-current was also potentiated by mercury in these neurons.

In *Helix* neurons glutamate evoked Cl-current was potentiated by lead ions, in case glutamate did not evoke maximal current, which is concentration dependent. Pretreatment with lead resulted increase of potentiation.

In *Lymnea* neurons GABA induced Cl-current was enhanced significantly in the presence of 0.01-1 μM $HgCl_2$ but higher than 10 μM mercury resulted in inhibition of the GABA evoked current. Mercury not only increased the amplitude of the current, but also the time to peak became shortened significantly.

Since the effective low metal concentrations in short application did not cause alone any current or only a very small one, the potentiation should be ascribed to modification of the receptor or channel properties occurring at the time of the agonist binding. Although there may be some differences between Cl-conductances gated by ACh, GABA and glutamate, it appears likely, that the mechanism of potentiation by heavy metals observed for different agonists is similar. Cl-channels activated by different agonists have a similar molecular structure, which differ only in the recognition site subunit. We suggest, that toxic heavy metals affect the activated Cl-channel at a subunit, different from the agonist recognition site.

The low heavy metal concentrations resulting in potentiation of ligand gated Cl-currents are close to concentrations occurring in the organism as a result of environmental pollution, consequently this phenomenon can be considered as an important factor in metal toxicity.

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REFERENCES

S.-Rózsa, K. and J. Salánki, (1987) Acta Biol. Hung. 38. 31–45.

S.-Rózsa, K. and J. Salánki, (1990) Environmental Monitoring and Assessment 14. 363–375.

Arakawa, O., M. Nakahiro and T. Narahashi, (1991) Brain Res. 551. 58-63.

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Preliminary note

RECEPTOR BINDING PROPERTIES OF A HEMORPHIN ANALOGUE IN RAT BRAIN MEMBRANE PREPARATIONS

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Partial enzymatic hydrolysis of proteins of nonneural origin may release peptide fragments with biological activity. Peptides acting on opioid receptors can be derived from β-casein (βcasomorphins), mitochondrial cytochrome b (cytochrophins) and hemoglobin (hemorphins). These nonclassical peptides called exorphins, differ structurally from the endogenous opiates since the N-terminal sequence Tyr-Gly is replaced by Tyr-Pro, moreover, two or three extra amino acids can be found on the N-terminus.

Previous investigations of bovine hemoglobin peptic hydrolysate (1.) revealed the existence of several opioid peptides including VV-hemorphin-7 (Val-Val-Tyr-Pro-Trp-Thr-Gln-Ar-Phe), which was found to be identical to fragments 31-40, and 32-41 of the β-chain of bovine and human hemoglobin, respectively. Opioid-like activities were proved using an electrically stimulated muscle contraction of isolated guinea-pig ileum (2).

We have determined the receptor binding profiles at the μ, δ and κ receptors of the peptide investigated (VV-hemorphin-7), using rat brain membrane preparations. The novel analogue bound preferentially to the μ opioid binding sites. In displacement experiments, the compound was found to exhibit moderate, still the highest affinity for the μ opioid binding sites measured by in vitro binding assays using tritiated DAMGO as a radioligand, while competition with the δ-specific [^{3}H]Ile^{5,6}-deltorphin II and [^{3}H]EKC

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with κ-preference showed lower affinities. So the peptide can be considered a relatively nonselective ligand showing highest affinity for the μ opioid binding site. Experiments are currently in progress on the CHO-μ_1, cell line representing, one of the μ opioid receptor subtypes.

Although exhibiting weak potencies to opioid binding sites, hemorphins have already been detected in extraordinarily high concentrations in blood plasma and pituitary gland (3). Therefore, their lower affinities could be compensated by their much higher concentrations *in vitro,* resulting in potential biological effects of comparable magnitude.

REFERENCES

Piot, J. M., Zhao, Q., Guillochon, D., Ricart, G. and Thomas, D. Isolation and characterisation of two opioid peptides from a bovine hemoglobin peptic hydrolysate. Biochem. Biophys. Res. Commun. 1992; 189 (1): 101–110.

Garreau, I., Zhao, Q., Pejoan, C., Cupo, A. and Piot, J. M. VV-hemorphin-7 and LVVhemorphin-7 released during *in vitro* peptic hemoglobin hydrolysis are morphinomimetic peptides. Neuropeptides. 1995; 28 (4): 243–250.

Glamsta, E. L., Marklund, A., Hellman, U., Wernstedt, C., Terenius, L. and Nyberg, F. Isolation and characterization of a hemoglobin-derived opioid peptide from the human pituitary gland. Regul. Pept. 1991; 34: 169–179.

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Preliminary note

SPINAL CORD NEURONS IN WHICH INTERACTION BETWEEN N-METHYL-D-ASPARTATE AND NEUROKININ-1 RECEPTORS MAY OCCUR

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N-methyl-D-aspartate (NMDA) and neurokinin-1 receptors (NK1-R) activated by aspartate/glutamate and substance P respectively, are reported to play a key role in the initiation and maintenance of hyperalgesia in the spinal cord. An interaction between NMDA and neurokinin receptors through the intracellular second messenger system has been suggested. This suggestion implies the co-expression of NMDA and neurokinin receptors on the same cell. The aim of this study was to show spinal neurons co-expressing NMDA and NK1-R.

Neurons expressing NK1-R were shown by immunohistochemical reactions using anti-NK1-R antibody (kindly provided by S. Vigna) in young rat spinal cord. The type and distribution of immunopositive perikarya were analysed and compared to those expressing NMDA, a-mamino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA) and kainate receptors shown previously (1).

In lamina I about 10% of the neurons were immunostained. The majority of immunopositive perikarya belonged to fusiform cells however, flattened cells were also present. In lamina II only 2-3% of the neurons showed immunopositivity. More than 80% of them were stalked cells while the others were 'cellules transverseux' of Cajal and large multipolar neurons. In laminae III and IV 5-6% of the neurons were stained. They belonged to the central cells and antenna cell type.

Comparison of these results to our previous findings suggests that the interaction between NMDA and NK1-R may occur in central and pyramidal

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cells of laminae III-IV. These results also indicate that there may be dorsal horn neurons which do not co-express NK1-R with any of the ionotropic excitatory amino acid receptors.

REFERENCE

Nagy et al. (1994) J. Comp. Neurol., 344:321–335.

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Preliminary note

THE ROLE OF SERUM AMYLOID P (SAP) COMPONENT IN ALZHEIMER'S DISEASE

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Serum amyloid P component (SAP) is a member of pentraxin family of plasma proteins (1). It has been isolated from postmortem brain tissue of patients with CNS dementias as Alzheimer's disease (2) and localised in different amyloid deposits (3).

SAP induces significant, time and concentration dependent cell death on the cells of the primary cultures of rat cerebral cortex. This effect develops slowly (8-10 hours) measuring by LDH leakage test, but the disintegration of the cell aggregates was observed after 4 hours by morphology (4). It suggests, that SAP, which has lectin like carbohydrate binding activity, can disconnect the cell-cell connections.

The effect of different glycosaminoglycans on the SAP-induced cell death was examined to test the role of its carbohydrate binding function. Significant inhibitory effect was detected and linear correlation was found between the measure of the inhibition (IC(50)) and the uronic acid content of the glycosaminoglycans.

The effect of SAP was also determined on primary glial culture of new-born rat brain. The measure of cell death was significantly lower on the glial cells than on the cells of the cortical culture, indicating the selective vulnerability of different cells.

These data suggest, that SAP may play an important role in the pathomechanism of Alzheimer's disease

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REFERENCES

Pepys, M. B., Dash, A. C., Markham, R. E. Thomas, H. C., Williams, B. D. and Petrie, A. (1978) Comparative clinical study of protein SAP (amyloid P component) and Creactive protein in serum. Clin. Exp. Immunol. 38. 119.

Coria, F., Castano, E., Prelli, F., Larrondo-Lillo, M., van Duinen, S., Shelanski, M. L., and Frangione, B. (1988) Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. Lab. Invest. 58. 454.

Kalaria, R. N. and Grahovac, I. (1990) Serum amyloid P immunoreactivity in hippocampal tangles, plaques and vessels: implications for the leakage across the blood-brain barrier in Alzheimer's disease. Brain. Res. 516. 349.

Urbányi Z., Lakics V. and Erdős. L. (1994) Serum amyloid P component-induced cell death in primary cultures of rat cerebral cortex. Eur. J. Pharmacol. 270. 375.

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ABSTRACTS

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QUANTITATIVE AND QUALITATIVE CHARACTERIZATION OF MICROGLIA IN THE RAT SPINAL CORD

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Qualitative and quantitative analysis of the resting microglia was done in the C6 segment of normal rat spinal cord. Using an immunohistochemical method, microglial cells were made visible by microglia-specific OX 42 antibody raised against CR3 complement receptor. Neurolucida Image-Analysis program was used for the morphological and numerical analysis. Microglial cells in the gray matter have round nuclei locating in the centre of the small soma. The profusely branching processes occupy a spherical space. In the inner half of this space, the density of the tiny secondary and tertiary branches is higher than in the periphery. There are no great differences in the length of the primary processes. The cells in the white matter have oval or rod-like nuclei and fewer processes. The longest processes are parallel with the fibres of the white matter depending on their orientation (radial or longitudinal). The number of OX 42 immunoreactive cells in the gray matter is twice higher than in the white matter. We revealed a mosaic-like territorial distribution of resting microglial cells, which provide an almost complete coverage of space in the gray matter. The distances of nearest nuclei are roughly equal. The mosaic in the gray substance appears as a pattern of approximately circular, closely placed polygonal areas. These areas are elongated in the white matter, and loosely spaced.

We suppose that the mosaic-like arrangement of microglial cells provides an optimum distribution of antigen receptors in need of immune defence.

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INVESTIGATIONS ON GLIAL REACTIONS TO LESIONS IN RAT FETUSES OF DIFFERENT AGES

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The reactive gliosis is supposed to be a limiting factor for the regeneration of the central nervous tissue. At the early stage of development, the central nervous system still has a fair regenerative capacity. The glial reaction to lesions has been demonstrated in newborn rats. Our previous studies showed that at E17 (17th embryonic day) telencephalic lesions do not inhibit the development of the corpus callosum and do not provoke reactive gliosis. In the present study our goal was to estimate the age when the brain tissue gains capacity of reactive gliosis.

At E17, E18, E19, E20 and E21, the embryos were exposed with the corresponding segment of uterus horn, and stab wounds were performed through the uterine wall into the developing cortex and then the embryos were repositioned into the abdominal cavity. Similar telencephalic lesions were performed on neonatal animals, too. From birth, animals were sacrificed on every postoperative day, and the brains were immersed into 4% buffered paraformaldehyde solution for three days. Series of vibratome sections were processed for immunohistochemistry against glial fibrillary acidic protein.

When neonatal or E21 rats were lesioned, a population of symmetrical and GFAP-immunopositive astrocytes appeared between the 2nd and 3rd postoperative days. After another day long processes developed toward the lesion site (asymmetrical astrocytes). At this age, no GFAP-immunopositive astrocytes were observed in the corresponding areas unlesioned. In the animals lesioned at E17 or 18, the lesion site was usually not recognizable at birth and no reactive gliosis developed. When the lesions were performed at E19, the results were usually similar to that observed after the E17 and E18. In some cases, large lesions were found in the diencephalon. Around these lesions, symmetrical and then asymmetrical astrocytes appeared during the 4th and 5th postoperative days, respectively. In the cortex, however, the lesion site was never recognizable. Lesions at E20 resulted frequently in recognizable damage of the subcortical structures, and in some cases, in the cortex. Reactive glia appeared on the 4th day.

In conclusion, we suggest that the responsivity of the astroglia to lesions developes during the 20 and 21 embryonic days, although the reactive gliosis cannot be manifest earlier than the first postnatal day. The growth of the developing brain repairs the small damages obtained before the critical age, whilst the large defects are fatal so the animals die *in utero* and there is no time for glial reaction. The phenomenon that the operations performed at E19 and 20 can result in visible damages and glial reactions after birth in the diencephalon without recognizable alterations in the overlaying cortex, can be explained by the retardation of the development of the cortex in comparison to that of the diencephalon.

This study was supported by an ETK grant of the Semmelweis University of Medicine.

DIFFERENT COUPLING OF SOMATODENDRITIC AND TERMINAL SEROTONIN AUTORECEPTORS TO THE $G_{I/O}$ PROTEINS IN RAT BRAIN

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Neuropharmacological and electrophysiological studies revealed that more types of serotonin (5-HT) receptors are involved in the regulation of serotonergic neural activity. Somatodendritic 5-HT receptors, located in the raphe area of midbrain, are of the 5-HT_{1A} subtype, whereas those that regulate neurotransmitter release at axon terminal levels, e.g. in the hippocampus, belong to the 5-HT_{1B} subtype. Recently we published our new in vitro release data about the role of various calcium and potassium channels in the regulation of somatodendritic serotonin release. (Ref.)

In this study the effect of N-ethylmaleimide (NEM), which inactivates $G_{i/o}$ proteins by alkylation, on the serotonin autoreceptor-mediated modulation of 5-HT release was studied. Slices from the raphe-area of midbrain and hippocampus of rats were prepared and preincubated for 30 minutes at 37°C in medium containing 30mmol/l NEM. After that slices were loaded with ^{3}H-5-HT, superfused continuously with Krebs-Ringer buffer and stimulated twice electrically. The effects of 5-HT_{1A} receptor agonist 8-OH-DPAT, which activates preferentially cell body 5-HT autoreceptors, and CGS 12066B, a selective 5-HT_{1B} agonist, were studied. Drugs to be tested were added to the medium from 15 minutes before second stimulation period. Fractional rate of tritium released during each fractions were calculated (S_2/S_1).

Our results indicate, that NEM pretreatment diminished the release-inhibiting effect of 8-OH-DPAT in the somatodendritic area of rat midbrain, while it did not modify the effect of CGS 12066B in terminal region. These results suggest that terminal 5-HT autoreceptors in the rat hippocampus may not be coupled to $G_{i/o}$ proteins, while somatodendritic ones are under the control of these proteins. This study provide further evidence that somatodendritic and terminal 5-HT autoreceptors also have different transducing mechanism.

REFERENCES

Bagdy, E. and Hársing, L.G.Jr: Neurochem.Res. 20,1409-1415, (1995).

HPLC ANALYSIS OF AMINO ACID NEUROTRANSMITTERS BY DIRECT INJECTION OF TISSUE PERFUSATE ON A 'BIO TRAP' COLUMN

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In principle, two different approaches can be used for the analysis of amino acid neurotransmitters in pmol/ml concentrations present in matrixes in tissue perfusate; (i) to enrich amino acids with the matrix or (ii) direct injection onto the column of the superfusate obtained in vitro or in vivo experiments. The most commonly used enrich procedure is the evaporation under reduced pressure. Every treatment of the sample prior to the chromatographic step decreases the "recovery".

The "bio trap" method is based on a direct injection of 1.0 ml volume of in situ precolumn derivatized 1-(alkylthio)-2-alkylisoindol amino acid adducts onto the column. This simple chromatographic guard column is able to enrich the samples and separate macromolecules from the low molecular weight components. "Bio trap" column can be used in the HPLC-system either manually coupled with a switching valve or in an automated system as an "extra valve".

The method developed by us was used for determination of gamma amino butyric acid (GABA) released from rat hippocampal slices in response to electric field stimulation. This direct injection method seems to be useful for determination of endogenous neurotransmitters released into superfusion fluid from in vitro brain slices.

EFFECT OF SCIATIC NERVE TRANSECTION ON THE LUMBAR SPINAL GANGLIA IN THE RAT

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Structural changes of the L_4-L_6 spinal ganglia were observed three to seven weeks following the complete transection and ligation of the sciatic nerve. The ganglia were embedded into Araldite and 1 mm thick plastic sections were stained with toluidine blue.

Both small (15-40 mm) and large size (35-65 mm) ganglion cells were stained, the former ones were distributed along the entire length of the ganglia, the latter type of cells clustered in the distal half of the ganglia. The transection of the sciatic nerve resulted partly in cytoplasmic changes and partly in loss of neurons. In a small proportion of the large neurons single or multiple vacuoles appeared in the cytoplasm. Neurons with vacuoles were most numerous 4 weeks following the surgery: at this time 3% of the large neurons were involved. Another type of the cytoplasmic changes was the appearance of densely stained granules which could be identified as large size mitochondria. Both kinds of cytoplasmic changes were seen exclusively at the side of the nerve transection.

Samples of cell counts (approx. 10% of the ganglion cells) showed a continuous reduction of ganglion cells during the observation period at the operated side.

THE ROLE OF α_2-ADRENOCEPTOR SUBTYPES IN LIPOPOLYSACCHARIDE-INDUCED FEVER RESPONSE IN RABBITS

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Exogenous pyrogens, e.g. bacterial lipopolysaccharides (LPS), are thought to stimulate macrophages to release endogenous pyrogens, e.g., TNFα, IL-1β, and IL-6, which act in the hypothalamus to produce fever. We studied the effect of different α_1- and α_2-adrenoceptor subtype antagonists, applied intraperitoneally, on the febrile response induced by LPS in rabbits. Evidence was obtained that prazosin, an α_1- and $\alpha_{2B/2C}$-adrenoceptor antagonist; WB-4101, an α_1- and α_{2A}-adrenoceptor antagonist; CH-38083, a highly selective α_2-adrenoceptor antagonist ($\alpha_1 : \alpha_2$ >2000); BRL-44408, an α_{2A}-adrenoceptor antagonist; and ARC-239, an $\alpha_{2B/2C}$- and also α_1-adrenoceptor antagonist, blocked the increase of colonic temperature of the rabbit produced by 2 mg/kg LPS administered intravenously without being able in themselves to affect colonic temperature. In addition, prazosin, WB-4101 and CH-38083 antagonized the fall in skin temperature that occurred at the time when the colonic temperature was rising in control animals injected with LPS. All these results suggest that norepinephrine, through stimulation of both α_1- and α_2 (α_{2A}- and $\alpha_{2B/2C}$-) adrenoceptor subtypes, is involved in producing fever in response to bacterial LPS.

THE EFFECTS OF STATIC MAGNETIC FIELDS ON RAT BEHAVIOR AND MEMORY

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Over the past 30 years, accumulating experimental findings suggest that living organisms are sensitive to magnetic fields. Experiments with animals show both behavioral and physiological responses to magnetic stimuli. With respect to potential health hazards, the effects of magnetic fields have gained increased importance in technical, medical and biological application. We, therefore, carried out several experiments to see whether long-term exposure (38 and 52 weeks, daily 4 hours) static magnetic fields (200 Gauss) can influence rat behavior and learning.

In the first experiment, we showed that static magnetic fields led to decline in 3rd generation rats of behavior and learning. In the second experiment with the exposure to chronic static magbetic fields we found that rat behavior at high environmental temperature (31°C) decreased. Also, there were significant differences between 3rd and the 4th generation rats in open field behavior pattern. On the other hand, static magnetic fields decreased the memory and learning of rats under operant conditions (delay-matching-to-sample).

In the present paper possible mechanisms of magnetic field effects on animal behavior are also discussed.

PHYSIOLOGICAL AND MORPHOLOGICAL DESCRIPTION OF PREMOTOR INTERNEURON-MOTONEURON PAIRS IN THE ISOLATED BRAIN STEM-SPINAL CORD OF FROG

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It is well established that neural circuits that generate the principal activity of spinal motoneurons are located in the spinal cord. In addition to the strong drive from the segmental motor apparatus, however, there is also abundant evidence for convergence of propriospinal input and descending commands from various brain stem nuclei on spinal motoneurons. In the experiments presented here we intended to identify propriospinal axon-motoneuron and reticulospinal axon-motoneuron pairs, and describe their physiological and morphological properties. Single axons in the ventromedial white matter and motoneurons in the lumbar or brachial spinal cord of frogs were impaled and recorded in an isolated brain stem-spinal cord preparation. Following physiological recordings the impaled axons and motoneurons were labelled with neurobiotin for a consecutive morphological analysis.

On the basis of their physiological and morphological properties the investigated axon-motoneuron pairs could be divided into three subgroups: (i) *Propriospinal neuron-lumbar motoneuron pairs*. The axons formed dense terminal arborizations at the level of the impaled motoneurons and established 14-24 close appositions with the medial dendrites of the labelled spinal motoneurons in close vicinity to the somata. The intraaxonal stimulation of the propriospinal axons evoked giant EPSPs in the motoneurons with a mean amplitude ranging form 1700 to 8000 *mV*. (ii) *Propriospinal neuronlumbar motoneuron pairs*. The propriospinal axons gave poorly arborizing collaterals to the spinal gray matter and the varicosities made 6-12 contacts with the impaled motoneurons. The contact areas were located on distant dendritic segments and were distributed over a wide area of the dendritic arbor. The stimulation of the axon evoked EPSPs with an amplitude of 150-400 mV recorded from motoneuron somata. (iii) *Reticulospinal neurons-brachial motoneuron pairs*. The cell bodies of the impaled reticulo-spinal neurons were located in the middle and inferior reticular nuclei of the medulla oblongata. The axons gave richly arborising collaterals at the level of the stained motoneurons and the varicosities of axon terminals established 6-7 close appositions with the dendrites of the labelled motoneurons. The contact areas were located both on proximal and distal dendrites and were distributed over a relatively wide area of the dendritic tree. Intraaxonal stimulation of the reticulospinal fibres evoked 160-300 mV EPSPs in the recorded motoneurons.

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NEUROCHEMICAL MARKERS FOR POLYAXONAL AMACRINE CELLS IN THE RABBIT RETINA

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Amacrine cells are the axonless interneurons of the vertebrate retina. Some amacrine cell types have wide dendritic fields, and in two recent studies (one with Golgi impregnation and the other with intracellular injection) four types of these wide-field amacrine cells have been found to bear axon-like (i.e. thin, cylindrical and often beaded) processes. In the present study we attempted to identify these cells by their neurochemical markers.

The following markers were investigated: (i) NADPH-diaphorase activity was visualised by enzyme-histochemical methods; immunocytochemistry was done to localise, (ii) 28 kDa calcium-binding protein (CaBP) and (iii) calretinin (CR)-immunoreactivities to specific populations of amacrine cells.

The results showed that NADPH-diaphorase positive cells can be divided into two classes. One is always weekly labelled, only the somata and the initial dendrites could be followed in the wholemount preparations. The other cell type was completely labelled. These latter cells are uniformly distributed in all parts of the retina. These cells have wide (up to 1 mm) dendritic fields and the dendrites of neighbouring cells overlap substantially. The beaded axon-like processes of these cells took their origin from the stem dendrites of the cells. These cells were found always in orthotopic position. This cell type has probably not been included in the polyaxonal amacrine cell catalogue of Famiglietti (1). From the calcium-binding proteins we investigated, CR was found to label ganglion cells and numerous narrow-field amacrines, so this protein was not present in any of the formerly identified axon-bearing amacrine cell type. CaBP, however, apart from being present in one type of horizontal and one type of bipolar celL was also present in a wide-field amacrine cell type. This amacrine cell was often displaced into the ganglion cell layer or occasionally into the inner plexiform layer and had processes extending as far as 1.5 rnm from the cell body. These long processes were smooth, straight and cylindrical. This cell type is probably equivalent with the PA2 amacrine cell described by Famiglietti (1).

None of the two above described polyaxonal amacrine cell type followed the usual pattern of neuron distribution of the rabbit retina (i.e. the cell density elevated only slightly towards the retinal centre in both cases, and did not forrn a sharp visual streak). The function(s) of these cells is(are) currently unknown, but their structural properties (and in the case of the NADPH-diaphorase cells also the neuroactive substance produced in them) make them suitable to modulate neural transmission over large areas in the inner plexiform layer of the retina.

This study was supported by an OTKA grant (F 16040).

References: (1) Famiglietti, E.V. (1992) J. Comp. Neurol. 316, 422–446.

LOCALIZATION AND FUNCTIONAL RESPONSES OF DOPAMINE CONTAINING NEURAL ELEMENTS AND DOPAMINE RECEPTORS IN RELATION TO LEARNING OF DOMESTIC CHICKS

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Dopamine is present in various avian brain structures including the nucl. tegmenti pedunculopontinus (substantia nigra) and the area ventralis tegmentalis of Tsai (ventral tegmental area). An important ascending dopamine pathway arises from the substantia nigra and projects on the lobus parolfactorius (LPO). The latter region has been implicated in the passive avoidance training of young domestic chicks.

To analyse the possible role of dopamine in memory formation, we first mapped the distribution of dopamine Dl and D2 receptor binding sites in the chick, using in vitro receptor autoradiography followed by computer-assisted densitometric analysis. High levels of Dl binding were found in the lobus parolfactorius and paleostriatum augmentatum (striatal regions), whereas the D2 binding was high in the former regions as well as in hippocampus. Following passive avoidance learning, significant bilateral elevation of Dl binding was detected in the LPO but not in other areas involved in memory formation (paleostriatum, IMHV, hippocampus). An increased D2 binding was found in the substantia nigra of trained chicks. Under similar training conditions, the concentration of dopamine metabolites, homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), as determined in tissue extracts of LPO by HPLC method, did not show significant alterations.

The results suggest that dopamine released from ascending nigral fibers terminating in LPO is instrumental in the passive avoidance response of domestic chicks. The principal mechanism of dopaminergic influence is likely to involve postsynaptic regulation of Dl receptors, rather than the alteration of presynaptic dopamine turnover.

CGRP-EXPRESSING INTERNEURONS, NITRIC OXIDE SYNTHASE AND THE α7 SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN THE PRIMATE PREFRONTAL CORTEX

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Areas 10 and 46r of the prefrontal cortex of adult Macaca mulatta monkeys contain a particular set of interneurons exerting calcitonin gene-related peptide (CGRP) immunoreactivity. CGRP-positive interneurons include wide arbor and small basket cells, double bouquet cells and fusiform neurons equipped with beaded dendrites and axons. Neither pyramidal nor chandelier cells express CGRP in the monkey prefrontal cortex. According to post-embedding studies, GABA is present in 50% of the CGRP-immunopositive cells, but only 35% of GABA-positive interneurons are CGRP immunoreactive. Immunocytochemical double staining proves that parvalbumin coexists with CGRP in wide arbour baskets and double bouquet cells. Nitric oxide synthase (NOS) is located in bipolar neurons throughout laminae 2-6 and in large multipolar nerve cells in lamina 5, as well as in fascicles of apical dendrites and in a fine varicous plexus in lamina 1. a-bungarotoxin-binding, co-localized or identical with the α7 subunit of the nicotinic acetylcholine receptor (nnAChR) is confined to small and wide-arbor basket cells, their axons proceeding parallel to pyramidal cell apical dendrites and constituting a fine meshwork throughout the cortex. The immunohistochemical patterns suggest structural interrelation between CGRP, NOS and nnAChR around apical dendrites of pyramidal cells. Though the role of CGRP in a unique population of local circuit neurons is unknown, it is assumed that CGRP-positive interneurons, together with nitric oxide released from NOS-positive structures and acetylcholine, binding to nnAChR-s, may contribute to the fine tuning of pyramidal cells.

REGENERATION OF THE CENTRAL NERVOUS SYSTEM IN OLIGOCHAETES. 1. 5-HT-, DA-, TH-IMMUNOREACTIVE ELEMENTS

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Recently, we have initiated a series of studies, dealing with the regeneration of the brain in earthworms *(L,umbricus terresfris* and *Eisenia fetida).* In the present study we followed the appearance of serotonin (5HT)- and tyrosin hydroxylase (TH)-immunoreactive neurons in the process of regeneration. Quantitative estimation (HPLC assay) of the 5HT and dopamine (DA) in the CNS was correlated to the results of immunostaining. In the first period of the regeneration after operation, a scar tissue can be seen at the site of the excised brain. On the 3rd postoperative day this tissue already contains 5HT-immunoreactive neurons, and by the 7th day TH-immunoreactive neurons and fibers also appear. During gangliogenesis, immunoreactive neurons are localised centrally in the loose scar tissue, whereas the fibers in the periphery of the preganglion. Later (between the 17th and 24th postoperative day) starts the migration of these neurons towards the periphery of the ganglion and they occupy their final position in the dorsomedial, dorsolateral and ventral parts. After the 20th postoperative day, a connective tissue capsule develops around the preganglion. We suppose that these neurons take their origin from the neuroblasts of the enteric plexus and/or within the ventral cord ganglia. In the course of the regenerative process, the number of 5HT-immunoreactive neurons increases in the pharyngeal plexus. Such change cannot be observed in the number of TH-immunoreactive nerve cells. HPLC assay of the 5HT and DA contents show a dynamic change. In the first period of regeneration the concentration of 5HT (10-17 day) and the DA (24-27 day) increase in the intact subesophageal ganglion. Later, their levels gradually decrease. In the newly formed brain, both the 5HT and DA content gradually increase *(Lumbricus 20.6; 29.5* pmol/sample, *Eisenia:* 33.8; 9.8 pmol/sample, respectively), but at the 56th postoperative day their concentration still differs from the control data, suggesting that the regeneration of the brain has not been finished for this time.

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REGENERATION OF THE CENTRAL NERVOUS SYSTEM IN OLIGOCHAETES. 3. PROCTOLIN-IMMUNOREACTIVE ELEMENTS

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The high degree of regenerative capability is a general feature of worms. This process concerns the body segments as well as their organs. Many experiments deal with the regeneration of the peripheral nerves. There are sporadic data on the regeneration of the central nervous system, that is the gangliogenesis, the origin of nerve cells as well as the migration of the neuroblasts. Following extirpation, the formation of the 8th ventral cord ganglion was studied in *Eisenia fetida,* by visualizing proctolin-immunoreactive elements. On the 3rd regeneration day, a migration of the proctolin-immunoreactive neurons starts from the surface of the intact ventral cord ganglia to the loose scar tissue. After the 7th postoperative day, these immunoreactive neurons are already present in the preganglion.

The process of the gangliogenesis is similar to the formation of the brain. It is supposed that the regeneration of the new ganglion is finished by the 72th postoperative day. During gangliogenesis, the number of proctolin-immunoreactive nerve cells do not change within the neighbouring intact ganglia nor in the enteric plexus.

Both the epithelial layer and the circular muscle layer of the body wall of the regenerating segments contain more proctolin-immunoreactive cells and fibres than the intact segments.

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KAINIC ACID EVOKES EPILEPTIFORM DISCHARGES IN THE CA3 AREA OF SLICE PREPARATIONS FROM X-RAY IRRADIATED RATS

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Newborn Wistar rats were X-ray irradiated. Six weeks later the CA3 area was tested for kainic acid sensitivity. Nissl and Timm stained sections were used to verify that radiation destroyed at least 85% of granule cells of the dentate gyrus.

The effect of kainic acid was examined in slice preparations from control and X-ray irradiated animals at the same age. Conventional extracellular recording techniques were used to assess responsiveness of CA3 pyramidal call layer with stimulation of the mossy fibers. Drog was applied in the bath for about 30 min. In slices both from normal md irradiated rats, perfusion of 500 nM kainic acid induced epileptiform discharges of he pyramidal cells. The evoked responses of the CA3 area was also facilitated. About 5 to lO min later, continuous application of the kainic acid depressed both the spontaneous and evoked responses in the CA3 area.

In conclusion: Loss of presynaptic (mossy fiber) kainic acid receptors does not prevent kainic acid induced epileptiform activity in the CA3 cell body layer. This suggests that matured kainic acid receptors at the postsynaptic site are sufficient to mediate the typical overexcitation of pyramidal cells. Therefore, immaturity of postsynaptic kainic acid receptors, instead of missing mossy fiber terminals, may explain the lack of kainic acid effect in the young (1-15 day old) rats.

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TRAJECTORY ANALYSIS IN FIRING GRANULE CELLS OF THE DENTATE GYRUS IN THE RAT HIPPOCAMPAL FORMATION

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Membrane potential of firing neurones continuously changes from the fall of an action potential to the rise of the next. This trace (trajectory) results from the interaction between after-potentials and subthreshold V-gated conductances and can indicate the complexity of contributing processes. Firing patterns evoked by current pulses in dentate granule cells in slice preparations were analysed. In the most common variant f-AHP decrease and spike duration increase were associated with declining slope of the post-AHP component of the trajectory. In some cells the trend turned to the opposite, the spike duration decreased and the f-AHP increased again until the waveform stabilised. In a rare variant seen in bursting cells, the trajectory went to the negative direction. The data suggest that interaction is more complex during the initial period of pulse induced repetitive firing while contribution of V-gated subthreshold conductances changes in a time-dependent way. Although trajectories are unexplored in many kinds of neurones, they seem to differ in pyramidal cells or interneurones from those seen in granule cells. Further details may help to complete functional properties of cell types and to correlate anatomical and functional classifications. Work continues also to find pathophysiological symptoms in trajectories.

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NUMBER AND DISTRIBUTION OF INTERNEURONS IN THE HIPPOCAMPUS OF THE RAT AND MONKEY

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NADPH-d histochemical staining can visualize relatively large subpopulation of the GABAergic interneurons in the hippocampus. Using this method and additional correlated immunohistological stainings we estimated the total number and distribution of the interneurons in the rat and monkey hippocampus.

From 50 mm thick serial sections of rat and monkey hippocampi every second (rat) and every third (monkey) sections were processed for NADPH-d histochemistry. All of these sections (43-48 from rats, 81 from monkey) were drawn with the aid of a Neurolucida system and all the NADPH-d positive neurons were plotted. To determine the ratio between the NADPH-d positive interneurons and the total number of GABAergic cells, we made colocalization with parvalabumin (PV) and calretinin (CR) which mark two large subpopulations of the local circuit neurons. In addition, cell counts were made at the same stereotaxic levels from sections stained for substance P (SP), PV, CR and NADPH-d.

It was found that in the rat the NADPH-d positive neurons form approximately 40% of the GABAergic interneurons whereas in the monkey, they make up only 20%. From these data and from our earlier data regarding the total number of principal neurons in rat and monkey (Seress, 1988), we could calculate the ratio of principal neurons / interneurons both in the dentate gyrus and in Ammon's horn.

In the rat dentate gyrus (600.000 granule cells) the ratio is 20:1 whereas in Ammon's horn (430.000 pyramid cells) the similar ratio is 10:1. In the monkey dentate gyrus (5 million granule cells) the ratio is 40:1 whereas in Ammon's horn (2.300.000 pyramid cells) the ratio is 13:1.

In conclusion, except for the dentate gyrus no significant difference was found between the ratio of GABAergic and principal cells in the rodent and primate hippocampus. The total number of interneurons appears to be significantly higher both in the rat and the monkey hippocampal formation as previously estimated.

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BLOOD-BRAIN BARRIER *IN VITRO:* PERMEABILITY STUDIES

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Cerebral endothelial cells regulate the entry of solutes, macromolecules and immune cells from the blood circulation to the brain tissue in the majority of vertebrates, providing a separate compartment for the central nervous system. Monolayers of endothelial cells derived from cerebral capillaries, cultured on inserts proved to be excellent objects for studying blood-brain barrier (BBB) permeability *in vitro*. Since astrocytes have been shown to be able of upregulating and maintaining certain BBB characteristics of cerebral endothelial cells, our studies were carried out on bovine brain capillary endothelial cells (BBCECs) cocultured with rat astrocytes.

Luminal application of histamine (10^{-5} and 10^{-4} M) resulted in no significant changes in the permeability of monolayers for [^{14}C]-sucrose or ^{3}H-inulin within 2 hours. Evans blue labelled bovine serum albumin flux through BBC ECs was, however, significantly ($p<0.05$) increased after the administration of histamine in both concentrations. Histamine increased the permeability of the BBB for albumin, but not for markers with smaller molecular weights, known as markers of paracellular route. Our *in vitro* data may suggest a selective induction of transcellular passage of albumin through the BBB. These observations are in accordance with *in vivo* data indicating increased pinocytotic activity after *intracarotid* injection of histamine.

One hour of treatment of BBCEC monolayers with either dibutyryl-cAMP or chlorophenylthio-cAMP decreased significantly ($p<0.05$) the flux of both sucrose and inulin. The transport for both tracers was significantly ($p<0.05$) lower in treated BBCEC monolayers grown above astrocytes, compared with that measured in monolayers without any astrocytic influence. Our data confirm the validity of previous findings obtained from *in vitro* culture systems and indicate that cAMP can rapidly reduce sucrose and inulin flux through the monolayers. Astrocytic influences can further strengthen the tightness of the intercellular junctions.

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IMMUNOCYTOCHEMICAL DOUBLE-LABELING STUDY OF LUTEINIZING HORMONE-RELEASING HORMONE AND GALANIN-CONTAINING NEURONAL ELEMENTS IN THE HUMAN HYPOTHALAMUS

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Luteinizing hormone-releasing hormone (LHRH) synthesized in the diencephalon plays a pivotal role in the regulation of gonadal activity. Recently, galanin (GAL) has been shown to be co-produced in vast majority of LHRH neurons and co-secreted from their nerve terminals in the median eminence of the female rat brain.

In order to elucidate whether the co-synthesis of these two neuropeptides also occurs in the human, the diencephalon from both sexes was processed for simultaneous LHRH and GAL detection. In immunocytochemical double-labeling experiments, fluorescein isothiocyanate (FITC) and Texas Red were selected to mark GAL and LHRH immunoreactive (IR) sites, respectively, using 30 μm thick, free-floating, frozen sections of four human hypothalami. For evaluation of GAL and LHRH immunoreactivities in axon terminals, consecutive pairs of semithin sections were collected from the infundibular regions and immunostained by means of post-embedding techniques.

GAL-IR neurons populated several hypothalamic nuclei. The most intensely labeled perikarya appeared in the infundibular nucleus, whereas the supraoptic and paraventricular nuclei displayed a moderate intensity of staining. A more diffuse distribution of GAL-ergic neurons characterized the preoptic area. Contrasting this image, the number of LHRH-IR neurons was lower and the neurons were concentrated in the preoptico-septal area and in the ventral part of infundibular nucleus. While most of GAL-IR neurons were multipolar, the LHRH-containing neurons also possessed bipolar and fusiform shapes. Application of the double-labeling immunofluorescent staining revealed simultaneously LHRH- and GAL-IR neuronal elements in the same diencephalic sections. One hundred LHRH-IR neurons from each diencephalon were tested for co-expression of GAL. The green and red fluorochromes distinctly labeled GAL and LHRH neuronal networks. None of the examined LHRH-positive neurons appeared to display GAL immunoreactivity in their cytoplasm. The lack of coexistence was also experienced in the hypophysiotrophic LHRH axon terminals contacting portal blood vessels in the median eminence.

These results seem to indicate that either GAL is not co-synthesized in LHRH neurons of the human brain or the amount is beyond the level of immunocytochemical detectability in the postmortem tissue.

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IN VITRO DEVELOPMENTAL STUDY ON THE SEIZURE-INHIBITORY EFFECT OF AN AMPAANTAGONIST

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In our previous in vitro experiments we have investigated the effects of glutamate antagonists on the excitatory postsynaptic potentials evoked by electrical stimulation in slices prepared from rats of different ages. Comparing the sensitivity of the young and adult animals in standard perfusion solution significant differences could be detected in the case of NMDA antagonist APV but not in the case of the AMPA antagonist.

The goal of the present study was to analyse the effect of an AMPA antagonist on the epileptic activity developed in Mg^{++}-free perfusion solution. The inhibitory effect of GYKI 53784 on the spontaneous and electrically evoked discharges was investigated using extracellular recording technique. We could not detect significant variance in the latency of the appearance of the spontaneous epileptic discharges, while the patterns of them were very different. Burst-like activity characterised the slices from young (1-4 weeks old) animals, and the antagonist was more effective in these cases. However significant differences could not be seen affecting the evoked responses of the same slices.

SELECTIVE INHIBITION OF ENHANCED SYMPATHETIC TONE IN URETHRA OF ANAESTHETIZED CAT

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The urethral smooth muscle tone is induced by the activity of its sympathetic innervation. An altered level of the latter is supposed to contribute to the symptoms of abnormal prostate growth. The use of selective alfa1-adrenoceptor antagonists offer an opportunity for pharmacological treatment of benign prostatic hypertrophy.

In cats anaesthetized with pentobarbital-Na, 35 mg/kg, i.p. or a mixture of α-chloralose (50 mg/kg) and urethane (400 mg/kg, i.p.), urethral constriction was elicited, 1) by i.v. infusion of phenylephrine, 2) by i.v. administration of adrenaline, 3) by administration of the ganglionic stimulant dimethyl-4-phenyl-piperazinium (DMPP) and 4) by a somatosympathetic reflex upon supramaximal electrical stimulation of the sciatic nerve. Intraurethral pressure, contractions of the nictitating membrane and blood pressure were measured and recorded with a Hellige or a Grass polygraph.

The selective alpha1-adrenoceptor antagonist alfuzosin in a dose of 50 μg/kg, i.v., did not influence the pressor reactions to adrenaline (5 μg/kg, i.v.), DMPP (40 μg/kg, i.v.) and sciatic nerve stimulation, while it antagonized the effects on the urethral pressure and on the nictitating membrane. In our experiments, the ED50 value of alfuzosin was 9.5 and 26.8 μg/kg, i.v. for urethral pressure and blood pressure, respectively.

Concerning the phenylephrine-induced contractions, our results are in good agreement with those of Lefevre-Borg et al. (Brit. J. Pharmacol. 109: 1282-1289, 1993). In the present experiments, however, it has been shown that alfuzosin antagonizes the effects of the sympathetic drive, elicited in various ways, to the lower urinary tract and to the nictitating membrane without affecting the effects on the systemic blood pressure.

DISTRIBUTION AND RELATIONSHIP OF LUTEINIZING HORMONE-RELEASING HORMONE AND NEUROPEPTIDE-Y-IMMUNOREACTIVE SYSTEMS IN THE HUMAN DIENCEPHALON

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Neuropeptide-Y (NPY) potentiates the effect of luteinizing horrnone-releasing hormone (LHRH) on luteinizing hormone secretion in several species including human. In addition to the pituitary sites, the interaction of NPY and LHRH systems may involve diencephalic loci. However, the morphological basis of this putative communication has not yet been elucidated in the human brain.

In order to reveal the putative interaction sites, the topology of LHRH and NPYimmunoreactive (IR) neuronal elements was mapped and their interrelationship was analyzed in the human diencephalon by means of immunocytochemical methods. For tracing LHRH-IR elements, antibodies generated both against LHRH and gonadotropin-releasing hormone-associated peptide (GAP) were utilized. NPY containing perikarya and fibers were widely distributed in the diencephalon with high densities in the preoptico-septal, periventricular and infundibular regions. Certain nuclei were infiltrated with dense networks of varicose NPY-IR fibers and axon terminals. The LHRH-positive perikarya were located mainly in the preoptico-septal, diagonal band of Broca, lamina terminalis, medial preoptic and infundibular areas of the brain. A few LHRH-immunopositive neurons and fibers were scattered in the mamillary region.

Overlapping between the NPY and LHRH systems was apparent in the medial preoptic and infundibular regions. Double-label immunohistochemistry revealed brown, LHRH-IR cells receiving black, silver intensified NPY-positive axon terminals. The putative innervation of LHRH neurons by NPY-IR fibers was also confirmed in 1 μm thick plastic sections by demonstrating juxtapositions. The present findings suggest a synaptic regulation of hypophysiotropic LHRH-synthesizing neurons by the NPY system and propose the existence of a hypothalamic integrative mechanism influencing gonadal action in humans.

LEUCOKININ- AND TACHYKININ-RELATED NEUROPEPIIDES IN THE MOLLUSCAN NERVOUS SYSTEM

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Distribution of leucokinin (LK)-, locustatachykinin (LomTK)- and substance-P (SP)-like immunoreactive (LI) neurons was compared in the central nervous system of *Lymnaea stagnalis* and *Helix pomatia*. Localization of LomTKLI neurons in the ganglia of the freshwater bivalve, *Anodonta cygnea* was also investigated. In both the *Helix* and *Lymnaea* CNS the immunoreactive neurons are concentrated in the cerebral and pedal ganglia, but the number of labelled neurons is higher in *Helix* by about an order than in *Lymnaea* (LKLI: 650-750 vs. 85-100; LomTKLI: 800-1000 vs. 150-190; 2100-2500 vs. 100 [Schot et al., 1982]). Both central neuropils and peripheral nerves are richly innervated by LKLI, LomTKLI and SPLI processes. LomTKLI neurons are mainly present in the neuropil of the *Anodonta* ganglia, the number of immunoreactive perikarya is low. In the periphery, LomTKLI and LKLI neurons show similar pattern of distribution in the *Helix* and *Lymnaea* intestine; both intrinsic and extrinsic immunoreactive elements participate in the innervation. Analyzing the membrane effects of LomTK, LK and anodontatachykinin (AncTK) on *Helix* nerve cells, both depolarizing and hyperpolarizing effects can be observed. Voltage-clamp experiments show the role of Ca- or K-currents in the effect of AncTK. Our results suggest that the different leucokinin- and tachykiniergic systems investigated are involved in different regulatory processes at central peripheral levels of the molluscan nervous system.

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REFERENCES

Schot et al., Cell Tissue Res. 216:273, 1982.

SODIUM AND ATP-DEPENDENT ACTION OF CORTICOSTERONE IN THE STRIATUM OF RATS

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Striata of rats were dissected, homogenised and purified with special case to avoid degradation of proteins or peptides. Under this condition the presence of sodium ion increased the B_{max} of labelled opiate antagonist, ^{3}H-naloxone, which effect could be further augmented by a preincubation of the membrane with ATP and isobutylmethylxanthin. ATPγS was ineffective when employed instead of ATP Environmental stress increased the ^{3}H-naloxone binding in the striatum as compared to group of rats submitted to A 4 days' handling. Corticosterone both in vitro and in vivo alters the binding characteristic of the opiate antagonist. The results may serve for explanation of the changes in emotional behaviour induced by stressful situations.

BEHAVIORAL DYSFUNCTIONS AND SELECTIVE DECREASE IN BRAIN MINERALOCORTICOID RECEPTORS AFTER PERINATAL ETHANOL EXPOSURE IN RATS

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The pathophysiological action of ethanol to modify adaptive behavioral responses to stress and the function of hypothalamo-pituitary-adrenal axis has been well documented. The long-term effects of prenatal ethanol exposure on brain corticosterone receptors and on adaptive and cognitive types of behavior were studied in adult male Wistar rats. Pregnant female rats were exposed to ethanol in drinking water (16% v/v) from the 11th gestational day up to the day of weaning (day 21 postpartum). Behavior of male offspring were tested at adult age of 5 months which was followed by biochemical measurements of corticosterone (CORT) receptors in the brain.

Characteristics of ^{3}H-corticosterone binding were determined in cytosol preparation of different brain areas, such as hippocampus, hypothalamus and corpus striatum, either by saturation analysis or by end-point measurement. Glucocorticoid (GR) and mineralocorticoid (MR) receptors were separately measured using RU 28362, a specific GR agonist. Non-aggressive intermale social behavior, discrimination and spatial learning performances were tested.

The results show, that the prenatal alcohol exposure resulted in a permanent decrease of corticosterone binding capacity in the hippocampus and hypothalamus. This decrement was due to a selective reduction of MR number in these structures, while the GR number remained unaltered. Striatal receptor sites were not altered, since MR capacity is very low in this area, and GR was unaffected by the treatment. A considerable impairments of adult adaptive behaviors were also observed among treated animals, a suppression of social activity and a substantial disturbance in discrimination and spatial learning. We concluded, that chronic ethanol treatment at prenatal age can cause longlasting selective alteration in brain MR sites, and these changes may contribute to the reduced behavioral activation and impaired memory functions.

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INTRACELLULAR Ca^{2+} MONITORING IN ISOLATED COCHLEAR OUTER HAIR CELLS

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Electromotility of cochlear outer hair cells (OHCs) is the candidate mechanism for sharp frequency selectivity and high sensitivity of hearing. These cannot be explained by the Békésy's travelling wave theory. According to the current view motor activities of OHCs modulate the stimulus input to the real sensory receptor cells: inner hair cells. Two types of shape changes of OHCs have been described *in vitro*. The slow, sustained shortening develops within minutes and is mediated by actin-myosin interaction presumably. The fast electromotile response provides a cycle-by-cycle energy feedback to the vibrating of the cochlear partition at the characteristic frequency. Both motile performances are probably dependent on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the present study we confirmed the effect of caffeine on $[Ca^{2+}]_i$ of OHCs using the Ca^{2+}-sensitive dye fura-2.

The isolated OHCs were incubated in perilymph-like solution with 6 μM fura-2/AM for 30 minutes. Caffeine was applied by pressure ejection through a micropipette placed close to the surface of the cell.

The caffeine increased $[Ca^{2+}]_i$ even in a Ca^{2+}-free medium, because caffeine released Ca^{2+} from intracellular stores. The increase was much more pronounced using higher concentration of caffeine (lOmM). Indirect visualisation of OHC motility was achieved by monitoring $[Ca^{2+}]$i. Under osmotically controlled circumstances the effect of caffeine (applied by pressure) was a visible OHC contraction, which was reversed by arresting the stimulation. Considering the time course of development (5-6s) and the extent of a maximal shortening, our investigations may reveal a special form of motility.

EFFECTS OF DERAMCICLANE ON THE CENTRAL AND PERIPHERAL SEROTONINERGIC SYSTEM

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Deramciclane, a new putative anxyolytic agent has been developed by EGIS Pharmaceuticals Ltd. The receptor binding studies has revealed that the compound has a relatively high affinity to central 5-HT_{1A} and 5-HT_3 receptors. On the basis of these findings we started to investigate extensively the effects of deramciclane on the central and the periferal serotoninergic systems in *in vivo* studies.

Deramciclane effectively antagonized the DOI induced head twitch reactions and reversed the hypomotility evoked by mCPP. The compound did not antagonise the tail flick reactions provoked by 8-OHDPAT. The 5-HTP induced reflex bradycardia (5-HT_3 effect) was not affected by the molecule. These results are with good accordance with the receptor binding studies.

In experiments investigating the action of deramciclane on the peripheral serotoninergic system we observed lower effectivity compared to central studies. Deramciclane antagonised the 5-HTP induced rat paw oedema and inreased capillary permeabilty only in relatively high doses. The difference between central and peripheral effectivity of deramciclane suggests that the psychotropic action of deramciclane may be void of untoward peripheral side effects.

NEUROPEPTIDES IN THE HUMAN CIRCUMVENTRICULAR ORGANS

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The circumventricular organs (CVOs) - the subfornical organ (SFO), vascular organ of the lamina terminalis (OVLT), subcommissural organ (SCO) and the area postrema (AP) are specialized structures within the central nervous system (CNS). In these regions, with exception of the subcommissural organ, the blood-brain barrier is absent, due to the fenestration in the capillary endothelium, and they may serve as open gates for circulating substances into the CNS. Intense neuronal networks and extrinsic connections have been demonstrated in CVO's of various species, but much less in the human. Current views on the significance of CVOs in human CNS are ambivalent. Indeed, the vertebrate CVOs have undergone profound changes during phylogenesis. Several of them show a structural regression, but some others (as demonstrated by serial histological sections) like SFO and the AP are well developed, exhibit fine structural organization.

The distribution of the neuropeptides and amino acids has been investigated in experimental animals. Extensive neuronal connections and fine networks of peptidergic fibers have been demonstrated in the SFO, AP and OVLT in rats, but there are only few data about their existence in human CVOs.

In the present study neuropeptide containing elements of the human CVOs (SFO, SCO, AP) have been investigated with avidin-biotin peroxidase immunohistochemical method in paraffin embedded and frozen sections. A number of neuropeptides (NPY, substance P, galanin, vasoactive intestinal polypeptide, methionin and leucine enkephalin, vasopressin, calcitonin-gene related peptide) were investigated in brains with short (up to 2 hours) *post mortem* delay. Since without blockage of the axonal flow (like colchicine treatment in experimental conditions), neural cells are immunostained quite rarely, the existence of peptid-ergic nerve fibers and terminals can be demonstrated in human brains. A fairly high number of various peptidergic fibers were found in the AP, especially substance P, NPY, angiotensin II and galanin. Scattered enkephalin-positive axons were also seen to penetrate into the AP. The subfornical organ, which contains several thousand cells in human, exhibited NPY-, substance P- and somatostatin-immunoreactive fibers. In the SCO, which mainly represented by small islands of specialized ependymal cells, few if any peptidergic fibers were observed only after NPY and substance P-immunostainings.

The presence of the variety of peptidergic fibers in the AP and the SFO in human indicates their intense neuronal connections with other brain structures. For the better knowledge of their functional significance, this study should be completed by further electron microscopic and *in situ* hybridization examinations.

THE MONOCLONAL ANTI-VIMENTIN ANTIBODIES VIM 3B4 AND V9 IN RAT AND CHICKEN BRAIN: A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY IN YOUNG AND ADULT ANIMALS

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We raised the question whether different vimentin isoforms exist in the brain of rat and chicken. Two monoclonal antibodies VIM 3B4 and V9 previously shown to be species-specific in cell lines were used in this study.

Immunoperoxidase technique was applied to study the cerebella in two age-groups of domestic chicken and rat.

By using VIM 3B4 we found an intense, specific staining of the Bergmann-glia, astrocytes and perivascular glia both in the young and adult chicken and in the 7-day-old rat, but not in adult rats where only the perivascular glia were immuno-positive.

The expression of the epitope recognised by the monoclonal antibody V9 was more restricted. The Bergmann-glia showed an intense immunostaining in the rat, but a weak staining appeared in chicken, independently of the age. Astrocytes showed an intense staining in the rat but were V9-negative in chicken in both age-groups. The perivascular glia were V9-positive in the adult only - both in chicken and rat.

We conclude that the expression of vimentin isoforms detected by the monoclonal antibodies VIM 3B4 and V9 shows not only a species-specificity but also differences according to the ontogeny.

EFFECTS OF AMPA ANTAGONISTS ON SPONTANEOUS ALTERNATION AND MOTILITY IN ISCHEMIZED GERBILS

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Temporary bilateral carotid occlusion (BCO) in the gerbil is a widely used model of global ischemia. This interventin usually causes severe tissue damage in the CA1 area of the hippocampus [1]. There has also been observed an increase in locomotor activity after the surgery which showed moderate correlation with the degree of CA1 neuronal cell loss [2]. The competitive AMPA antagonist NBQX has been shown to prevent hippocampal tissue damage as well as hyperactivity [2]. Since the hippocampus plays the substantial role in the spatial memory we examined the effect of BCO on memory functions in the gerbil too. For this purpose we measured the so called spontaneous alternation (SA) behavior of ischemized gerbils in a Y-maze. The aim of our study was to investigate the effect of NBQX and the non-competitive AMPA antagonist GYKI-52466 on the hippocampal tissue damage as well as on the behavioural changes induced by BCO.

Male mongolian gerbils (60-70 g) were subjected to bilateral common carotid artery occlusion for 5 minutes under ether anesthesia. Ischemized gerbils received either vehicle or NBQX 3×30 mg/kg ip. 30-60-90 min after reperfusion or GYKI-52466 4×15 mg/kg ip. 30-45-60-75 min after surgery. Four days after the operation gerbils were tested in a Y-maze for 5 min. Total arm entries and percentage of spontaneous alternations were registered as measures of hyperactivity and memory function, respectively. After the behavioural testing animals were anaesthetized and perfused through the heart with a fixative solution. Alternate coronal sections of brains were stained by silver impregnation and rated for neuronal damage.

Five minutes' occlusion caused CA1 cell death in the hippocampus, a decrease in SA, and an increase in total arm entries. NBQX and GYKI-52466 increased the proportion of SA responses parallel with with their neuroprotective action on CA1 one neurons. However, hyperactivity was decreased only by NBQX. We conclude that the SA response can be used as a behavioural correlate of hippocampal memory function and that both types of AMPA antagonist can protect CA1 neurons against ischemic damages.

1. Kirino, T. Brain Res. 239; 57-69, 1982.
2. Judge, ME et al. Neuroscience Letters 133; 291-294, 1991.

LONG-TERM DEPRESSION IN AREA CA3 OF RAT HIPPOCAMPUS

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There is a well accepted hypothesis in the field of neurobiology that at the cellular level, memories are stored as long-term alterations in the strength of synaptic transmission. These alterations are used as a model of the synaptic plasticity. One mechanism that increases synaptic strength is called long-term potentiation (LTP). There is an opposite phenomenon too, use dependent decreases of the synaptic strength which can persist for hours, namely long-term depression (LTD). Recently, there is a new form of LTD described, which is present only during a critical period of the postnatal development. The goal of our study was to analyse the electrophysiological mechanisms of this type of LTD.

Excitatory postsynaptic currents (EPSCs) were recorded in CA3 pyramidal cells of hippocampal slices of 7 to 12- day old rats at room temperature (21-23°C) using the whole-cell configuration of the patch clamp technique. The EPSCs were evoked by extracellular stimulation of the mossy fibres. A pipette (3-4 MΩ) filled with physiological extracellular solution was used for stimulation. Pulses of 50-100 ms were delivered by a stimulus isolator. All experiments were performed in the presence of NMDA receptor antagonist CPP (20 μM). A high- frequency stimulation train to the mossy-fibres induced a stable and long-lasting decrease in amplitude of the EPSCs. The maximal depression was monitored immediately after the train then the amplitude slowly increased in 10 minutes until reached a stable level that was maintained for rest of the experiment. Experiments were performed measuring paired-pulse facilitation too, which has a clear presynaptic site of action. The facilitation of the second response comparing to the conditioning one was not affected suggesting that LTD expression occurs at site different from that involved in paired-pulse facilitation. This type of LTD therefore it seems likely to be a postsynaptic phenomenon. To test whether changes in the concentration of the free intracellular calcium can play a role during the induction of LTD, experiments were performed using patch pipettes contained the Ca-chelator (BAPTA). In neurons in which Ca^{2+} was chelated by BAPTA the high-frequency stimulation train failed to induce LTD, suggesting that induction of this type of LTD is mainly postsynaptic.

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STRUCTURAL BASIS OF A DUAL EFFECT OF ACETYLCHOLINE ON HIPPOCAMPAL INHIBITORY NEURONS

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Type 2 muscarinic receptor (m2) is expressed in non-pyramidal cells of the hippocampal formation. (Levey et al., J.Neurosci., 15:4077-4092, 1995). Cell bodies immunoreactive for m2 have horizontal dendrites, and are located at the stratum oriens/alveus border of the CA1 region, whereas in CA3 the positive neurons are multipolar. Axon terminals immunopositive for m2 are abundant in stratum pyramidale, they surround somata of pyramidal cells in a basket-like manner. Our aim was to identify the types according to calcium binding protein and neuropeptide content - and GABA immunoreactivity of m2-positive neurons, and the source of m2-positive axon terminals. GABA was shown to be present in all m2-immunoreactive interneurons, but coexistence of m2 with calcium binding proteins parvalbumin, calbindin and neuropeptides VIP, CCK or somatostatin was rare throughout the hippocampal formation. Only the calcium binding protein, calretinin, showed colocalization with m2 in 15% of the cells. Using retrograde transport of PHAL-gold conjugate some of the m2-positive cells were found to project to the medial septum. At the electron microscopic level all m2-positive axon terminals were shown to be immunoreactive for GABA, and to establish synaptic contacts with axon initial segments and somata of pyramidal cells. Pre-embedding immunogold staining for parvalbumin combined with pre-embedding immunoperoxidase staining (using DAB as chromogen) for m2 revealed that m2-positive axon terminals in stratum pyramidale contain this neurochemical marker. These results suggest that there is a differential axonal and soma-dendritic localization of m2 receptor on different GABAergic interneuron types.

ADRENERGIC INNERVATION OF GALANIN-CONTAINING NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF THE RAT

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Galanin (GAL), a 29 amino acid peptide, is widely distributed in the central nervous system (CNS). It plays a pivotal role in the regulation of neuroendocrine processes, in control of body weight and may operate as a neuromodulator in synaptic transmission. The hypophysiotrophic areas of the diencephalon are densely populated in the rat by GAL-immunoreactive (IR) structures. The paraventricular nucleus (PVN) of the rat hypothalamus controlling the operation of the anterior and posterior lobes of the pituitary gland and integrating autonomic functions displays a high GAL content. At the cellular level, both parvi- and magnocellular neurons are IR for GAL. The PVN also receives significant phenylethanolamine-N-methyltransferase (PNMT)-IR input from the adrenergic C1-C3 cell groups of the brain stem.

In order to elucidate the putative interrelationship between the GAL-ergic and adrenergic systems in the PVN, immunocytochemical studies were conducted at the light and electron microscopic levels in colchicine-treated male rats. In the posterior magnocellular subnucleus (pm), the majority of the cells displayed GAL immunoreactivity, while the number of GAL-ergic perikarya situated in the periventricular (p) and medial parvicellular (mp) subnuclei was lower. The distribution of PNMT-IR varicose axons was very dense in the parvicellular subnuclei, especially in the mp subnucleus. In contrast, the pm subnucleus displayed only scattered adrenergic fibers. By means of immunocytochemical double labeling, overlapping of GAL-IR and PNMT-IR structures was demonstrated in the PVN. In the mp and p subnuclei, GAL-containing neurons were embedded in a rich adrenergic fiber-network. Axo-somatic and axo-dendritic juxtapositions were revealed both in thick (30μm) and semithin (1μm) sections. The GAL-synthesizing cells in the pm subnucleus, however, received only few adrenergic axons. At the ultrastructural level, PNMT-IR axons were found to establish synapses on GAL-IR cell bodies providing morphological evidence of neuronal communication between the two systems.

These data seem to indicate, that adrenergic fibers from the C1-C3 cell groups of the hind brain heavily innervate the GAL-IR neurons in the p and mp subnuclei of the PVN, while adrenergic innervation of magnocellular GAL-IR neurons is sparse. By using these anatomical connections, the adrenergic system may modulate the GAL-driven regulation of adenohypophysis and the control of body weight.

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BEHAVIORAL, BIOCHEMICAL AND HISTOCHEMICAL CHANGES AFTER CHRONIC ETHANOL ADMINISTRATION IN RAT

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It is a firmly established notion that ethanol modulates basic neuronal functions by acting on several different intracellular processes such as, for example, the release of neurotransmitters, the firing rates of nerve cells and local Ca^{2+}-buffering mechanisms which are involved in maintaining a physiologically adequate $[Ca^{2+}]_i$ balance. Since Ca^{2+} plays a key role in ethanol induced neurotoxic events, changes in the regulation of local Ca - buffering mechanisms, such as those of the fast, transient binding of Ca^{2+} to Ca^{2+}-binding proteins (CaBPs), may effectively influence ethanol mediated neurotoxicity. Furthermore, chronic ethanol administration directly influences the chemical properties of biological membranes *via* the generation of free radicals. In this respect, free oxigen radical scavenging enzymes may play protective roles against ethanol induced tissue damage. In the present study we therefore investigated the effects of chronic ethanol ingestion on adaptive animal behavior, activities of antioxidant enzymes as well as specific neuronal and glial CaBPs.

Chronic ethanol administration was carried out for 4, 7, 14 and 21 consecutive days. Rats were housed individually and the group of ethanol-treated animals was given a 7% ethanol containing standard liquid diet (chocolate flavor), while control animals were offered the same diet except that ethanol was substituted with an amount of sucrose equivalent to the calories available in ethanol. Chronic ethanol ingestion exerted a marked suppressant effect on the open-field behavior of rats which resulted in significantly decreased ambulation and rearing activities accompanied with an increased latency before starting exploration. Free oxigen radical scavenging enzyme activities, namely glutathione peroxidase, glutathione reductase and catalase, showed a permanent derangement of these antioxidant systems with greatest changes after 4 days of chronic ethanol intoxication as it was measured in blood samples. Furthermore, significantly increased levels of lipid-peroxidation processes were displayed by blood and plasma malonyl dialdehyde contents. Moreover, chronic ethanol administration induced persistent alterations of the intracellular Ca^{2+} homeostasis of both nerve and glial cells as these were displayed by the changes of the immunocytochemically detectable amounts of CaBPs, parvalbumin, calbindin-D28k and S-100, respectively.

Our present results provide further indications on the toxic effects of chronic ethanol ingestion that directly influence animal behavior and induce profound alterations of protective mechanisms both on the periphery and in the central nervous system.

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MODULATION OF LIPOPOLYSACCHARIDE-INDUCED TUMOR NECROSIS FACTOR-α AND NITRIC OXIDE PRODUCTION BY DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS IN MICE

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The effects of various agonists and antagonists of dopamine D_1 and D_2 receptors on lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) and nitric oxide (NO) production was investigated in mice. Pretreatment of animals with bromocryptine or quinpirole, agonists of dopamine D_2 receptors caused a blunting of both the TNF-α and NO responses to LPS injected intraperitoneally. Sulpiride, an antagonist of dopamine D_2 receptors, decreased LPS-induced TNF-α plasma levels in a dose-dependent manner and inhibited the LPS-induced NO production by peritoneal macrophages. Bromocryptine or quinpirole, agonists of dopamine D2 receptors, blunted both the TNFa and NO response to LPS. SCH-23390, an antagonist of dopamine D_1 receptors did not alter the LPS-induced TNF-α production, but inhibited the LPS-induced NO production. These results indicate that while the D_2 subtype of dopamine receptors is involved in the modulation of both the LPS-induced TNF-α and NO production, dopamine D_1 receptors only regulate the production of NO. Since several drugs possess effect on dopamine D_2 receptors, the present observations may be of clinical relevance.

RESPONSES OF NEURONS TO TASTE STIMULATIONS IN THE PREFRONTAL CORTEX OF THE RAT

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The prefrontal cortex (PFC) is involved in various perceptual, motivational and learning mechanisms and it is considered to be essential in neural mechanisms controlling feeding behaviors. Lesions of the sulcal PFC result in decrease of body weight and food intake, while lesions of the medial PFC (mPFC) produce finickness and disturbance of conditioned taste aversion learning. Dissection of intrinsic corticocortical projections in the PFC also produce deficits in the association of taste and place cues. Despite the abundance of literature on feeding related PFC functions, the specific roles of its neurons in gustatory information processing are to be defined. To investigate gustatory responsiveness of PFC neurons, their extracellular single unit activity was recorded during intraoral taste stimulations.

Fourteen anesthetized CFY rats were used and single neuron activity in the mPFC was recorded by glass microelectrodes. Two concentrations of five basic tastes (sweet, salty, sour, bitter and umami, respectively) and a complex tastant (orange juice) were administered into the mouth through an intraorally oriented tube. Data were computerized, stored and analized off-line after a unit separation procedure. Taste responsive neurons were found in the mPFC in almost all rats examined. The propotion of gustatory neurons in the mPFC was 17%. Taste sensitive cells were predominantly narrowly tuned and responded only to one (78%) or two (22%) tastants. Activity changes were mainly firing rate suppressions (78%), and one-third of gustatory neurons showed habituation during repeated applications of the same stimulus.

Although the orbital frontal cortex is widely reported to be involved in gustatory functions in primates, our results, are among the first data indicating the role of mPFC in the central processing of taste information in the rat. Present results also suggest that the mPFC is an important cortical area in the higher level, motivational coding of gustatory information.

FETAL BRAIN TISSUE IMPLANT PROTECTS THE BRAIN FROM ISCHEMIC INJURY IN RAT

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Preventing acute ischemic injury and apoptosis of neural elements is the main task in stroke therapy. A novel experimental method is cell or tissue transplantation into the brain to prevent cell death. We have demonstrated the protective effect of fetal brain tissue implanted into the brain prior to unilateral permanent middle cerebral artery (MCA) occlusion in rat.

Solid telencephalic tissue harvested from embryonic rat neocortex has been implanted stereotaxically into the left lateral putamenal area of adult Long-Ewans rats (group 1, n=21). On the 7th postoperative day (24 hours prior sacrificing the animals) the ipsilateral MCA has been occluded. The control animals escaped the primary operation (group 2, n=6) or mechanical cortical injury has been performed (group 3, n=6). Brains have been sectioned in 4 standardized coronal plains (A,B,C and D, respectively).

We have demonstrated the integration of the graft using Nissl stain after histological processing (n=3). The vascularization of the graft has been sufficient and no extravasation of the infused horse radish peroxidase has occurred indicating an intact blood brain barrier (n=3). The extent of infarction has been detected after incubation of the brain slices with 2,3,5 triphenyl-tetrazolium-chloride and measured by computed planimetric method. The values in the control animals (group 2,3) have been 12.3%, 12.6% in plane A, 10.6%,10.3% in plane B, 11.0%, 10.8% in plane C and 9.9%, 10.7% in plane D, respectively, whereas the values in the transplanted animals (group 1) have been significantly lower: 5.6% in plane A, 7% in plane B, 5.5% in plane C and 1.3% in plane D, respectively.

Solid fetal telencephalic graft implanted in the lateral putamenal area has integrated to the host environment. The graft has significantly reduced the extent of the ischemic lesion in the hemisphere where the MCA has been occluded.

The various neurotrophic factors that have probably been released from fetal tissue and/or the graft-induced neural gene and protein expression could have remodelled the ischemic cascade in our experiments.

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KINDLING OF THE PERFORANT PATHWAY RESULTS IN EPILEPTIC BEHAVIOUR BUT NO SPROUTING OF THE MOSSY FIBERS IN THE RAT HIPPOCAMPUS

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Kindling - a commonly used model of the human temporal lobe epilepsy - has been used to investigate the causal relationship between electrophysiological and morphological alterations in temporal lobe epilepsy. Previously it has been described, that kindling of the perforant pathway results in an increased excitability of the dentate gyrus, and in an aberrant connectivity among granule cells due to sprouting of mossy fibers. However, it is not clear whether the increased excitability of the hippocampal neurons is caused by the excess excitatory connections, or sprouting is a consequence of hilar cell loss, that is caused by the kindled seizures. Moreover, the significance of granule cells in the kindling process is also unknown.

In order to answer these questions two groups of animals were used: 1. normal adult animals; and 2. adult rats that were irradiated at birth and through irradiation the number of granule cells were reduced by 70-85%. Both groups were kindled to reach 30 seizures of stage 5. The input-output characteristics of the hippocampi were recorded both at the beginning and the end of the kindling procedure.

By the end of the kindling (two months) the animals were sacrificed and stained for the Timm method. Neither the normal nor the irradiated animals showed the characteristic signs of mossy fiber sprouting despite of the changes in excitability of the hippocampus that was revealed by electrophysiological recordings. Furthermore the irradiated rats needed 50% more kindling sessions to reach 30 (stage 5) seizures.

The present results indicate, that kindling can be a useful model of the human temporal lobe epilepsy as far as the electrophysiological alterations and behavioural signs are concerned. On the other hand the morphological alteration of sprouting, described in the literature, may develop only after several months of epileptic activity, and is possibly the consequence of the hilar cell loss, and not the direct granule cell excitation.

The relative difficulty to kindle irradiated rats, compared to controls, indicates that granule cells are actively involved in the kindling process.

STUDIES ON AMPA RESPONSE INCREASING EFFECT OF DL-2-AMINO-5-PHOSPHONO PENTANOIC ACID (APV) BY CORTICAL WEDGE METHOD

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The effect of APV, a widely used competitive antagonist of NMDA type glutamate receptors, was studied on depolarising responses to AMPA in slices of cingulate cortex of rat. Experiments were carried out on the cortical wedge preparation (Harrison and Simmonds, 1985). In most of the experiments we used magnesium-free (Mg-free) artificial cerebrospinal fluid (ACSF). Depolarisation of neurones was evoked by 1 minute perfusion of AMPA and was recorded as change in d.c. potential between the compartments containing the white and grey matter. Two experimental protocols were used. In the first, after having stable responses to 40 μM AMPA, a dose-response curve was obtained on each slice in the presence or absence of test compound(s). In the second type of experiment effect of compound(s) was tested using only 40 μM AMPA. In both cases responses were evaluated as percent of the control response to 40 μM AMPA.

50 μM APV shifted the dose response curve leftward, yielding almost 40 % increase in response to 40 μM AMPA ($p<0.057$, unpaired t-test, $n=7$). Similar potentiating effect of APV was observed in the second type of experiment with 40 μM AMPA (41% increase, $p<0.05$; paired t-test, $n=12$). Potentiating effect of APV was dose-dependent. We tested dizocilpine, a non-competitive NMDA ion channel blocker, and 3-((RS)-2-Carboxypiperazin-4-yl)-propyl- 1phosphonic acid (CPP) a more potent competitive NMDA antagonist. They also possessed potentiating effect which, however, was less pronounced (29 and 10 % respectively) even at the relatively high concentration of 10 μM. In Mg-containing solution no potentiation could be observed. The potentiating effect of APV in Mg-free ACSF was blocked if the solution contained 0.5 μM tetrodotoxin (TTX) or 10 μM scopolamine or 10 μM atropine. These results suggest that increase in AMPA responses in the presence of APV is caused by activation of a neuronal circuitry that seems to involve cholinergic transmission. Perfusion of the non-selective cholinergic agonist carbachol neither modified responses to AMPA nor influenced potentiating effect of APV.

Our results indicate that AMPA receptor mediated responses, under certain circumstances, are influenced by the state of NMDA receptors and a cholinergic mechanism is involved Elucidation of the exact mechanisms of this interaction needs further investigation. In contrast with previous suggestions in the literature we found that in some cases depolarisation induced by excitatory amino acids in cortical wedges are different in TTX containing medium from those devoiding TTX.

Harrison, N.L. and M.A. Simmonds, 1985, Quantitative studies on some antagonists of N-methyl-D-aspartate in slices of rat cerebral cortex, Br.J.Pharmacol. 84, 381.

ROLE OF NEURONAL CELL ADHESION MOLECULE (PSA-NCAM) IN NEURO-GLIAL PLASTICITY

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Sex hormones are involved in the physiological regulation of several aspects of behavior and neuroendocrine events. It is generally admitted that such effects are mediated mainly by direct steroid actions on neurons but recent studies have revealed that astroglia are also affected by gonadal steroids in developing and adult animals. It was shown that growth and branching of astroglial cell processes is affected by gonadal hormones since in monolayer hypothalamic cultures treated with 17β-estradiol, GFAP-immunoreactive astrocytes with epithelioid shapes are progressively transformed to complex forms with two or more cell processes. This effect was dependent on a direct contact of astroglia with living hypothalamic neurons.

The present study shows that somata and processes of neurons in such cultures were immunoreactive for polysialic acid (PSA); astroglia were immunonegative. PSA appears to participate in the estradiol-induced shape changes since treatment with endoneuraminidase, an enzyme that specifically removes PSA from the cell surface, abolished PSA immunostaining and prevented the 17β-estradiol-induced morphological changes of astroglia. In contrast, treatment with endoneuraminidase did not affect astroglial shape changes induced by basic fibroblast growth factor (bFGF), nor those induced by the addition of neurons to glial cultures. These results suggest that PSA on neuronal membranes, probably linked to the highly sialylated isoform of the neural cell adhesion molecule, is necessary for the expression of certain hormonally-regulated neuro-glial interactions.

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DOUBLE- AND TRIPLE-LABEL DETECTION OF VARIOUS PROTEIN AND mRNA COMPOUNDS IN HYPOTHALAMIC NEURONS WITH COMBINED USE OF IMMUNOCYTOCHEMICAL (ICCH) AND IN SITU HYBRIDIZATION (ISH) TECHNIQUES

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Novel technical approaches are described, which enable simultaneous application of immunocytochemical (ICCH) and various in situ hybridization (ISH) techniques within the same tissue sections.

Tissue processing steps include perfusion-fixation of rats with 4% paraformaldehyde, infiltration of brains with sucrose, snap-freezing of tissue pieces, cryosectioning and longterm storage of floating sections in cryoprotectant solution. Prehybridization, hybridization and posthybridization conditions allow for both synthetic oligoDNA and in vitro transcribed cRNA probes to be used simultaneously for dual-label ISH. Moreover, ISH does not interfere with subsequent ICCH detection of antigens, thus multiple labeling using combined ISH-ICCH techniques can be performed. The following examples for double- and triple-labelings are presented:

1. Simultaneous use of antisense oligoDNA and cRNA probes for dual-label ISH: Proopiomelanocortin mRNA-containing neurons of the hypothalamic arcuate nucleus were detected with a digoxigenin-labeled cRNA probe, whereas, preprogalanin mRNA-expressing neurons of the same region were visualized by means of autoradiography following application of a ^{35}S-labeled oligoDNA probe.

2. Simultaneous application of ICCH and ISH methods for demonstration of neuronal contacts: Galanin-containing axons were visualized immunocytochemically by means of a silver-gold intensified diaminobenzidine chromogen, and thereafter, thyrotropin releasing hormone (TRH) mRNA was detected by non-isotopic ISH. Neuronal contacts established between galaninergic fibers and thyrotropin releasing hormone neurons were revealed.

3. Simultaneous use of dual-label ICCH and isotopic ISH methods for triple-labeling: In this technique, c-Fos protein was detected immunocytochemically by means of black nickel-DAB chromogen in the nuclei of luteinizing hormone-releasing hormone (LHRH) neurons, whose perikarya, in turn, were immunolabeled with the brown diaminobenzidine (DAB). Following dual-label ICCH staining, preprogalanin mRNA was visualized by use of isotopic ISH and autoradiography. Galanin expression was registered in 70-75% of the LHRH neurons expressing the c-Fos oncogene.

These data indicate that combined ISH-ICCH methods provide multiple information of high physiological impact regarding selected neuronal networks of the brain. (Supported by grant OTKA T016354).

SIGNIFICANCE OF THE SECOND MESSENGER MOLECULES IN THE REGULATION OF CEREBRAL ENDOTHELIAL CELL FUNCTIONS: AN OVERVIEW

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Several receptors for different vasoactive substances have been shown to be coupled to the microvascular adenylate cyclase and to increase the intraendothelial amount of cyclic AMP. As a consequence of elevated intraendothelial cyclic AMP, changes in permeability, in endothelial growth and differentiation, in nitric oxide and endothelin secretions and in a hyperpolarization-activated current carried by both Na^+ and K^+ ions have been reported. Elevations of cyclic AMP concentrations may be important in regulating the endothelial barrier in two-ways: (i) by narrowing the paracellular pathways for small molecular weight substances, and (ii) activating the transendothelial transport for macromolecules. Recently, significantly elevated cAMP levels have been found in microvessels from Alzheimer's disease compared to nondemented elderly controls. Guanylate cyclase, the synthesizing enzyme of cyclic GMP was found to be activated in brain microvessels by Ca^{2+}, but not influenced with calmodulin or carbamyl choline. The enzyme was seen to respond to atrial natriuretic peptide (ANP) in a dose-dependent manner. ANP specifically inhibits amiloride-sensitive sodium uptake into isolated cerebral capillaries in vitro and ameliorates brain oedema formation. Large increases in microvascular cGMP level was detected by Marsault and Frelin on the effect of exogenous nitric oxyde (NO) donor molecules (sin- 1 and sodium nitroprusside). The finding indicates that brain capillary endothelial cells, in contrast to aortic ones, may act as recipient cells for NO produced by neurons.

Changes in intraendothelial Ca^{2+} concentrations can be trasmitted to calcium/calmodulin-dependent protein kinase II and protein kinase C (PK C), or both Substance P can stimulate PK C translocation, phorbol esters increase hexose uptake, and fluid-phase endocytosis. The role of PK C in the activation of ICAM-1 expression on endothelial cells was also evidenced. In a recent study, several isoforms of PK C were seen in freshly purified microvessels except for presence of PK C-g isoform; primary cultures of endothelial cells expressed PK C-α,-β,-δ,-ε and -η isoenzymes, whereas the immortalized cell line expressed only PK C-α,-δ,-ε, and -η. The rat aortic endothelium contained PK C-α, and -δ isoforms, only.

Recently, Vigne *et al.* presented evidence for the operation of cross talk among cyclic AMP, cyclic GMP and Ca^{2+}-dependent intracellular signalling mechanisms.

EFFECT OF ISCHAEMIC CONDITIONS ON NEUROTRANSMITTER RELEASE FROM RAT HIPPOCAMPAL SLICES

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It has been shown that adenosine-triphosphate (ATP) is released by axonal activity from rat habenula and, during LTP, from hippocampal slices. It has been suggested that ATP is an excitatory neurotransmitter in rat central nervous system. It has been well established that the neurotoxic effect of ischaemic conditions is partly caused by the excitatory neurotransmitters which are released under these conditions from neurons. On the other hand, numerous data indicate that adenosine is also released from neurons or non-neuronal cells during ischaemia and functions as a neuroprotective modulator. We wished to know if ATP could release from hippocampal slices during ischaemic conditions and adenosine could originate from released ATP and/or it is released by its own right via adenosine uptake system. The ATP release was evoked by supramaximal electric field stimulation, using a microvolume perfusion system. The released ATP was measured by a luciferin-luciferase assay. ATP was released in response to electrical field stimulation. Hypoxia alone could release a considerable amount of ATP, while hypoglycemia or ischaemia had a weaker effect. Adenosine was released concomitantly and measured by HPLC. Our data suggest that ATP and adenosine could release from rat hippocampal slices during ischaemia.

LEARNING AND BEHAVIOURAL RESPONSES TO DOPAMINERGIC AGENTS IN DOMESTIC CHICKS

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Pecking response elicited by conspicuous visual stimuli in newly hatched chicks is an adaptive prerequisite for learning to discriminate between edible and noxious substances. Pecking at a bitter tasting bead results in lasting memory formation in telencephalic areas and a suppression of pecking at the same object. Such learning was associated with significant elevation of dopamine D1 receptor binding in the lobus parolfactorius (LPO). Following this finding, we tested (1) the involvement and specificity of telencephalic dopamine receptors in the learned suppression of pecking and (2) the possible involvement of -putative subtelencephalic- dopaminergic mechanisms underlying the unconditioned pecking response.

In experiment 1, specific D1 antagonist, SCH 23390, administered directly into the LPO prior to training, significantly attenuated learning, whereas the D2 antagonist, sulpilide, had no such effect. The D1 agonist, apomorphine, had no effect on the conditioned suppression of pecking despite its being a potent enhancer of unconditioned pecking.

In experiment 2, we studied the subtelencephalic mediation of the pecking response after complete telencephalic ablation. There was a ten-fold increase in the frequency of spontaneous pecking in decerebrated chicks, substantiating the existence of a tonic inhibitory input from telencephalon. The potent dopamine agonist, metamphetamine administered i.p., enhanced craniocervical stereotypies (pecking, biting) in both control and lesioned birds. However, while drug-elicited biting in non-operated birds was performed in vacuum (i.e. not targeted), decerebrated birds responded on the drug by visually targeted bites. Since dopaminergic agents act differentially on the subtelencephalic mediation of pecking response (enhance) and the telencephalic mediation of conditioned suppression of pecking (passive avoidance), we suggest the existence of a dopaminergic mechanism specific to the avoidance learning paradigm.

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF THE ESTROGENRECEPTIVE NEURONS AND THEIR AFFERENT CONNECTIONS IN THE RODENT BRAIN

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Neurons of the medial preoptic area (MPOA) are involved in regulating estrogen-influenced functions of the limbic system by relaying gonadal steroid messages. Genomic effects of estrogens are exerted via specific receptors (ER) which reside in different types of neurons in the MPOA. In order to determine the intracellular location of estrogen receptors, pre-embedding immunoelectronmicroscopical studies were carried out in the MPOA of female rats. The receptor loci visualized by silver-intensified diaminobenzidine (DAB) were mainly detected in the nuclei of immunoreactive (IR) neurons. Some expression occurred in the perinuclear cytoplasm, whereas the nucleoli were immunonegative.

For revealing the chemical identity of neuronal fibers impinging on this estrogen-sensitive neuronal network, an immunocytochemical double-labeling technique was used at light and electron microscopic levels. Either the ER and neurotransmitter/neuropeptide content of the estrogen-receptive neurons or ER and neurochemical composition of afferents to ER-immunoreactive (IR) neurons were detected by means of DAB and silver-intensified DAB chromogens. ER-immunoreactivity was found to coexist with galanin (GAL) and glutamic acid decarboxylase (GAD), respectively. The intensity of GAL-immunoreactivity and the number of GAL-IR cells increased, while GAD-immunoreactivity did not change in response to estrogen treatment.

Among the putative regulatory neuropeptides/neurotransmitters which might influence -via synaptic mechanisms- the functions of ER-producing neurons, neuropeptide-Y, adrenocorticotropin, galanin, substance P, adrenaline, serotonin and GABA were detected in axon varicosities juxtaposed to ER-IR neurons. In case of NPY, galanin, substance P and GAD-IR axons, the appositions proved to be synapses.

These findings contribute to our knowledge of neuronal networks involved in the regulation of estrogen-dependent functions of the brain by delineating the specific afferents and chemotypes of the ER-containing neurons in the preoptic area of the brain.

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STUDIES ON THE INTERACTION BETWEEN AMPA RECEPTOR MODULATOR ANIRACETAM AND GYKI 52466 IN RAT HIPPOCAMPAL SLICES

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Both positive and negative modulators of AMP-type glutamate receptors exist, and some data indicate a common site of action for these two types of drugs. In the present experiments the effects of the negative AMPA receptor modulator GYKI 52466, and the positive modulator aniracetam were studied on synaptic responses in the CA1 region of slices of rat hippocampus. Further, the interaction between these two compounds was investigated. Field potentials were evoked by stimulation of the Schaffer collateral-commissural pathways and recorded extracellularly from the pyramidal and radial layers simultaneously.

The depressant effect of GYKI 52466 (10-80 μM) both on the slope of population EPSPs and on the amplitude of population spikes (PS) was dose-dependent. Aniracetam had only weak and inconsistent potentiating effect at high concentrations (500-1000 μM), either in the presence or in the absence of the GYKI compound. In contrast with GYKI 52466, aniracetam preferentially acted on the long-latency components of the responses. In some cases it also affected the PS peak amplitudes and the early phase of EPSP.

When aniracetam was applied first, and dose-response curves for GYKI 52466 were determined (for inhibition of the early components) in the presence of 125-1000 μM of the positive modulator, we failed to detect any significant difference between these IC_{50} values and the one obtained without aniracetam. The lack of antagonism was also observed when a wide range of stimulation intensities was used.

Although some experimental data indicate an antagonism between aniracetam and GYKI 52466, in vivo, our data suggest that there is no significant interaction between the two types of AMPA modulators under our experimental conditions.

INGESTION-RELATED INCREASE OF EXTRACELLULAR GLUTAMATE IN THE GLOBUS PALLIDUS: 'ON-LINE' STUDIES WITH A NEW BIOSENSOR

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To monitor and/or control the composition of extracellular fluid (ECF) in the brain are important ways to obtain information on (and potentially influence) central regulatory functions. Various constituents of the ECF can be determined in two ways: 1) by implanting 'direct reading sensors' into the brain and 2) by methods sampling the ECF for *ex vivo* analysis (such as microdialysis). The latter is the most popular technique for the above purpose, its disadvantage, however, rests in the several minutes time resolution. 'Real-time' measuring of any component of the ECF in correlation with various, especially feeding-associated behavioral actions has for long been an 'unachievable' goal for neuroscientists. Here we report of the first results of our recent investigations on 'on-line' detection of ingestion-related changes of extracellular glutamate in the rat pallidum. Examinations were performed in the globus pallidus that has already been shown to be involved in the central control of food intake, and, more specifically, in motivational regulation of feeding-associated gustatory information processing as well. In these studies, a new, commercially available biosensor, a special 'dialysis electrode' has been employed to monitor and follow up alterations of extracellular glutamate levels in correlation with food and fluid intake actions. Attempts have also been made to elucidate possible relationship between consumption of pleasant or aversive fluids and changes of extracellular glutamate concentrations. Findings of these 'pilot-experiments' indicate specific involvement of glutamatergic mechanisms in central processing of feeding associated (in particular gustatory) information.

EFFECT OF DOPAMINERGIC LESION ON THE HOMEOSTATIC DRINKING

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The homeostatic behaviors (drinking, eating) have characteristic temporal pattern. The satiation which occurs at restoration of homeostatic deficit is followed by a temporal pattern of the drinking. Midbrain Central Pattern Generators (MCPG) are responsible for the shaping of temporal pattern and these generators are controlled by some limbic structures. The Nucleus Accumbens (NAC) is an important limbic structure in this control and its role occurs in different frequency and delays in the drinking pattern. The NAC plays important role in the initiation and stopping of consummatory actions.

In recent work we explored the functional relationship between NAC and MCPG and the effect of NAC on the temporal pattern of drinking. Recent results support our earlier hypotesis (Neurobiology 1995 3(1) p.73) that at least two central generators are involved in the shaping of drinking pattern (BOUT LICK gen., NON-BOUT LICK gen.) therefore the NAC exerts functional, presumably inhibitory influence on NON-BOUT LICK generator only.

SUBSTANCE P RECEPTOR-IMMUNOREACTIVE NEURONS IN THE RAT HIPPOCAMPUS

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The rat hippocampus consists of three fundamentally different types of non-principal neurons: Two of them terminate in different domains of the pyramidal cells, one in the perisomatic region and the other in the dendritic region. The third type is specialized to contact other interneurons. In this study, we aimed to identify the types of interneurons immunoreactive for Substance P Receptor (SPR), by examining the colocalization of SPR and various neurochemical markers known to label interneurons with established termination pattern. The results revealed that, at least to some extent, all three distinct subpopulations of hippocampal non-principal neurons express SPR. In many cases the colocalization of SPR and the given marker varied significantly across the subfields of the hippocampus. Nearly all cholecystokinin-containing perisomatic inhibitory cells contained SPR in all subregions, whereas parvalbumin-positive cells showed SPR immunoreactivity only in basket cells of the dentate gyrus. The somatostatin and neuropeptide Y content of large spiny SPR-positive cells in the dentate gyrus and CA3 identified them as dendritic inhibitory cells. The dendrites of these cells form a dense meshwork in the hilus and in str. lucidum of the CA3 region. In the CA1 region only very few somatostatin-positive cells colocalized SPR, however, many neuropeptide Y-immunoreactive cells contained the receptor. Another marker of dendritic inhibitory cells, the calcium binding protein, calbindin, was present only in large aspiny SPRpositive cells in CA3 str.oriens. Calretinin selectively labels the third inhibitory cell type, and 40% of these small, multipolar cells also colocalized SPR. However VIP-positive neurons with a similar target selectivity to calretinin-positive cells never contained SPR. All SPR-positive cells were immunoreactive for GABA, except some spiny somatostatin-containing neurons in the hilus, which probably project to the contralateral hippocampus.

Based upon the known termination pattern of the colocalized markers, we conclude that SPR-positive interneurons participate in various inhibitory processes, such as perisomatic inhibition (cholecystokinin-containing cells), feed-back dendritic inhibition (somatostatin/neuropeptide-

DISTRIBUTION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IMMUNOREACTIVE CELLS IN THE BRAIN STEM OF CAT AND RAT STUDIED BY IMMUNOHISTOCHEMISTRY

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Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a recently discovered member of the secretin family. PACAP was isolated from ovine hypothalami (Miyata et al., 1989). With the use of immunohistochemistry it has been demonstrated that PACAP is widely distributed in the central and peripheral nervous systems. The PACAP immunoreactive elements were observed in various areas of the forebrain (Köves et al., 1990), in the hindbrain regions such as the area postrema, nucleus of the solitary tract, dorsal motor nucleus of the vagus, parapyramidal area, ventrolateral medulla, nucleus ambiguus (Légrádi et al., 1994), and in primary sensory neurons (Moller et al., 1993). The PACAP immunoreactive perikarya were readily observed in intact cats, but in rats we had to use colchicin treatment for demonstrating cell bodies (Köves et al., 1994).

In the present experiment we have investigated the localization of PACAP immunoreactive elements in various areas of the brainstem of intact cats and colchicine treated rats. The rats were injected by colchicine intra-cerebro-ventricularly and were sacrificed two days after the injection. PACAP immunoreactive cell bodies were observed in the same areas of the hindbrain described previously and we have found further groups of cell bodies exhibiting PACAP immunoreactivity including motor nucleus of the hypoglossal nerve, the superior olivary complex, the nucleus of trapezoid body, the vestibular nuclei, the pontine gigantocellular reticular formation and in the motor nucleus of the trigeminal nerve. In the mesencephalon PACAP immunoreactive cells were found in the motor nucleus of the oculomotor and trochlear nerves, in the mesencephalic nucleus of trigeminal nerve, as well as in the substantia nigra and in the red nucleus. The most prominent PACAP immunoreactive fiber networks were observed in the interpeduncular area and in the ventral and lateral regions of the central gray matter of the mesencephalon.

ENTORHINAL CORTICAL FIBRES IN THE STRATUM LACUNOSUM MOLECULARE TERMINATE ON DENDRITES OF PARVALBUMIN CONTAINING NEURONS LOCALIZED IN CA1 AND CA3 AREAS OF THE RAT HIPPOCAMPUS

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A major hippocampal input, the perforant path to the CA1 region, is critical for sustaining physiological patterns, such as hippocampal theta and concurrent gamma activity. Additional physiological data suggest that entorhinal cortical efferents directly influence the activity of interneurons localized in the Ammon's Horn's pyramidal layer. To verify the morphological basis of this hypothesis, combined experiments of anterograde tracing and degeneration studies were performed. For studying the neuronal connections between entorhinal cortex axons and parvalbumin (PA) - immunoreactive dendrites in the stratum lacunosum moleculare (slm), 1) light microscopic double-immunostaining for PA and the anterograde tracer PHAL injected into the entorhinal cortex; 2) light- and electron-microscopic analysis of cleaved spectrin-immunostained hippocampal sections following entorhinal cortex lesion: and 3) an electron microscopic study of PA-immunostained hippocampal sections after entorhinal cortex lesion were performed.

Our morphological analysis demonstrates, that all of the PA-immunoreactive dendrites in the stratum lacunosum moleculare are synaptic targets of entorhinal cortical fibres, forming asymmetric synaptic contacts. These PA-immunopositive dendrites represent processes of GABAergic, inhibitory basket and chandelier cells.

The present findings support the hypothesis that entorhinal cortex activation results in inhibition of pyramidal cells, by way of direct excitation of chandelier and/or basket cells.

EFFECT OF A SEROTONIN AGONIST ON THE CGRP-INNERVATION OF THE CEREBRAL DURA AND ON THE C-FOS EXPRESSION IN THE CAUDAL TRIGEMINAL NUCLEUS IN AN EXPERIMENTAL MIGRAINE MODEL

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The supratentorial cerebral dura of the albino rat is equipped with an extremely rich sensory innervation by nociceptive axons displaying calcitonin gene-related peptide (CGRP) immunoreactivity. According to our earlier studies, electrical stimulation of the Gasserian ganglion which can be regarded as an experimental model of migraine headache, induces conspicuous (3-4 x) increase in the sizes of club-like perivascular nerve endings, followed by the rupture of these terminals. The aim of the present investigations was to reveal the immunohistochemical equivalents of the effects of the anti-migraine drug Sumatriptan (Imigran, Glaxo). We found that i.v. injection of Sumatriptan, prior to electrical stimulation of the Gasserian ganglion, prevents rupture of the perivascular terminals and induces accumulation of CGRP in preterminal portions of the axons, in consequence of which immunopositive varicosities of the preterminal portions increase in size and number. Since CGRP level of blood plasma increases during migraine attack, and since Sumatriptan exerts its anti-migraine effect by virtue of its 5-hydroxitriptamine agonist action, it is assumed that free serotonin receptors are needed to induce release of CGRP which, in turn, induces painful vasodilatation; this process is inhibited by the binding of Sumatriptan to 5-HT-1_D receptors. Electrical stimulation of the Gasserian ganglion induces immunohistochemical signs also in the caudal trigeminal nucleus, insofar as the number of nerve cells expressing *c-fos* protein increases; this, however, is not abolished by i.v. administration of Sumatriptan which does not pass the blood-brain barriere. Accordingly it appears that neurobiological effects visualized by immunohistochemical tools drugs in the future.

PARTIAL COHERENCE ANALYSIS OF HIPPOCAMPAL GAMMA OSCILLATIONS

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We examined extracellular generation and spatial distribution of gamma frequency activity in the hippocampus of the awake rat. Multiple site recording techniques with silicon probes (16-site arrays) combined with wire electrodes were used. The power at gamma frequency was greater during theta than during immobility and slow wave sleep. Partial coherence analysis revealed separate stratum lacunosum-moleculare (lac-mol) and pyramidal layer (30 - 60 Hz). Mathematical elimination of the components coherent with electrical activity in the hilus from signals recorded at different depths in CA1 did not effect the coherence between gamma and lac-mol. and str. oriens indicating that these oscillators were independent from the DG oscillator. Independence of the lac-mol and the pyramidal oscillators was suggested by marked separation of coherence between CA1 electrodes placed above or below the pyramid layer. Similarly, the coherence between oscillators in the hilus and the hippocampal fissure remained significant when allowance was made for gamma oscillations in CA1. The granule cell layer also separated coherent oscillations in the hilus and in the molecular layer. Following surgical removal of the entorhinal cortex (EC), the active generators of gamma activity were found in str. radiatum and pyramidale. The coherence between these oscillators could be eliminated by partialization on the signal recorded from CA3c region suggesting that the latter is transmitted from the CA3c region. We conclude that the intact brain layers II and III of EC are coupled with gamma oscillators of DG and CA1 regions, respectively.

These results have originally been presented at the Soc. for Neurosci. Annual Meeting in November, 1995.

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THE ROLE OF NITRIC OXIDE IN EFFECTS OF NATRIURETIC PEPTIDES ON LEARNING IN RATS

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The effects of pretreatment with nitro-arginine was investigated on the natriuretic peptides-induced memory consolidation in passive avoidance learning.

It has been shown in our Departments that L-arginine treatment facilitates the consolidation in one-way passive avoidance learning. Nitro-arginine prevents the effect of L-arginine. In previous experiments we have shown also that atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and the C-type natriuretic peptide (CNP) is able to facilitate the consolidation of passive avoidance learning.

Nitro-arginine treatment was carried out immediately after the learning trial and 30 min later the peptied was administered. All treatment were given intracerebroventricularly via the lateral brain ventricle in freely moving rats.

ANP, BNP and CNP facilitated the consolidation of passive avoidance learning, however nitro-arginine prevented the action of ANP, BNP and CNP.

The results suggest that in the facilitation of consolidation of passive avoidance learning and memory processes nitric oxide is one of the possible mediators.

EFFECT OF N-CYCLOPROPYLMETHYLNORAZIDODIHYDROISO-MORPHINE (CAM) ON THE COPULATORY ACTIVITY IN MALE RATS

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CAM is a semisynthetic morphine derivative possessing a κ-agonist, μ-antagonist property (Fürst, S., Friedmann, T. and Knoll, J.: in Regulatory Roles of Opioid Peptides, eds: Illés, P. and Farsang, C., 358-377, 1988.).

We considered sexually active male rats which displayed full scale of copulatory patterns (mounting, intromission and ejaculation) with receptive females at least twice out of four weekly mating tests (test duration: 30 min).

CAM in a dose of 50 μg/kg s.c. completely inhibited the copulatory activity of the males and in this respect CAM proved to be about hundred times as potent as morphine. The inhibition of copulatory activity still persisted even with 10 μg/kg CAM s.c. which was indicated by an increase of intromission and ejaculation latencies.

Naloxone by itself in a dose of 1 mg/kg s.c. failed to inhibit copulation but 0.5 mg/kg s.c. completely antagonized the inhibitory effect of 50 μg/kg CAM.

Furthermore (-)deprenyl, an irreversible, selective inhibitor of MAO-B enzyme (Knoll, J.: Acta Neurol. Scand. Suppl. 95, 57, 1983), given in a dose of 0.25 mg/kg s.c. either 30 minutes or one week before the administration of CAM, could partially reverse the inhibitory action of 50 μg/kg CAM. Other drugs influencing the dopaminergic systems (e.g. apomorphine, amphetamine) were unable to antagonize the copulatory inhibitory effect of CAM.

Further experiments are necessary to decide which types of opiate receptors are involved in the copulatory inhibitory action of CAM and what kind of interaction exists between (-)deprenyl and CAM leading to the observed effect.

ASTROGLIAL REACTIONS TO INJURY IN VIVO AND IN VITRO

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As a consequence of mechanical, chemical or infectious injuries of brain tissues, astroglial cells display characteristic functional changes. Increases in the mitotic and locomotory activities as well as in phagocytotic capacity, the redistribution of intermediate filament proteins and production of cytokines are the signs of reactivation of glial cells.

Astroglial cells can produce scar tissue around the traumatized region which can fulfill some of the protective functions of the blood brain barrier. Such elementary protective reactions, however, may cause serious alterations in the microenvironment preventing functional neuronal regeneration. Moreover, they can induce widespread inflammatory processes resulting in severe pathophysiological effects.

In vivo, while astrocytic reactions can be followed by immunocytochemical and histochemical methods, the molecular and cell biological responses in reactive gliosis cannot be directly studied. Brain cell cultures provide appropriate tools for detailed biochemical and cell biological studies.

In the present study, astroglial responses to mechanical injuries were investigated both within the brain and in cultures of embryonic and postnatal rat brain cells.

Among the events of "reactive gliosis" the changes in viability, the TNFa-production and the alterations in the distribution of intermediate filament proteins have been assayed.

CHOLINERGIC MODULATION OF THE EVOKED RESPONSES OF THE RAT BARREL CORTEX

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Several experimental and clinical evidence testify an important role to the cholinergic innervation of the cortex in maintaining normal cognitive functions. It is widely accepted that acethylcholine (Ach) has in most cases a facilitatory effect on neurones in the CNS. ACh either applicated experimentally or released from terminals of stimulated neurones in nucleus basalis magnocellularis (NBM), in sensory cortical areas generally increases the amplitude of responses evoked by sensory stimuli, without inducing any change in background activity. The aim of the present study was to examine the effects of muscarinic agonists and antagonists on the somatosensory responses. The posteriomedial barrel subfield of the primary somatosensory cortex of rodents contains unique and easily identifiable segregation of neurones, called barrels in layer IV, which correspond to the individual whiskers situated on the contralateral face. 6-8 big whiskers -cut to a length of 10-11 mm- were moved forward or backward using a multiangular electromechanical stimulator. Stimulus waveforms were ramp-and-hold trapezoids. Extracellular recording and pressure microinjection techniques was used to study the effects of drugs on evoked responses. Peristimulus time histograms (PSTHs) were produced then diagrams were created. Acetylcholine, acetyl-L-carnitine, carbachol and scopolamine in 10-3 - 10-4 M concentrations induced transient (2-5 min) facilitoly and/or inhibitory effects on different components of evoked responses in the barrel cortex neurones, while background activity remained unchanged in most cases. Morphological studies also showed that the atropine application resulted in nearly complete block of the [H^3]glycine labelling in the vibrissa stimulated barrel cortex. These results suggest that ACh is a powerful neuromodulatory agent in the rat somatosensory cortex.

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP), A RECENTLY DISCOVERED MEMBER OF THE SECRETIN FAMILY, IS A NEW HYPOPHYSIOTROPHIC PEPTIDE

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It has been demonstrated that PACAP, the latest discovered member of the secretin family, has an important role in the regulation of anterior pituitary functions. The source of PACAP may be the hypothalamus or the pituitary itself. PACAP was demonstrated in the hypothalamus, ME and in the pituitary portal blood. PACAP was also revealed in the anterior pituitary with RIA, HPLC and with the demonstration of its mRNA. The level of PACAP mRNA in the anterior pituitary is highest during the proestrous LH surge. In our immunohistochemical studies we were able to demonstrate relatively large rounded or oval PACAP immunoreactive cells in the anterior pituitary of male and female rats. The shape and the distribution of PACAP immunoreactive cells were very similar to that of gonadotrophs; however, the number of PACAP cells was less than that of LH or FSH cells. In female rats with various stage of estrous cycle another PACAP positive cell population was observed. These cells had smaller diameter. Double labeling revealed that the large PACAP cells also exhibited LH immunoreactivity and those with small diameter stained for prolactin as well.

It is not clear how PACAP influences the pituitary hormon secretion. Because PACAP is present in the LH and prolactin cells and its mRNA is highest in the afternoon of the proestrous stage in female rats, we have studied the effect of PACAP on LH and prolactin surges preceeding the ovulation. Intracerebroventricular administration of PACAP just prior to the critical period in proestrous stage inhibited the expected ovulation, although iv administration of PACAP in same dose had no effect. PACAP antiserum did not influence the ovulation when it was injected intracerebroventricularly or intravenously, and it was not able to provoke ovulation when it was blocked by Nembutal. When PACAP blocked the ovulation, the LH and prolactin surges, which normally preceeded the expected ovulation, were also inhibited.

Our results support the view that PACAP has an important role in the control of LH and prolactin secretion and this effect is, at least partially, mediated through the hypothalamus. The role of the locally produced PACAP has to be elucidated.

EXPRESSION OF GLUTAMATE RECEPTORS IN CEREBRAL ENDOTHELIAL CELLS

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Activation of glutamate receptors has been shown to mediate a large number of neuronal processes such as LTP and ischemic damage. Beside neurons and glia, glutamate receptors may occur on cerebral endothelial cells (CECs) as well. The aim of our study was to determine which glutamate receptors are expressed in CECs and to find intracellular processes which can be initiated by the activation of these receptors.

By using RT-PCR we have shown that CECs express NMDA receptors (NR1 subunit which is necessary for the formation of functional NMDA receptors - and NR2A-C subunits), AMPA receptors (GLURl-4 subunits) and metabotropic receptors (mGLUR). NMDA receptor subunit NR2D and the kainate receptor GLUR6 could not be detected in CECs.

Exposure of the cultures to 2mM glutamate, a well established mediator of ischemic damage, for 30 min increased significantly the phosphorylation of CAM-PK II even after 10 and 60 min recovery time. This effect could be prevented by the NMDA blocker MK-801. Moreover, exposure to glutamate induced a raise in the intracellular Ca^{++} level as revealed by electron microscopy using the oxalate-pyroantimonate method.

The presence of multiple glutamate receptor types may confer a finely tuned responsiveness of the CECs to glutamate. In addition, our results suggest that NMDA receptors may take part in the reactions of CECs in ischemic brain oedema.

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DIFFERENTIAL SUBCELLULAR DISTRIBUTION OF THE PRESYNAPTIC METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES mGluR2/3 AND mGluR7 IN THE RAT HIPPOCAMPUS

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Glutamate is the main excitatory neurotransmitter in the brain that acts through both ionotropic and metabotropic glutamate receptors-(mGluRs). mGluRs mediate slow responses to glutamate through intracellular signal transduction via G-proteins. So far, 8 different subtypes of mGluRs have been identified (mGluRl-8). They are divided into three subgroups based on their sequence similarities, signal transduction mechanisms, and agonist selectivities. Antibodies specific to mGluR2/3 and mGluR7 were used for immunocytochemical analysis in order to determine the distribution and precise subcellular location of these metabotropic glutamate receptors, and to investigate whether they are colocalised within the same neuronal elements in the rat hippocampus.

Both receptor subtypes were found presynaptically. Axons constituting the perforant path and mossy fibers contain both mGluR2/3 and mGluR7 receptors, whereas the axons of pyramidal cells are labelled only for mGluR7. Electron microscopic immunogold analysis revealed that mGluR7 receptors are restricted to the presynaptic membrane specialisation of asymmetrical synapses, whereas mGluR2/3 receptors are present both peri- and extrasynaptically in axon terminals as well as in the preterminal portion of the axons, but never in the presynaptic membrane specialisation. Preembedding double labelling for mGluR2/3 (peroxidase) and mGluR7 (immunogold) revealed the segregation of these receptors within the same terminals.

In summary, the present study shows a differential distribution and subcellular location of the presynaptic mGluR2/3 and mGluR7 receptors in the rat hippocampus, suggesting their different roles in the regulation of glutamate release. The presence of mGluR7 in the presynaptic grid suggests a role in the suppression of calcium channels and/or vesicle fusion whereas mGluR2/3 in the axonal membrane may regulate the spread of the action potential.

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NEUROPROTECTION BY NOVEL GLYCINE SITE ANTAGONISTS AGAINST NMDA-INDUCED CELL DEATH IN RAT CORTICAL CULTURES: A COMPARATIVE STUDY

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The neuroprotective efficacy of novel, potent antagonists acting at the glycine site of the NMDA receptor was compared to their displacing potency to rat brain membranes. 5,7-Dichlorokynurenic acid (DCKA), LY-294,619, L695,902, and ACEA-1021 protected rat cortical neurones against 1 mM NMDA-induced cell death, with half-maximal protective concentrations of 7 μM, 0.5 μM, 27 μM, and 0.03 μM, respectively. The same compounds, in the above order displaced ^{3}[H]DCKA with IC_{50} values of 0.087 μM, 0.015 μM, 11 μM and 0.006 μM in a concentration dependent manner. The ratios for neuroprotective and displacement IC_{50} values differed to a major extent.

L-695,902 and ACEA-1021 showed emphasised in vitro neuroprotection with an order of magnitude lesser protection/displacement ratios of 2.4 and 5, compared to DCKA and LY-294,619, which have ratios of 80 and 33. Our data suggest that glycine-site antagonists can be classified into two types on the basis of their relative neuroprotective efficacy.

EFFECTS OF MORNING AND EVENING ADMINISTRATION OF METHOXAMINE ON SLEEP AND EEG

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The intracerebral concentrations of neurotransmitters, including monoamines, and the sensitivity of their receptors display circadian fluctuations. Diurnal changes in the level of monoamines and their effects on sleep may contribute to the circadian regulation of sleep-wake activity. Circadian variations have been described in the effects on sleep of various monoaminergic agonists, such as apomorphine, a dopaminergic agonist, and ritanserine, a serotoninergic agonist. Similar variations were found in the effects of other drugs acting on monoaminergic systems, e.g. reuptake blockers. Effects on sleep of drugs acting on noradrenergic systems may be influenced by circadian fluctuations of noradrenaline sensitivity. Effects on sleep of an α_2 adrenoceptor agonist, dexmedetomidine display circadian variations. In the present study, we compared the effects of the morning and evening administration of an α_1 adrenoceptor agonist, methoxamine (MTX).

Male Sprague-Dawley rats with implanted EEG electrodes were kept at a 12 h light - 12 h dark cycle, and the same conditions were maintained during the experiment. At light or dark onset, saline was administered intraperitoneally on the control day, and 1 or 4 mg/kg MTX on the experimental day. The sleep-wake activity was recorded for 12 h postinjection. The EEG effects of the drug were also studied by means of spectral analysis (fast Fourier transformation).

During the first 3-h period following administration of 1 mg/kg MTX, a reduction in REM sleep (REMS) and a tendency to a decrease in non-REM sleep (NREMS) were noted in the morning, but no change in sleep was found if the drug was given in the evening. There was a slight decrease in the total power of the EEG during W in the first hour postinjection in the morning. The higher dose (4 mg/kg) of MTX suppressed both REMS and NREMS during the first 3-h period postinjection in the morning, but only the amount of REMS was reduced in the evening. Spectral analysis of the EEG revealed reductions in power densities in all frequency bands during wakefulness (W). During NREMS decreases in power densities in all frequency ranges were found in the first hour postinjection, which where followed by increases in power densities in the low-frequency range. These changes in the EEG were similar in the morning and in the evening.

Our findings confirm the earlier papers reporting suppressions in sleep after MTX administration. In the present study, we found circadian variations in the sensitivity to MTX; the effects on sleep of the drug were more pronounced in the morning. Diurnal changes in noradrenaline sensitive systems may explain the time of day dependency of the effects of MTX on sleep. However, differences in alertness between day and night may also contribute to the diurnal variations in the sleep suppressive activity of MTX.

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EFFECTS OF RETINOIC ACID ON NEURAL DEVELOPMENT, *IN VITRO*

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Retinoic acid (RA) was used to induce neurogenesis in several neural progenitors, such as primary neuroectodermal cells obtained from early embryonic brain vesicles, immortalized cell lines derived from p53-deficient embryonic brain vesicles, cell lines isolated from perinatal transgenic mice carrying a conditionally active neuron-targeted Tag oncogene and embryonic carcinoma cell lines P19 and PCC-7. Treatment with RA resulted in production of neurons and astroglial cells in early embryonic wild-type precursors, as well as in immortalized cell lines except those derived from brains of perinatal animals. The neuronal phenotypes and the morphological patterns produced by such RA induced cells varied according to the types of progenitors. The findings indicate that RA, while is needed for the initiation of neuron formation, does not directly interfere with cell-fate decisions made by induced cells.

In brain cell cultures derived from late embryonic or fetal brain tissues, RA did not induce the formation of neurons from progenitors, but modified the glutamate homeostasis of cultured cells. By enhancing the glutamate concentration in the fluid environment of cells, RA could promote the elongation of neuronal processes.

The data indicate that RA and its nuclear receptors play multiple roles in neural development which might result in age- and cell type-dependent reactions in the CNS.

MOLECULAR CHANGES CAUSED BY CHRONIC OPIOID EXPOSURE

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The phyisiological phemomena of tolerance and dependence provoked by prolonged exposure of opiates is fairly well known. The molecular mechanisms underlying these reactions thogh yet intensively studied, are poorly understood, and the experimental results are often contradictory. Data suggest that multiplicity of opioid receptors, efficacy of opioid agonists, coupling to G-proteins, adenylate cyclase and transport of opioid receptor proteins, interaction with other transmitter systems, are all involved in the decreased opioid sensitivity.

Concerning changes at the opioid receptor level, the results in the literature are rather contradictory. Down-regulation of μ or δ receptors was primarily measured in *in* vitro systems, while up-regulation, down-regulation or no change were either detected with radioligands *in vivo*.

In our studies with chick embryonic neuronal cell cultures *in vitro*, treatment with kappa-opioid agonists at relatively small concentration and within a short period caused down regulation of ligand binding capacity of intact cells, while the immunocytochemistry, applying the KA8 monoclonal antibody in permeabilized cells, demonstrated no significant change. This suggests that a receptor redistribution occurs from the cell surface to intracellular structures.

In contrast, no desensitisation and down-regulation was detected for cell surface μ opioid receptors in rat brain due to chronic morphine injection. However, *in vivo* morphine treatment caused 50% *increase* in the receptor concentration in the microsomal fraction. Functioning μ opioid receptors are coupled to pertussis-toxin sensitive G-proteins (Gi/Go) in the plasmamembrane fraction of rat brain, while they are uncoupled in the microsomal fraction. We also detected that the level of G-proteins increased in both subcellular fractions in the tolerant animals.

Taken together, our data provide further support for the notion that multiple molecular changes do occur upon chronic opioid exposure.

MODULATION OF LIPOPOLYSACCHARIDE-INDUCED TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-6 PRODUCTION BY ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS IN MICE

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The effect of various agonists and antagonists of adenosine A_1 and A_2 receptors on lipopolysaccharide (LPS)-induced tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6) plasma levels was investigated in mice using ELISA.

Pretreatment of animals with N6-cyclopentyladenosine (CPA), a selective agonist of adenosine A_1 receptors caused a dose-dependent inhibition of LPS-induced TNF-α plasma levels, which was not prevented by 8-cyclopentenyl-1,3-dipropylxanthine (DPCPX), a selective antagonist of these receptors. DPCPX alone did not alter the TNF-a response evoked by LPS. Similarly to CPA, CGS-21680, a selective agonist of A_2 receptors dose-dependently suppressed the LPS-induced TNF-a production. Interestingly, the selective A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) did not block, but potentiated the effect of CGS-21680 on LPS-induced TNF-α release. However, DMPX administered alone had no effect on the LPS-induced TNF-α production. Finally, LPS-induced IL-6 plasma levels were not affected by either CPA or CGS-21680. Since TNF-α has been implicated as a key mediator of immune-mediated and inflammatory processes, adenosine receptor agonists might be of therapeutical use in the treatment of inflammatory diseases and/or septic shock.

KAINATE-INDUCED INHIBITION OF OUTWARD POTASSIUM CURRENTS IN CULTURED CHICK TELENCEPHALIC NEURONS

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The effect of kainate on the outward currents of cultured chick telencephalic neurons was investigated by whole cell- and nystatin-perforated configurations of the patch clamp technique. Besides opening AMPA/kainate receptor coupled ion channels kainate produced an additional effect, with a slower time course: an inhibition of the voltage-activated outward currents. This inhibition is due to the inhibition of potassium channels, since it was absent when potassium ions were replaced for caesium ions in the pipette, and 5 mM TEA was present extracellularly.

Both the current directly evoked via non-NMDA glutamate receptors and the inhibition of outward potassium currents were blocked by the non-competitive non-NMDA receptor antagonist GYKI 53784. In itself the antagonist did not cause a depression of potassium currents.

The inhibition is decreased in calcium-free medium, or after incubating the cells in BAPTA-AM before experiments, and also by the presence of 300 μM Cd^{2+} in the extracellular medium. Increasing the intracellular calcium concentration by the ionophore ionomycin also resulted in an inhibition of outward potassium currents. These results show that the mechanism of the inhibition of outward potassium currents by kainate involves the elevation of intracellular calcium concentration.

THE DISTRIBUTION OF NADPH-DIAPHORASE POSITIVE NEURONS IN THE CENTRAL NERVOUS SYSTEM OF *PORCELLIO SCABER*, LATR. (CRUSTACEA, ISOPODA)

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The presence of several biogenic amines within the central nervous system of isopods has been documented by the use of immunocytochemical and biochemical techniques, but no data are available on the occurrence of fixation sensitive NADPH-diaphorase (which is probably identical to nitric oxid synthase) in their neurocytes.

In this study we investigated the presence and distribution of NADPH-diaphorase positive neurons in the ventral nerve cord ganglia of *Porcellio scaber*. Whole-mount enzyme histochemistry revealed that the ganglia contain several NADPH-diaphorase positive neurons. The number, size and position of labelled cells are highly variable from ganglion to ganglion. With the exception of the terminal ganglion these cells show symmetrical distribution. A prominent feature of the positively reacting neurons in the terminal ganglion is a distinct group of unpaired cells at the midline. The intersexual difference in the number and position of unpaired cells suggest that they innervate the genital organs. For establishing their exact function further investigations are needed.

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ON THE ORIGIN OF EMOTIONS:
— psychophysiological approach —

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The problem: Does expression, recognition and physiological background of basic emotions support their view as inborn complex patterns? Darwin supposed (1872) and P. Ekman proved (1972) the universality of expression and perception of emotions. Lately Field (1981) succeeded to make 36 hours newborns to imitate the expression of three basic emotions. In the present study we tried to check the universality of this innate capacity to spontaneously express and to imitate all of basic emotions, further by the aid of a psychophysiological index (heart rate) to map the 'meaning' or connected patterns of these emotions.

Methods: In two experiments the subjects were 28 (Experiment 1) and 16 (Experiment 2) newborn infants, respectively, ranging in age from 3.5 hours to 40 hours. Each infant was shown several gestures to imitate in a random order and all potential responses, including free interactions were videotaped and the records were later analysed and rated by 30 independent observers, unaware of the hypothesis of the study. In Experiment 1 the infant's heart rate changes were also measured using disposable chest electrodes.

Results and conclusions: Most of the infants were able not only to imitate but also express spontaneously all of the six basic emotions (happiness, sadness, surprise, disgust, anger, fear). Imitation of anger was accompanied by significant HR acceleration while spontaneous disgust by marginally significant HR deceleration. Patterns and reaction time of both spontaneous and imitated surprise were quite similar to eye brow flash well known from human ethology (Eibl-Eibesfeldt, 1973). Results placed into the frame of *Homo imitans* (Meltzoff and More, 1977) and *Homo provocans* (Nagy and Molnár, 1993) dimensions of inborn social competence support the presence of intersubjectivity (Trevarthen, 1973) as a basic and inborn index of empathy, which can serve as a basis of the suggested so-called emotion reading.

PLASTIC CHANGES IN NEUROKININ-1 RECEPTOR EXPRESSION OF SPINAL CORD NEURONS IN INFLAMMATORY HYPERALGESIA

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Neurokinin-1 receptors (NK1-R) are suggested to play an increased role in the synaptic transmission between nociceptive primary afferents and spinal cord neurons in pathological conditions. Up-regulation of NK1-R mRNA and increased effectiveness of NK1-R antagonists has been shown in inflammatory hyperalgesia. However, there are no data available on the changes in the receptor expression itself. Here we studied the changes in NK1-R expression of dorsal horn neurons in inflammatory hyperalgesia.

Ultraviolet irradiation was used to induce skin inflammation in rats hind paw. At the peak of the heat hyperalgesia spinal cord neurons expressing NK1-R were shown using anti-NK1-R antibody (Vigna et al., J. Neurosci. 14:834). Intensity measurements and analysis of immunopositive perikarya were done in control and inflammed animals.

On the ipsilateral side both the intensity of NK1-R staining and the number of immunopositive perikarya decreased particularly, in the superficial laminae. There was no change in the type of the immunopositive perikarya.

Our results suggest that NK1-R expression is lowered or less detectable in the dorsal horn of the spinal cord in inflammatory hyperalgesia.

DEVELOPMENT OF A MICROPROCESSOR-CONTROLLED UNIVERSAL BIOLOGICAL AMPLIFIER

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There is a big choice of different amplifiers from a number of manufacturers in commercial circulation. We decided to design our own construction due to a few special requirements emerging in our laboratories, and because we could not find all these functions built into one single equipment.

The most important difference (in comparison to other models) is that our BioAmp is a programmable amplifier, but it has no sampling circuits at all. In other words, it is controlled by a built-in microprocessor, but it has got only analogue amplifier circuits. This feature is indispensable when we use averaging techniques for processing its output signal. The built-in computer is optically isolated from the amplifier stages.

Although it is a programmable equipment, it does not need a separate computer to work. According to this fact, it can be used as a stand-alone usual amplifier (while possessing the standard RS-232 connector to communicate with a PC if it would be necessary). Its microprocessor-based control panel supports high level of reliability and easy usage.

The amplifier has got a multi-purpose connector for external preamplifiers. This method gives an opportunity for use of other type of input modules to meet all the future demands.

Our BioAmp is designed with programmable analogue filter modules (HP and LP) to avoid every kinds of periodic noises. In other amplifiers Double-T notch filters are applied because that kind of filter can be manufactured easily. In this device, however, especially efficient (more than 40 dB) Wien-Robinson notch filters are employed to offer very low phase-distortion.

CONNECTIVITY ESTIMATIONS FROM ERROR PROPAGATION ON NEURAL UNITS (AN OPTIMIZATION STRATEGY FOR NEURAL NETWORK ASSOCIATIVE MEMORY STORAGE)

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For the increase the number of storable patterns in an associative neural network, we need to code the arbitrary original patterns to a more plausible inner representational form. This inner representation has to be larger and sparse, than the original ones, for the sake of enhanced storage capacity. This representation has to fulfill some information content dependent constrains. Modeling the generation the inner representation we can make assumptions also on the connectivity of its generator interface neural network: For the use of optimal associative memory storage it is necessary, that the initial error does not increase too much during this coding. The statistical analysis of the "error propagation" on a McCulloch-Pitts neuron shows, that from this point of view the optimal convergence onto an inner representational neuron has to be the minimal. If we make further assumptions on the error distribution on the input patterns, then we can make further constrains on the coding interconnections structure. If we assume that the error on the initial patterns prefers locality, that is it occurs in patches, and assume the minimal wiring, then the topographic ordering of the representational neurons decrease significantly the error propagation. The comparison of the morphologically measured convergence data to the model-implied-optimal-wiring is discussed. This method can lead to the design of even practically useful associative neural networks.

STUDIES ON CALMODULIN II AND PARVALBUMIN GENE EXPRESSIONS IN ISCHEMIC BRAIN AND HEART

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Ischemia causes a marked upregulation of expression in a wide variety of genes, including Ca^{++}-binding proteins. The aim of this study was to quantify the changes of expression in two Ca^{++}-binding proteins, calmodulin II and parvalbumin, in rat heart and brain tissues following coronary occlusion.

The left anterior descending coronary artery was occluded in 18 anesthetized rats. After 20 minutes the hearts and cerebral cortices were removed from 7 rats which survived the treatment. Heart tissues were excised either from the areas surrounding the left anterior descending coronary artery or from areas not affected by the occlusion. The heart tissues and cerebral cortices removed from 8 anesthetized, non-treated animals served as absolute controls. Total RNA was extracted by the guanidium isothiocyanate method. Poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose column chromatography. Northern-blot analysis with radiolabeled cRNA probes was applied to measure the changes in expression of calmodulin II and parvalbumin genes. RNA probes specific to the mRNAs of the respective Ca^{++}-binding proteins, and also the murine b-actin gene, as internal standard, were prepared by *in vitro* transcription using [^{35}S]-UTP. Autoradiography and computer-assisted image analysis of the blots were employed to analyse the results.

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THE EFFECTS OF C-TYPE NATRIURETIC PEPTIDE ON STRIATAL DOPAMINE METABOLISM IN RATS. MICRODIALYSIS STUDY

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The effects of different doses of C-type natriuretic peptide (CNP) were studied on striatal dopamine (DA), DOPAC, homovanillic acid (HVA) and 5-hydroxyindole acetic acid (HIAA) release.

The CNP was administered into the lateral brain ventricle. The transmitter release was measured in pentobarbital-urethan anesthetized rats, in samples taken by microdialysis from the striatum with the aim of HPLC-electrochemical detector.

The DA release with the dose of 1 mg showed a peak at the 140 min. The level was somewhat higher in all time studied, however the increase did not reach statistically significance level. Five hundred ng and 2 μg had no action.

The DOPAC level showed a dose-related increase already at 40 min. The highest level was observed at 120 min with the dose of 1 μg. There was no difference between 500 ng and 2 μg of CNP administration.

The HVA level showed a tendency to increase with the dose of 500 ng and 2 μg, however the increase was not significant.

The HIAA levels showed a steady rise up to the 180 min, followed by a decline. One mg dose of CNP had no action, while 2 μg showed a constant higher level compared to the control. This difference however was not statistically significant.

The data indicate the CNP neuropeptide is able to modulate the activity of the catecholaminergic and serotoninergic system. This modulation could have a physiological implication related to the behavioural action of CNP observed in our Department.

EFFECTS OF ZINC IONS ON THE TRANSMITTER RELEASE

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Treatment with 0.1 or 0.25 mM $ZnCl_2$ irreversibly blocked neurotransmission in the *Torpedo* electric organ by inhibiting acetylcholine (ACh) release. Neither the ACh content and compartmentation, nor the ATP and phosphocreatine stores were affected in Zn^{2+}-treated tissue. In synapses where transmission was blocked by Zn^{2+}, stimulation still provoked an accumulation of calcium. In Zn^{2+}-treated synaptosomes, ACh release was inhibited, as measured either on a KCl depolarization, or by using a Ca^{2+} ionophore.

On the other hand, successive application of a Ca^{2+} ionophore and Zn^{2+} efficiently triggered ACh release from synaptosomes. Actually Zn^{2+} mimicked the two fundamental actions of Ca^{2+} on release, which are, i) to trigger at a relatively high concentration the immediate activation of release and ii) to cause a more slowly developing desensitization of release. Zn^{2+} was even more powerful than Ca^{2+} for both actions.

In morphological studies it was found that the blocking of ACh release by zinc ions did not cause any significant fine structural alterations in the nerve-electroplaque junctions. Electrical stimulation of the Zn^{2+} treated synapses, however, resulted in a significant loss of synaptic vesicles. The appearance of vesicles in different phases of fusion with the presynaptic membrane indicates that the exo-endocytotic process is blocked in these circumstances. In freeze-fracture replicas made from both quick-frozen and fixed material we could also demonstrate a high number of vesicle openings (pits). The effect of zinc was very similar to that observed following the treatment with diamide, a thiol oxidating agent. This indicates that in both cases certain vesicular docking proteins with high cysteine content might be affected.

EFFECTS OF THE EXCITATORY AMINO ACID ANTAGONISTS NBQX AND GYKI 5 466 ON SPINAL REFLEX ACTIVITY, IN RATS

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Until recently has been thought that non-NMDA-type glutamate receptors mediate monosynaptic, while NMDA-type glutamate receptors mediate polysynaptic segmental spinal reflexes, as supported by Block & Schwarz, and Turski et al. On the contrary, based on their results in spinal rats, Farkas & Ono suggested that AMPA receptors play the main role in both of these reflexes.

In the present study the pharmacological sensitivity of two spinal segmental renexes was investigated in rats using electromyographic recording from the anterior tibial muscle after electrical stimulation of one hind paw (flexor reflex, polysynaptic) and from the plantar foot muscle afler percutaneous electrical stimulation of the tibial nerve (Hoffmann reflex, monosynaptic).

The effects of GYKI 52466, a non-competitive AMPA receptor antagonist 2,3-benzodiazepine compound, and NBQX, a competitive AMPA antagonist quinoxaline compound, were studied. Male SPRD rats weighing 250 to 320g were anesthetized with chloralose (25 mg/kg i.p.) plus urethane (lg/kg i.p.). Rectal temperature was maintained at 37.8±0.5°C. GYKI 52466 was applied in doses of 1-4 mg/kg in physiological saline, i.v. The effects of NBQX on spinal reflexes was studied at consecutive i.v. administrations (maximum cumulative dose: 32 mg/kg). Stimuli were applied continuously. Myographic responses were integrated and undercurve areas were recorded by a paper-chart recorder.

The ED_{50} values for inhibition for the polysynaptic flexor and the monosynaptic Hoffmann reflex by GYKI 52466 were very similar (ED_{50}s: 2.2 and 1.6 mg/kg respectively). NBQX was effective in similar doses, being slightly more potent in inhibiting flexor reflex.

Our findings are in good agreement with those of Farkas & Ono, and with our former data obtained in cats. However, they are in conflict with the results of Turski et al., who found that NBQX was much more potent in inhibiting Hoffmann reflex than flexor reflex, in mice. This contradiction can probably be explained by the differences in the anesthesia and species.

REFERENCES

F. Block, M. Schwarz; Eur. J. Pharmacol. 256: 149-153. (1994)
S. Farkas, H. Ono; British Journal of Pharmacology 114: 1193-1205. (1995)
L. Turski et al.; J. Pharmacol. Exp. Ther. 260: 742-747. (1992)

BIOLOGICAL EFFECTS OF SHORT β-AMYLOID PEPTIDE FRAGMENTS

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Elevated concentration (10^{-7} M) of β-amyloid peptides containing 40 or 42 amino acids are characterized by neurotoxic effects. The similar biological effects have been detected in our laboratory using the β-amyloid peptide 25-35 undecapeptide and the 31-35 pentapeptide in mixed neuron-glia culture. Whereas natural β-amyloid peptide shows very low water solubility and a high tendency for aggregation, we have synthesized β-amyloid peptide fragments and analogues with higher solubility and lower aggregation tendency. For searching natural binding sites (receptors) tritiated 25-35 and 31-35 β-amyloid peptides were synthesized.

In our first experiment mixed neuron-glia cultures obtained from superior cervical ganglion of fetal rat were saturated with Indo-I fluorescent dye for investigation of effects of β-amyloid peptides on Ca^{2+} flow. Each β-amyloid peptide raised the intracellular Ca^{2+} concentration. The 31-35 β-amyloid peptide caused only a short Ca^{2+}-signal at 10^{-8} M concentration, however, using higher concentration (10^{-6}, 10^{-5} M) the Ca^{2+} level of the cell remained high without recovery during the observation period (5 minutes). The 25-35 undecapeptide showed similar effects but at lower concentration. Structurally similar peptides containing the same amino acids in randomized sequence had no effect on Ca^{2+} concentration.

In vivo experiments were performed for investigation of the effect of β-amyloid peptides on rat sciatic nerve applying perineural cuffs containing 500 μM concentration of these peptides. The 25-35 pentapeptide caused degeneration and was proven to be neurotoxic, nevertheless, another randomly selected amyloid precursor protein fragments were inactive.

In behavior studies after the injection of the water soluble $Phe(SO_3H)^{24}$ 25-35 β-amyloid peptide into the basal nucleus of rat brain, a significant hyperactivity was detected from day 5 to day 14 of experiment showing the characteristics of neurotoxins. However, the treated animals were hypoactive in a new environment. Memory disturbances were also observed in treated animals in a different experiments.

ASCENDING PROJECTIONS FROM THE ROSTRAL VENTROMEDIAL MEDULLA OBLONGATA IN THE RAT

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Stimulation of the rostral ventromedial medulla (RVM) that includes the nucleus raphe magnus and the adjacent nuclei of the reticular formation produces inhibition of nociceptive neurons in the spinal dorsal horn, resulting in a powerful attenuation of pain behaviour. A growing body of experimental evidence suggests that in addition to the spinal dorsal horn, the RVM also influences the function of supraspinal centres involved in nociceptive information processing and pain control. Consequently, in the present study, we investigated the course and termination patterns of ascending fibers arising from the RVM. Following microinjections of the anterograde neural tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) into the RVM, ascending fibers and terminals were revealed in several areas of the brain stem, diencephalon and telencephalon. We demonstrated a significant projection from the RVM to the pontine parabrachial area, locus coeruleus, periaqueductal gray, anterior pretectal nucleus, parafascicular and ethmoidal thalamic nuclei, ventromedial areas of the hypothalamus, nucleus of the solitary tract, and medial septal nucleus. The findings suggest that the RVM may exert a profound effect on nociceptive information processing at various spinal and supraspinal centres and play important roles in the modulation of sensory-discriminative and affective-motivational aspects of pain behaviour.

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STUDY OF THE INTRACORTICAL COMPONENTS OF THE ACOUSTIC 'STEADY-STATE' RESPONSE IN CAT

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Oscillatory brain activity in the 40 Hz range (gamma band) is a characteristic phenomenon in various sensory areas of human and mammalian cerebral cortices. The auditory steady-state response (ASSR) is a sinusoidal electrical response to periodically presented auditory stimuli. It appears when the rate of the stimuli is sufficiently rapid to produce overlapping of the auditory evoked potentials (AEPs) to individual stimuli. In the present study, we investigated the surface and intracortical distribution of the ASSR.

Acoustic stimuli consisted of 0.1 ms square-wave pulses delivered through a bone-conductor at presentation rates ranging between 1 and 50/s- AEPs and ASSR were recorded from chronically implanted arrays of epidural electrodes, while chronically implanted 16 contact multielectrodes were used to record intracortical field potentials. The responses were recorded in freely moving cats during wakefulness and slow wave sleep. Spontaneous EEG activity was also recorded in both condition to compare spontaneous gamma band activity with ASSR. The spatial and spectral distribution of the ASSR and the spontaneous gamma activity was similar, the amplitude maximum appeared at 32-42 Hz. In slow wave sleep the ASSR amplitude decreased while the early components of the AEPs remained almost unchanged.

Recording the intracortical activity simultaneously from 16 consecutive depth of the cortex, the intracortical sinks and sources could be localized by one dimensional current source density (CSD) analysis. The peak latency of the earliest surface positive - deep negative component of the AEP was 10-12 ms on the surface and 9-11 ms in the deepest layers. At about 20-25 Hz repetition rate the surface positive component disappeared while the amplitude of the deep negative component only slightly decreased. At 30-46 Hz rate a high amplitude sinusoidal oscillation developed in the superficial layers, the negative phase of which had a peak latency of 12-15 ms. The primary deep negative component was still present. The CSD analysis revealed that in the waking animal the primary sink in the fourth layer was similar to that which appeared with low repetition rate stimulation. However, with ASSR high amplitude sink-source oscillation developed in the supragranular layers, with a 10-15 ms delay related to the input sink. In slow wave sleep this superficial sink-source oscillation was missing.

The data indicate that intracortical neuronal circuits of the supragranular layers of the auditory area may be responsible for the ASSR as well as for the spontaneous gamma band activity.

STUDIES ON MUSCARINIC M1, M2 AND M3 BINDING SITES IN ADULT RAT ANTERIOR PITUITARY GLAND

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Muscarinic binding sites have been described in the anterior pituitary (Mukherjee et al., 1980, Schaeffer et al., 1980), but their functional role has not been elucidated. The discovery of muscarinic receptor subtypes made it possible to explain the controversial findings with non-selective drugs; as the presence and simultaneous activation of two or more receptor subtypes with divergent physiological actions may result in unpredictable effects. The muscarinic receptor subtypes in the rat pituitary have not been characterized before. Binding of [^{3}H]-QNB, [^{3}H]-pirenzepine, [^{3}H]-AFDX 384 and [^{3}H]-4DAMP were studied at 25 °C in membrane preparation of male rat anterior pituitary gland. In competition experiments atropine (non-selective), pirenzepine (selective M1), 4-DAMP (selective M1-M3) and methoctramine (selective M2) - muscarinic receptor antagonists were used to characterize the muscarinic receptor binding sites. [^{3}H]-QNB binding was more potently antagonized by 4-DAMP (K_i=5.5xlO^{-12}) than by atropine (K_i=7x10^{-11}) and by pirenzepine (K^i=8x10^{-9}); methoctramine was 62.5 fold less potent than pirenzepine (K_i=5x10^{-7}). [^{3}H]-pirenzepine binding was potently antagonized both by pirenzepine (K_i=2x10^{-8}) and by 4-DAMP (Ki=3x10^{-9}). [^{3}H]-AFDX 384 was displaced by atropine (K_i=2x10^{-8}) and methoctramine (K_i=10^{-7}), whereas [^{3}H]4-DAMP binding potently antagonized by 4-DAMP (K_i=4.6x10^{-12}), by atropine (K_i=7x10^{-9}) and by pirenzepine (K_i=1.9x10^{-8}). Based on these results we assume that both M1 and M3 muscarinic receptor binding sites are present in rat anterior pituitary gland but their potential physiological role in regulation of pituitary GH and PRL secretion remains to be investigated.

REFERENCES

Mukherjee, A., Snyder, G., McCann, S. (1980) Characterization of muscarinic receptors in intact rat pituitary cells. Life Sciences 27, 475-482.

Schaeffer, JM., Huesh, AJW. (1980) Acetylcholine receptors in the rat anterior pituitary gland. Endocrinology 106, 1377-1381.

THE PROJECTIONS OF THE ROSTRAL VENTROMEDIAL MEDULLA AND PONTINE A5 CELL GROUP TO THE INTERMEDIOLATERAL CELL COLUMN IN THE SPINAL CORD OF THE RAT

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The spinal projections of neurons in the rostral ventromedial medullary region (RVM) and AS pontine cell group (AS) that play a major role in the regulation of autonomic functions in general and of cardiovascular control in particular were studied in the rat by using the anterograde neural tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L). After injecting PHA-L into the RVM or AS, numerous labelled axon terminals were detected in the intermediolateral cell column (IML) of the thoracic spinal cord. To study the postsynaptic targets of these descending fibres, sections were stained for both PHA-L and calbindin-D28K (CaB), a calcium-binding protein, that has been reported to be a marker of spinal autonomic preganglionic neurons. CaB-immunoreactive neurons in the IML were found to receive contacts from fibres descending from both the RVM and AS. Synaptic contacts between RVM or AS terminals and CaB-immunoreactive structures were identified in a correlative electron microscopic study.

Combining the PHA-L labelling with the immunocytochemical detection of neurokinin1-receptor (NK1-R; kindly provided by S. Vigna) a well-known receptor for substance P, it was found that autonomic preganglionic neurons receiving monosynaptic contacts from VMM and A5 terminals express a strong immunoreactivity also for NK1-R. Synaptic contacts as well as GABA- and glycine-immunoreactivity of the presynaptic terminals were identified in a correlative electron microscopic study by using postembedding immunocytochemical methods.

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF GABA IMMUNOREACTIVE STRUCTURES OF THE ISTHMIC NUCLEUS IN *RANA ESCULENTA*

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The isthmic nucleus is a main relay station of the visual pathway in the frog. It receives information from the ipsilateral optic tectum and projects to both tecta. Majolity of the isthmic cells was found to be cholinergic. Recent immunocytochemical data however raise the issue that other transmitters are also involved in the organisation of the projections of this nucleus. Data exist stating that a large number, maybe all of the isthmic cells are serotonergic. GABA-immunoreactive cells were also described formerly. This is a report of the experiments which aimed at to give more detailes al out the GABA- and SHT-immunoreactivity in the nucleus.

For the experiments we used samples of the isthmic nucleus deriving from the frog *Rana esculenta*. Some of them were filled retrogradely with HRP from the contralateral optic tectum. Light microscopic observations were done after ABC immunohistochemistry on cryostat sections. For ultrastructural studies samples were processed with the postembedding immunogold method.

A considerable GABA-positive cell-population were located partly in the rim coltex and partly in the medulla. In transverse sections these latter cells were found scattered in the medulla and many of them seemed to be located along a diagonal dividing the neucleus from the medial superior towards the lateral inferior borders. GABA-positive profiles studied Ln the electron microscope formed synapses on both immunopositive and negative profiles and vice versa. HRP-filled contralaterally projecting neurones does not seem to bear GABA-positivity. Neither the filled dendrites nor the somata and the axons were immunopositive. The filled structures received synapses overwhelmingly from profiles containing round clear vesicles without GABA-immunoreactivity. These terminals most probably derive from the ipsilateral tectum. The terminals with pleomorphic vesicles could not be marked by GABA-immunocytochemistry. Significant serotonin immunopositivity could not be detected in any region of the nucleus.

The results indicate that GABA does not have a considerable influence on the contralaterally projecting cells, since GABA-immunoreactive terminals rarely synapse upon them. We plan to verify with immunocytochemistry for glutamate,that the contralaterally projecting cell population gets mainly excitatory input from the ipsilateral optic tectum while the local GABA-circuitry exerts effect mainly on the organisation of the reciprocal ipsilateral tectoisthmotectal pathway.

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THE LOCALIZATION OF LAST-ORDER PREMOTOR INTERNEURONS IN THE LUMBAR SPINAL CORD OF RATS

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There is a strong evidence that neural circuits underlying certain motor behaviours are located in the spinal cord. Such local central pattern generators are thought to coordinate the activity of motoneurons through specific sets of premotor interneurons, some of which (last-order interneurons) establish monosynaptic contacts with motoneurons. In the experiments presented here we intended to identify and localise interneurons that can likely be regarded as last-order premotor interneurons. After small iontophoretic injections of neurobiotin and biotinylated dextran into the lateral motor column (LMC) or adjacent white matter at L3-L6 segments, retrogradelly labelled spinal interneurons were investigated in the lumbar spinal cord of rats. Following injections centred within the LMC, labelled interneurons were revealed in 3-4 segment long compartment of the spinal gray matter ipsilateral to the injection site. With the exception of the very close vicinity of the injection site where stained perikarya were distributed throughout the entire extent of the ventral horn, labelled neurons were confined to a narrow horizontal layer of laminae V-VI and the dorsal regions of lamina VII. In cases the tracer was injected into the lateral white matter adjacent to the LMC, labelled perikarya were observed within the same location but in a substantially lower number. Following injections into the subpial region of the white matter, however, very few, if any, labelled perikarya were revealed. Our results suggest that most of the premotor interneurons that establish synaptic contacts with a specific set of motoneurons is distributed in a 3-4 segment long section of the spinal cord, and they are mostly confined to a narrow horizontal layer of the intermediate gray matter in rats. The findings also indicate that most of the last-order interneurons may form synaptic appositions with somata and proximal dendrites of motoneurons, while distal dendrites may receive synaptic contacts from segmental interneurons only in a limited number.

FMRFAMIDE IN THE NERVOUS SYSTEM OF *LUMBRICUS TERRESTRIS*

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FMRFamide immunopositive elements of the nervous system of earthworm *(Lumbricus terrestris)* have been studied by means of quantitative immunocytochemistry utilizing Sternberger's peroxidase-antiperoxidase technique and Neurolucida programme.

It has been found that all parts of the *Lumbricus* central and peripheral nervous system contain FMRFamide immunopositive cell bodies. A rich immunoreactive fibre network is also detected throughout the nervous system.

The cerebral ganglion contains about one hundred of FMRFamide immunopositive nerve cells. In the cranial part of the cerebral ganglion the cells are mainly situated in dorsomedial and lateral positions, only few of them are found in the medial, central and ventral parts of the ganglion. In the caudal part of the ganglion the cells become more clearly distributed into two main distinguishable groups, dorsal intermediate and lateral ones. The central neuropil contains a rich FMRFamide positive network, some of the fibers run transversely crossing the midline. In the vicinity of the emergence of the connectivum a few smaller cells are detectable. A portion of fibers of the connectivum show strong staining and they either reach the subesophageal ganglion or join the stomatogastric ganglia. Immunopositive cells are found in the stomatogastric ganglia as well.

In the subesophageal ganglion approximately 50 immunoreactive nerve cells were detected. In the cranial part of the ganglion these cells are in ventromedial position, in the caudal part they extend to the lateral part as well, surrounding the central neuropil in an arch-like form.

The immunopositive cells of the segmental ganglia show a similar distribution to that of the subesophageal ganglion, the number of the cells is however smaller.

Each segmental nerve contains faintly stained FMRFamide immunoreactive fibers. These fibers can be traced in nerves between the two muscle layers of the body wall, and among the muscle fibers a fine immunoreactive network can be detected. Similar fiber network is found in the walls of the pharynx and the foregut.

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THE ROLE OF C_6-OH AND C_{14}-OH GROUPS IN THE OPIOID RECEPTOR SELECTIVITY OF N-CPM-NORMORPHINE DERIVATIVES

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The agonist and antagonist properties of N-cyclopropylmethyl(N-CPM)-normorphine, N-CPM-norisomorphine, N-CPM-dihydronormorphine, N-CPM-dihydronorisomorphine and their C_{14}-OH substituted pairs were observed on the electrically evoked contractions of the longitudinal muscle of guinea pig ileum (GPI) and mouse vas deferens (MVD).

The replacement of CH_3 group by CPM group on the N atom in morphine, isomorphine, dihydromorphine and dihydroisomorphine increased the agonist activities in the GPI; this agonist activity could be antagonized by a low concentration of norbinaltorphimine (K_e: 0.23 nM) and high concentration of naloxone (K_e: 6.2–7.1 nM) suggesting that the agents are κ-agonists in the GPI. The C_{14}-OH substitution of the N-CPM compounds markedly reduced this agonist activity, the inhibition of the contractions did not reach 50%.

In the MVD the investigated compounds showed very weak agonist activities (maximal inhibition was not higher than 20%).

The antagonist potencies of the compounds were measured for μ and δ opioid receptors using normorphine and D-Pen^2-D-Pen^5-enkephaline (DPDPE) as selective agonists on the receptors, respectively. N-CPM-normorphine, N-CPM-norisomorphine, N-CPM-dihydronormorphine and N-CPM-dihydronorisomorphine produced strong antagonist affinities for μ-receptors (K_e: 0.89–1.87 nM). The C_{14}-OH substitution slightly decreased the μ antagonist potencies (K_e: 2.1–5.43 nM). The epimerization of the C_6-OH group, regardless of the C_{14}-OH substitution did not markedly alter the antagonist affinities for μ-receptors. The N-CPM and C_{14}-OH-N-CPM derivatives produced weaker affinities for δ- than μ-receptors but all $C_6\beta$-OH derivatives had higher δ-receptor affinities (K_e: 22.28–29.56 nM) than their $C_6\alpha$-OH pairs (K_e: 70.78–89.9 nM). The C_{14}-OH substitution of the N-CPM derivatives did not change the δ-receptor affinities of the parent compounds.

DISTRIBUTED AND DEDICATED NEURAL NETWORKS IN REGULATION OF VISCERAL FUNCTIONS IN *HELIX POMATIA L.*

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The visceral functions were shown to be regulated by overlapping neural networks including multifunctional neurons and distributed to various ganglia of CNS in *Helix pomatia L.* (Gastropoda, Mollusca). The neurotransmitters and some peptides were found to filter sensory information and tō take part in reorganization of the temporarily formed neural assemblies (S.-Rózsa, 1979, 1987).

The aim of the present investigation was to study how alterations on the firing mode of neurons contribute to the filtration of the sensory information and network reorganization and how multiple signal molecules modulate the firing pattern of neurons in an overlapping neural networks.

The experiment were performed on semi-intact preparation of *Helix pomatia L.* including CNS, cardio-renal respiratory and genital systems with their connecting nerves. Using current clamp and voltage clamp methods firing patterns of the central neurons, their connectivity, membrane conductance or ionic currents were recorded as well as the activity of the regulated peripheral organs and their nerves.

The results showed that sensory information from visceral organs converged in a large population of neurons in CNS and the same neurons are involved into the regulation of the above three visceral systems.

Reorganization of the neural networks was accompanied by alterations in the firing pattern of the neurons. Most of the identified neurons altered their firing pattem from tonic (regular) to phesic (bursting) type depending on the combination of signal molecules present or activation of their synaptic connections. The appearence of a slow membrane oscillations or burst firing were shown to tum the neurons insensitive to input activation and contributed to shifting the neurons from one regulatory networks to another one, e. 9. from the regulation of heart activity to that of pneumostome or genital organs. The signal molecules such as 5HT, DA, Ach, GABA, FMRFamide and opioid peptides are involved into the network reorganization.

The results showed that because of the large number of neurons involved in the regulation of visceral organs, this circuit can be interpreted more efficiently as a distributed network also including dedicated regulatory units.

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MORPHOLOGICAL PATTERN FORMATION DURING *IN VITRO* NEUROGENESIS

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Neuroectodermal progenitors give rise to neurons and glial cell types in course of multistep processes. Neuron formation can be induced by all-trans retinoic acid (RA) in several progenitor cell lines. Besides the uniform inducing effects, treatment with RA results in a variety of morphological patterns formed by differentiating cells. It might be due to cell to cell interactions which can cause alterations in the timing and the location of the events of differentiation.

In order to elucidate the developmental capacity of the progenitor lines and also, to get some insight into the significance of cell to cell interactions in neurogenesis, the morphological patterns formed by induced neuroectodermal cells of different origin were characterized and compared. The morphological patterns formed during induced neurogenesis in cultures of P19 embryonic carcinoma cells were compared to those formed in NE-4C immortalized precursor cell line established from p53-deficient embryonic mouse brain vesicles. The changes in the frequency of various patterns in time were also followed within the induced cell populations.

RA could induce neural differentiation in both types of progenitors. The induced neuronal phenotypes, however, seemed to be highly dependent on the stem-cell population. The finding indicated that RA, while was needed for initiation of neuron formation, did not decide on neural cell-type determination.

STRUCTURAL INTEGRATION OF HOMOTOPIC OLFACTORY BULB TRANSPLANT

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Solid grafts of olfactory bulb from 17.5 day-old embryonic progeny of Tg GAD-*LacZ* #1 mice expressing bacterial β-galactosidase markergene in the relay neurons of olfactory bulb were transplanted into non-transgenic neonatal mice after unilateral bulbectomy. As a consequence of complete bulbectomy, no remnant of the host olfactory bulb was found 2 months after surgery. A bulb-like structure had developed in the bulbar cavity which consisted of neuronal elements from the transplanted tissue and olfactory axons of host origin. The grafted relay cells could be readily identified by the presence of the β-galactosidase in the whole cytopasm including dendrites and axons. Dendritic branching patterns of relay cells were clearly recognized within glomeruli-like structures. To visualize relations between grafted relay cell dendrites and olfactory nerve endings we carried out double staining using enzyme histochemistry of β-galactosidase and immunohistochemistry of the olfactory marker protein. OMP positive bundles of olfactory axons had invaded the transplant and formed numerous glomerulus-like structures in random manner. Double-stained glomerular profiles were found providing light microscopic evidence for the termination of grafted relay cell dendrites within newly formed olfactory glomeruli. In addition, axons of relay neurons could also be followed through the olfactory pedunculus towards the primary olfactory cortex where they terminated in the piriform cortex.

Electron microscopically, immunolabelling of relay neurons expressing β-galactosidase in grafts enabled identification of the peripheral segments of the dendrites and synaptic connections made by them. We found numerous synaptic contacts between endings of olfactory sensory axons and dendritic branches of labelled relay neurons within the olfactory glomeruli in grafts. In addition, relay cells formed reciprocal synapses with unlabelled granule cell dendrites in the graft neuropil resembling the external plexiform layer. Similar reciprocal synapses were seen also on the soma of the grafted relay neurons. These synaptic contacts indicates that local circuits are reestablished between transplanted relay neurons.

The present study provided the first direct evidence for organotypic innervation patterns established by grafted relay neurons. These cells receive synaptic input from olfactory sensory axons of host origin, are involved in dendro-dendritic contacts like in intact olfactory bulb and grow axons which terminate in the primary olfactory cortex of the host.

LIPOPROTEIN(a) AS A MODULATOR OF HUMAN BRAIN ENDOTHELIAL FIBRINOLYTIC ACTIVITY

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Vascular endothelium has a central role in the maintenance of haemostatic balance. Endothelium can modulate fibrinolysis by secreting of tissue plasminogen activator (t-PA) and inhibitor (PAI-l) in a complex manner. On this way endothelial cells participate in the cleavage of local fibrin clot.

Lipoprotein(a) (Lp(a)), a highly atherogenic low density lipoprotein is an independent risk factor for coronary heart disease and ischaemic stroke. Lp(a) shows a close structural homology with plasminogen and interfers with fibrinolysis by competition with plasminogen at the site of plasminogen receptors.

The effect of Lp(a) on the expression of t-PA, urokinase-type plasminogen activator (u-PA) and PAI-1 was measured in cultured human brain microvessel endothelial cells (HBEC).

Endothelial cells from human brain capillaries were isolated and cultured. The endothelial origin of the cells was characterized by Dil-Ac-LDL uptake and presence of von Willebrand factor. The experiments were performed on primary cell cultures. The cells were incubated with human thrombin (90 nmoVl) and Lp(a) (450 μg/ml). The control cells were incubated without stimuli to determine the basal activity.

Levels of t-PA, PAI- 1 and u-PA antigens were measured by enzyme immunoassays (Coaliza t-PA, Coaliza PAI-l from Chromogenix, Mölndal, Sweden, and Technoclone u-PA ELISA from Technoclone, Vienna, Austria) in the conditioned media.

HBEC synthetized t-PA, u-PA and PAI-1 (19.6 ng/ml, 10 ng/ml and 184 ng/ml7 respectively) during the 72 hour period of time. These basal values were modulated by Lp(a): t-PA was decreased (-37 %), u-PA was decreased (-86 %). The concentration of PAI-l was relatively unchanged. After the incubation with thrombin the Lp(a) resulted in same effects: t-PAand u-PA were decreased (-65 %) and (-15 %), respectively. PAI-1 level was l14 %.

Lp(a) is a possible link between thrombosis and thrombogenesis by the alteration of fibrinolytic activity.

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EFFECT OF CA^{2+}-CHANNEL ANTAGONISTS ON NEUROTRANSMITTER RELEASE FROM RAT HABENULA

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The habenula, as the chief relay nucleus of the descending dorsal diencephalic system, is an important link between limbic and striatal forebrain and lower diencephalic and mesencephalic centres. Its role in integrative behavioural functions, and in psychotic disorders, such as stimulant-induced schizophrenia has been put forward. Beside glutamate, acetylcholine, GABA and peptides, ATP is the latest discovered mediator of this nucleus: electrophysiological, neurochemical and enzymecytochemical evidence favours its fast neurotransmitter function.

The main objective of this study was to investigate, whether Ca^{2+}-influx via different subtypes of Ca^{2+}-channels is involved in the initiation of depolarization induced ATP and [^{3}H]acetylcholine ([^{3}H]ACh) release from rat habenula slices. According to the current view, N, P and an incompletely identified type (class E) of voltage-dependent Ca^{2+}-channels has been attributed to trigger vesicular exocytosis in the mammalian central nervous system. To test the involvement of these channels neurotoxins of carnivorous invertebrates, and inorganic Ca^{2+}- antagonists were used.

Endogenous ATP, measured by luciferin-luciferase assay, and [^{3}H]ACh released simultaneously in response low frequency (2Hz, 2.5 msec, 360 shocks) axonal stimulation from the habenula slices. The N-type Ca^{2+}-channel inhibitor w-conotoxin GVIA reduced dose dependently (0.01 - 0.1 μM) the stimulation-evoked release of ATP and [^{3}H]ACh. Similarly, the P-type Ca^{2+}-channel blocker w-agatoxin IVA (0.05 mM), and the inorganic Ca^{2+}-channel antagonist Cd^{2+} (0.02 mM) exhibited inhibitory effect on the outflow of both neurotransmitters. On the other hand, Ni^{2+} (0.01 mM), the putative class E - antagonist had only weak effect. Long-term perfusion (90 min) of the slices with Ca^{2+}-free Krebs' solution inhibited the evoked release of neurotransmitters, showing that ATP and [^{3}H]ACh is released by a $[Ca^{2+}]_O$ -dependent way.

In summary, it can be concluded that both N and P type Ca^{2+}-channels are involved in the electrical-stimulation induced ATP and [^{3}H]ACh release in the rat habenula. Since a high correlation could be observed between the effect of different Ca^{2+}-antagonists on the release of ATP and [^{3}H]ACh, our results supports the idea, that ATP and ACh is released from common nerve terminals i.e. it may function as a co-transmitter with ACh in the rat habenula.

RECEPTOR-MEDIATED REGULATION BY HISTAMINE OF THE ACID PHOSPHATASE ACTIVITY IN CULTURED CEREBRAL ENDOTHELIAL CELLS

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Unlike most organ systems, the central nervous system is separated from the blood by a protective cellular barrier. The walls of the brain microvessels represent this cellular interface with specialized morphological, physiological and biochemical properties. Acid hydrolase activity was first demonstrated by Baranczyk-Kuzma et al. (1989) in primary cultures of cerebral endothelial cells (CEC). However, it remained to be seen if acid hydrolase activity could be modified in or released from the CEC by vasoactive substances. The present study was designed to check the possible effects of histamine on the activity of different molecular forms of acid phosphatase. We have used an immortalized cell line (RBE4) of CEC, which was produced (Durieu-Trautmann et al., 1993) by transfection of primary rat CEC with the plasmid *pEA-neo,* containing the adenovirus ElA encoding sequence followed by the neomycin resistance gene. Acid phosphatase activity was measured by the rate of hydrolysis of p-nitrophenylphosphate Cp-NPP) at 37°C in the appropriate buffer for 1 hour. Histamine (in 10-5 M concentration) increased the total activity of acid phosphatase (from 60.24 ± 4.79 mU/mg protein to 72.02±2.04 mU/mg protein) in the CEC. This effect of histamine on the activity of endothelial acid phosphatase could be inhibited by ranitidine (H_2-receptor blocker) in 10-6 M concentration, but not with pyrilamine, a H_1-receptor blocker.

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PARVALBUMIN CONTAINING FIBRES INNERVATE CALBINDIN D28k-IMMUNOREACTIVE NEURONS IN THE INTERMEDIATE LATERAL SEPTUM OF THE RAT

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In the septal diagonal band complex, botlh parvalbumin (PA) and calbindin (CaBP)-immunoreactive neurons co-contain GABA as neurotransmitter. In the same area, a large number of homologous synaptic connections between GABA-containing neurons can be observed. In order to further characterize their neurochemical nature, as well as the extrinsic and/or intrinsic origin of these GABA terminals the following experiments were performed: 1) correlated light and electron microscopic double-immunostaining for CaBP and PA on septal vibratome sections of control rats; 2) light microscopic immunostaining for PA of septal sections after surgical isolation of the septum from its telencephalic or 3) hypothalamic afferents; and 4) PA-immunostaining of complete series of brain sections two days following HRP injection into the border between the lateral and medial septal areas. The results demonstrated that a group of CaBP-immunostained, non-somatospiny cells located between the lateral and medial septal areas are surrounded by PA-immunoreactive baskets. These basket-forming axon terminals establish symmetric synaptic contacts on cell bodies and dendrites of their targets. The cells of the origin of PA-immunoreactive axons are not in the medial septum, but in the diagonal band nuclei. The observations indicate that a portion of the GABA-GABA synaptic connections inside the septal complex represents functional interaction between parvalbumin- and calbindin-containing neurons. In addition, only a population of PA-containing GABAergic neurons of the medial septum-diagonal band complex projects exclusively to the hippocampus, while others, which may also send axons to the hippocampus, terminate on septal calbindin cells.

REACTIVE CELL TYPES AROUND STAB WOUNDS IN THE AVIAN BRAIN

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Macrophages/microglia and reactive astroglia are the classic participants of the post-lesion demarcation and removal of damaged tissue in the vertebrate CNS. We have previously described a type of reactive glia following lesions in the chicken but not in the rat brain (Székely et al., Anat. Anz. Suppl. 170:689, 1991). These cells displayed spontaneous peroxidase positivity (SPP) with one or two thick or thin processes running to the lesion surface. For further characterization, adult zebra finches, two-day-old chicks and adult rats were anaesthetized, placed in a stereotaxic frame and one of the telencephalic hemispheres was lesioned with a sterile hypodermic needle. The survival times varied between 2 and 14 days, then the animals were perfused with a fixative containing 4% paraformaldehyde and 0.5% glutaraldehyde. Coronal sections were cut from the region of the lesion and used in the subsequent light and electron microscope studies. In zebra finches, SPP cells were discernible and they showed NADPH-diaphorase (ND) reactivity. Also macrophages/microglia, reactive for both ND and *Griffonia* lectin staining, appeared close to the lesion. In young chicks, SPP cells were found in the vicinity of the stab wound and they also formed clusters around small vessels in the periphery of the lesion. Some large, vacuolar, DAB positive cells were present in these groups, more frequently in young birds, surrounded by lightly stained cells with an arbor of fine processes. Ultrastructurally the vacuoles represented dense inclusions of decomposing nuclei of avian red blood cells.

In rats, no similar cellular response was detected following lesions, in particular, we did not observe SPP cells or perivascular reactive cell types, apart from the appearance of macrophages/microglia and the GFAP positive astroglial cordon demarcating the lesion. SPP cells of the bird may represent a species (class ?) - specific reactive cell type or the sustained presence of such cells in the avian brain may be associated with the difficulty in postlesion removal of nucleated erythrocytes.

COMPARISON OF THE EFFECTS OF NEUROPROTECTIVE DRUGS ON FOCAL CEREBRAL ISCHEMIA MODEL

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Occlusion of middle cerebral artely (MCA) is a widely used technique to induce focal cerebral ischaemia in rats or mice. To quantify brain injury after focal cerebral ischaemia the infarct volume is usually determined. In the present study, we made an attempt to use the infarct area on the mouse brain surface after MCA occlusion to test the effect of neuroprotective drugs.

The occlusion of middle cerebral artely was performed according to Backhaus et al. Fourty-eight hours after the occlusion the brains were perfused transcardially with tetrazolium chloride (TTC) and the unstained infarct area was determined by means of computerized image analyzing system. The infarct volume was calculated from the infarct area on coronal slices and the distance between succeeding slices. Good correlation was observed between the infarct area and the infarct volume ($r=0,80$; $p < 0.001$ $n=49$). Following reference drugs were used: MK-801, memantine (NMDA antagonists); flunarizine (calcium antagonist); dimethyl-thiourea, idebenone, (antioxidant); and vinpocetine. All these drugs, administered i.p. 30-60 min before MCA occlusion at three different doses, were able to decrease the infarct area.

The activity of the drugs in our mouse model was similar to those published using rat models of focal and global cerebral ischaemia. These findings show that the present mouse model, with a simple technique for measuring the infarct size, is suitable for screening purposes.

REFERENCES

Backhaus, C.; Karkautly, C.; Welsh, M.; Krieglstein, J: A mouse model of focal cerebral ischaemia for screening neuroprotective drug effects. J. Pharmacol. Methods 27: 27–32, 1992.

EFFECTS OF CYTOKINES AND OPIOID PEPTIDES ON THE GABA-INDUCED IONIC CURRENTS OF *LYMNAEA* NEURONS

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The aim of the present study was to demonstrate whether opioid peptides and immune messengers can modulate the ionic currents activated by classical neurotransmitters. The action of opioid peptides and cytokines was studied and compared on the GABA-induced ionic currents of intracellularly perfused and dialysed neurons of *Lymnaea stagnalis L.* using whole-cell clamp method. The GABA-induced inward currents were reduced or blocked by metenkephalin (10^{-7}-10^{-5} M) and morphiceptin (10^{-7}-10^{-5} M) in a dose dependent manner. The reversible inhibition of the GABA-induced slow inward current produced by met-enkephalin or morphiceptin was naloxone (10^{-5}-10^{-4}M) sensitive, whereas the irreversible block of the fast GABA-response was not antagonised by naloxone.

Interleukin-2 and interleukin-4 (IL-2 and IL-4) are lymphokines secreted by distinct groups of Th cells and have somehow opposite immune effects. The two cytokines modulated the GABA-currents in an opposite manner: while IL-2 inhibited GABA-response at 2-100 U/ml concentration, IL-4 (0.2-100 U/ml) augmented them. Both effects were reversible and did not affect the reversal potential of the currents. Slight and opposite modulation of temporal kinetic parameters of the currents was also observed.

Molluscan neurons prove to be a valuable tool in studying the communication and interaction between various signal systems.

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IMMUNOCYTOCHEMICAL LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTORS mGluR1a AND mGluR5 IN THE DEEP CEREBELLAR NUCLEI OF THE MOUSE

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We have investigated the immunolocalization of two metabotropic glutamate receptors (mGluR1a and mGluR5) in the medial and interposed cerebellar nuclei of 30-day-old mice by pre-embedding light and electron microscopy using antibodies produced and characterised by Görcs et al. (1993, 1995).

Immunolabelling with anti-mGluR1 a antibody was present in a neuronal population of smaller diameter in both cerebellar nuclei investigated, although, in the interposed nucleus only a proportion of the small neurons were found to be immunopositive. In the neuropil of these cerebellar nuclei the immunostaining appeared exclusively postsynaptically, in dendritic profiles of different diameter. In the large dendrites the DAB immunoprecipitate was rather flocculant whereas in the smaller ones the immunolabelling appeared to be more condensed. Both the thick and the thin dendrites received massive input from cortico-nuclear Purkinje axons, from local interneurons, as well as multiple, "en passant" synaptic contacts from mossy collaterals. In the interposed nucleus mGluR1a immunolabelled dendrites received also climbing fiber collaterals, in addition to more complex axodendritic contacts. One of these was a glomerular formation, in which large axon terminals were surrounded by numerous small-medium size mGluR1 a immunopositive dendritic profiles. Another morphological sign of the massive excitatory input in this nucleus was the appearance of unusually large mossy terminals, surrounding and contacting 34 small immunolabelled dendritic profiles or dendritic spines.

mGluR5 immunoreactivity was present only in a sub-population of the smaller size neurons, both in the medial and in the interposed nuclei. These cells had a narrow cytoplasmic rim and contained only a few cytoplasmic organelles. In the neuropil of the medial cerebellar nucleus mGluR5 immunoreactivity appeared also postsynaptically in medium or smaller size dendrites, receiving mostly inhibitory axonal input, the majority of which were varicose axons of Purkinje cells, which frequently formed "en passant" synapses with thin dendrites. In addition, inhibitory axon terminals of local interneurons (having pale axoplasmic matrix) were occasionally also contacting mGluR5 positive dendrites. The only, presumably excitatoly axon tenninals synapsing with immunolabelled dendrites were relatively small axonal endings resembling to recurrent axon collaterals of the large neurons of this nucleus. In contrast to the medial nucleus, in the interposed nucleus the dominance of inhibitory axonal input to the mGluR5 immunopositive dendrites could not be seen, the ratio of inhibitory and excitatory type of axon terminals being about the same.

REGENERATION OF THE CENTRAL NERVOUS SYSTEM IN OLIGOCHAETES. 2. GABA-IMMUNOREACTIVE ELEMENTS

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It is known that after extirpation of ganglia of the ventral cord or the cerebral ganglion regeneration takes place in oligochaetes. The complete regeneration of the cerebral ganglion, however, requires that the pharynx must be left intact. Therefore it seems probable that there are neuroblasts within the organs in the adult earthworms and they might be transformed into an active stage following extirpation. In our earlier work, GABA-immunoreactive nervous elements have been mapped in both the central and peripheral nervous system of the earthworm. In the present study, the appearance and formation of the new GABAergic elements (cells and fibres) in the regenerating nervous tissue were investigated. The new GABA-immunoreactive neurons appear, similarly to the serotonergic neurons, by the 3rd postoperative day (20-22 neurons), and later their number gradually increases. The development of the new brain can be divided into two distinct stages, as the appearance of the new GABA-immunoreactive neurons and their migration within the preganglion. In the first period of the regeneration (1 h-3 day), the number of GABA-immunoreactive neurons increases gradually, probably by division of the neuroblast cells situated in the dorsal pharyngeal wall. These cells move along the pharynx to the site of the preganglion. The migration of the outer neuroblast, situated on the surface of the ventral cord ganglia or within the ganglia, has not yet been observed. The structure of the newly formed brain becomes similar to the structure of the intact ganglion by about the 72-76th day.

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A NEW MODEL OF BACTERIAL MENINGITIS IN 14-DAY-OLD RATS WITH MEASUREMENTS OF THE VASOPRESSIN mRNA LEVELS IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI

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Background/aim: Despite the newly introduced programmes of vaccination, bacterial meningitis (BM) remains one of the main clinical problems during the early period of human life. The disease is very often accompanied by disturbed water and salt balance leading to brain oedema. The aim of the study was to develop an animal model of BM in suckling rats with measurements of the arginine vasopressin (VA) mRNA levels in hypothalamic brain nuclei.

Subjects: Three groups of intraperitoneally pentobarbitone anesthetized, male Wistar rats (number of animals: 12, in each experimental group).

Interventions: Group 1: Cerebrospinal fluid (CSF) was obtained from the cisterna magna, and 200 ng/bwkg *Escherichia coli* O111 B4 endotoxin, dissolved in artificial CSF, was given intracisternally (IC). Group 2: Pure artificial CSF was given IC. Group 3: sham-operated rats. Four hours later cisternal CSF was sampled and white blood cells (WBC) and protein concentration (PC) were determined. Plasma (P) and urine (U) osmolality (O) were also measured. Expression of the VA mRNA in the nucleus supraopticus (NSO) and paraventricularis (NPV) was investigated on brain cryostat sections by in situ hybridization analyses.

Results: A highly significant ($p < 0.001$) pleocytosis and elevation in CSF PC developed in group 1 (520, ± 113 WBC/ml, 2.12, ± 0.43 g protein/l) compared both to values measured in group 2 (4.4, ± 1.6 WBC/ml, 0.22, ± 0.08 g protein/l) and in group 3 (6.5, ± 2.2 WBC/ml, 0.18, ± 0.1 g proteinA). Significantly ($p < 0.05$) increased VA mRNA levels were observed in the NSO (percent change 120.3, ± 2.4 %) in group 1, compared to values measured in group 2 (100%) (data represent mean ± SD, Student's t-test). There were no significant differences regarding to VA mRNA levels in the NPV, and in the O values measured in P and U (data not shown).

Conclusion: IC injection of endotoxin resulted in CSF changes typical to BM in suckling rats, similarly to our previous findings obtained on newborn pigs (Temesvári et al. 1993 Pediatr Res 34:182-186). Moreover, the above data indicate, that during the very early stage of BM, an enhanced VA gene expression occurres in NSO, which phenomenon may have a pathogenetic role in the development of the accompanying brain oedema.

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FUNCTIONAL ANATOMY OF NEURONAL MICROTUBULE-ASSOCIATED PROTEIN 2

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Neuronal microtubule-associated proteins (MAPs) are morphogenetic effectors which play a crucial role in the formation and transformation of cytoskeletal structure. Their function is controlled by their level of expression and by posttranslational modifications such as phosphorylation/dephosphorylation and proteolysis. We use their most abundant representative, MAP2, to provide an example of how fine details of such a posttranslational control can be studied.

As MAP2 is multiply phosphorylated *in vivo*, specific antibodies are needed for the selective monitoring of a particular site in the molecule. We raised antibodies against both the phosphorylated and dephosphorylated forms of a proline-rich segment of MAP2 (P peptide) which is likely to be involved in proline-dependent phosphorylation events and used them for studying this epitope. We found that a significant phosphorylation in high-Mw MAP2, and dephosphorylation in low-Mw MAP2, occurs at this epitope postnatally in rat brain. In *in vitro* studies we identified glycogene synthase kinase 3 as the kinase and protein phosphatase 1 as the phosphatase most likely to be responsible for these changes.

A similar approach with Alzheimer type overphosphorylated tau protein revealed that this pathology may be due to the dysfunction of protein phosphatase 2A.

AMYLOID PRECURSOR PROTEIN IMMUNOREACTIVE STRUCTURES IN THE RAT CEREBELLUM DURING ONTOGENESIS

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The amyloid precursor protein (APP) is a transmembrane glycoprotein of neurons and glial cells. Previous findings indicate that APP promotes the differentiation of glial cells and possesses neurotropic and neurotrophic affects under *in vitro* conditions. Therefore the aim of the present investigations was to study the appearance and localization of APP in the course of postnatal development of the rat central nervous system.

Rats ageing 1, 3, 7, 14, 21 and 30 days were used in these experiments. The peroxidase-antiperoxidase technique was utilized for the demonstration of APP-immunoreactivity in 25 μm thick cryostat sections of the brain. Antibodies directed against either the N-terminal segment of APP or the complete cytoplasmic domain (anti-"Jonas") were used.

Processes of radial glial cells directing the migration of neurons showed strong immunopositivity during the first two postnatal weeks in cerebellar and cerebral cortex and hippocampus; positivity of cell bodies was also seen. The staining intensity decreased gradually from week 3 and by the end of the first postnatal month the staining pattern similar to that seen in adults. *Neuronal* APP immunoreaction in perikarya of Purkinje cells appeared during the second postnatal week. Later it became stronger but remained much fainter than processes of radial glial cells. Astrocytes, especially perivascular astrocyte feet also showed APP-immunoreactivity. Capillary endothelial cells were also immunopositive. The reaction product has been detectable in perivascular microglial cells and in ramified microglia during the early period of ontogenesis Ependymal cells lining cerebral ventricles showed conspicuous immunopositivity.

Results of immunochemical investigations and immunohistochemical study indicate that the APP content (different molecule isoforms) of central nervous system reaches its peek by the second week of postnatal life, at the critical period of neuro- and synaptogenesis. Our results would suggest that APP plays an important role in migration of neurons binding to extracellular fibronectin and laminin as a cell adhesion molecule (CAM), in differentiation and maturation based on its neurotropic and neurotrophic effect, and stabilization of synapses during the postnatal ontogenesis.

POSSIBLE ROLE OF THE SEPTOHIPPOCAMPAL GABAERGIC PATHWAY IN THETA RHYTHM GENERATION

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The septohippocampal pathway includes in addition to the cholinergic projection, GABAergic fibers which terminate specifically on hippocampal interneurons. We examined the functional effects of this pathway using septohippocampal slices from rats.

We examined the effect of septal stimulation on spontaneous IPSPs recorded with KCl filled electrodes from CA3 hippocampal pyramidal cells in the presence of CNQX (20 μM, APV (100 μM) and atropine (1±M). IPSP frequency was reduced by 73 ± 8% during trains (20-50 Hz, duration 0.2-1 s) of septal stimuli (n=16 slices). Averages of responses to single septal shocks (n=4 cells) revealed a hyperpolarisation at membrane potentials of -65 to -80 mV while IPSPs were depolarising at these potentials. In contrast, at potentials positive to the IPSP reversal in records with CsCl-filled electrodes, averages showed a depolarisation (n=3 cells). With septal stimulation monophasic hyperpolarizing IPSPs could be evoked in nonpyramidal cells. IPSPs evoked in non-pyramidal cells showed the characteristics of $GABA_A$ events, no $GABA_B$ component was detected. We aimed to investigate the effect of the septal stimulation on the action potential generation of the hippocampal pyramidal cells. With the stimulation of the medial septum (5 pulses at 20 Hz) action potentials could be evoked in hippocampal cells, but the activation of the medial septum did not evoke action potentials after each stimuli. 40% of the stimulations was found to be effective. Medial septal cells are know to have intrinsic oscillatory activity. Theta pattern stimulation (3 pulses at 5 Hz) was used to model the activity of the rhythmically discharging septal GABAergic cells. With this experimental paradigm the spontaneous IPSPs recorded from pyramidal cells could be suppressed at this frequency. This effect could be maintaned up to several seconds. The average of the traces showed that the rhythmic suppression of the spontaneous IPSPs resulted in an intracellular membrane potential oscillation in pyramidal cells at theta frequency. The average firing rate of the pyramidal cells did not change during the rhythmic septal stimulation, but all their action potentials were time-locked to the suppressed inhibition. These results suggest that one functional effect of the septohippocampal GABAergic pathway is disinhibition which may synchronize large populations of pyramidal cells at theta frequency.

DEPRESSION AND CELLULAR IMMUNITY IN MULTIPLE SCLEROSIS PATIENTS: A PSYCHONEUROIMMUNOLOGICAL STUDY

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Alterations of immune responses have been suggested to play role in the pathogenesis of several nervous system disorders including multiple sclerosis (MS). Previous studies demonstrated impaired natural killer (NK) cell activity in MS patients. To find possible correlation between immunological and mental functions in this disease, we investigated 50 patients with a definite diagnosis of MS. Progression of the disease was determined by Kurtzke expanded disability status scale (EDSS). A quick, widely used diagnostic test (Zung-test) was filled by all subjects to determine their ability of concentration, attention and the presence or absence of depression. The single cell cytotoxicity test was used to detect NK cell activity from blood samples of the patients and their age and sex-matched controls. All MS patients were free of any steroid drug treatment at least one month before the dates of examinations and in remission period of the disease. Depression was present in one fifth of the MS patients. NK cell activity, compared to patients without depression, decreased significantly in this population. There was no correlation among the Kurtzke EDSS and NK cell activity scores, nor between the depression of patients and their EDSS scores. Our results on the correlation of NK cell activity with mental condition in the MS further support findings of previous investigations suggesting that the immune system and the nervous system influence each other. To elucidate pathophysiological significance and to cover up the background of these findings dynamic, time-scheduled series of investigations are needed.

REPERFUSION BRAIN INJURY - EFFECT OF CYTOKINS AND HEMOSTATIC FACTORS ON ICAM-1 EXPRESSION IN HUMAN BRAIN ENDOTHELIAL CELL CULTURE

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Cerebral ischemia is caused by reduced blood supply at the microcirculatory level. The main goals of the therapy are to reestablish brain microcirculation in the affected region by thrombolysis and to prevent reperfusion injury. However, there is a paucity of information on how hemostatic-fibrinolysis factors and cytokines interact with human brain microvascular endothelium. Intercellular adhesion molecule-1 (ICAM-1) is expressed by endothelial cells and appears to be important in tight leukocyte binding and in their passage into the brain parenchyma. Leukocyte plugging in the vascular bed resulting from induced upregulation of adhesions is an important factor in the development of reperfusion injury.

We have studied the effect of cytokins (TNF-α, IL-lα) and hemostaticfibrinolytic factors (thrombin, plasmin, miniplasmin) on ICAM-1 expression in cultured endothelial cells prepared from human brain microvessels.

The cells were isolated and cultured with standard procedure and characterized by the uptake of DiI-AcLDL and by the presence of von Willebrand factor. For the experiments the cells (4×10^3) were seeded on 96-well microplates or on glass coverslips and cultured till confluence. The cells were treated with TNF-α, ILlα, thrombin, plasmin and miniplasmin. After 7-31 h of incubation the medium was removed and the expressed ICAM-1 was detected by cellular ELISA (with indirect immunoperoxidase staining) on microplates or visualized by Avidin-biotin method on glass coverslips. The control cells were incubated without stimuli.

TNF-α (135 U/ml) has resulted in significant ICAM-1 overexpression compared to the control (7 h: 143%; 11 h: 181%; 26 h: 175%; 31 h: 250%). IL-1α (25 U/ml) has also caused significantly increased ICAM-l expression (7 h: 136%; 11 h: 144%; 26 h: 146%; 31 h:195%). The changes have not been dependent on concentration in the range tested (TNF-α 2.7 - 270 U/ml and IL-lα 2.0 - 50 U/ml).

Thrombin (10-130 nmol/l) could have increased the ICAM-l expression consistently but not significantly (10 nmol/I: 113%; 130 nmol/1: 116%). Plasmin (20 nmol/l) and particularly miniplasmin (20 nmol/l) has reduced the ICAM-l expression (87% and 80%, respectively).

HBEC supply continuous basal ICAM-1 expression. The level of ICAM-1 is upregulated by cytokins and modified by thrombin, plasmin, miniplasmin. The interaction of these factors with the endothelial cells initiates the reperfusion injury of brain tissue by the increase of ICAM-1 expression..

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EFFECT OF INTRAAMYGDALOID BOMBESIN INJECTION ON FOOD INTAKE IN THE RAT

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Bombesin-like peptides are supposed to play a role in preabsorptive satiety. Bombesin (BN) originally isolated from amphibians - has a mammalian form, called gastrin-releasing peptide (GRP), which shares a similar C-terminal decapeptide with BN. Since BN reproduces all the known effects of GRP, this peptide is commonly used in mammalian experiments. One significant action of intraperitoneally or intracerebroventricularly (i.c.v.) applied BN is the inhibiton of feeding in either food-deprived, nondeprived, or sham-fed laboratory animals. GRP receptors in the rat brain were found by immunohistochemical and autoradiogrphical investigations with significant amounts in the hypothalamus and amygdala. The role of hypothalamus in feeding processes is well established. The amygdaloid-lesion studies suggested functional parallelism between lateral hypothalamus (LH) and the dorsomedial-central part of the amygdala (ACE), and on the other hand between the ventromedial hypothalamus (VMH) and the basolateral amygdala (ABL). It has also been shown that the amygdaloid centres only modify the hypothalamic feeding-related mechanisms.

The effects of BN have been tested on central regulatory processes by i.c.v. applications with the exception of LH, where the direct BN injection decreased food intake. On the basis of the role of amygdala in feeding processes and the immunohistochemical data, one may suppose, that centrally applied BN has an effect through amygdaloid receptors on food consumption. In the present experiments the effects of intraamygdaloid BN injection on food intake were examined. We applied 10 and 40 ng BN through formerly stereotaxically implanted bilateral chronic cannulas into the ACE or ABL of male CFY rats after 24 hour food-deprivation. The solid food consumption was measured. Locally applied BN caused transient, dose-dependent inhibition in food intake in both structures. In the case of ACE food intake reduction occurred during the first 20 minutes after 40 ng BN application, and it was followed by a "compensatory overeating" in the third 20 minutes. Interestingly, in ABL even the lower dose of BN decreased food intake in the first 20 minutes. Compared to controls, a significant food intake reduction was caused by 40 ng BN injection in the ABL lasted for the end of the first 60 minutes. No "compensatory overeating" was recorded after ABL injection. Effects of BN in the amygdala were eliminated by prior i.c.v. application of a BN antagonist in both structures.

In conclusion, our experiments demonstrated, that locally applied BN effectively inhibits feeding in both observed areas of the amygdala. However, the intensity and characteristics of BN effects are different in the amygdaloid hunger- (ACE) and amygdaloid satiety "centre" (ABL).

STUDIES OF THE GENE EXPRESSION OF CALCIUM-BINDING PROTEINS IN RAT BRAIN

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Calcium is a key element in intracellular signalling under both physiological and pathological conditions. Individual cells regulate their intracellular calcium levels by means of intricate systems, including that of calcium binding proteins. Many of these proteins share a common structural feature, i.e. they are constituted by a set of EF-hand modules, an ancient calcium-binding domain. In spite of their extensive structural homology, the functions of the members of the EF-hand family may differ significantly; some proteins act as triggers (e.g. calmodulin), whilst others serve as plain calcium buffers (e.g. parvalbumin). A number of drugs are known to act by intervening in the cellular calcium homeostasis. For example, ethanol changes the intracellular calcium level in neurons. Because of the ethanol's widespread use, it is of great importance to determine its effects on systems regulating neuronal calcium levels. Thus, we decided to investigate the withdrawal-induced alterations in the gene expression of the EF-hand-type calcium-binding proteins of different function, calmodulin and parvalbumin, in the brain of rats chronically treated with ethanol.

Unique sequences of calmodulin II and parvalbumin genes were chosen and amplified by means of PCR techniques. The isolated sequences served as templates for *in vitro* transcription producing [^{35}S]-labeled sense and antisense cRNAs. These were used as probes for *in situ* hybridization reactions performed on brain slices of control and chronically ethanol treated rats. Autoradiographic films were exposed to the slices, developed and evaluated on an image analysis system.

Supported by the U. S. - Hungarian Science and Technology Joint Fund under Project JFNo. 410

CALRETININ-IMMUNOREACTIVE STRUCTURES IN THE OPTIC TECTUM OF FROG, *RANA ESCULENTA*

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The presence of calcium-binding proteins in nerve cells is very important in regulating calcium homeostasis. Furthermore, they may also serve as specific neurochemical markers of neuron populations in the central and peripheral nervous system. Previous studies carried out on in the optic tectum of frog species showed that parvalbumin-immunoreactivity was entirely absent from this structure and 28 kDa calcium-binding protein, calbindin was found to be restricted to the mesencephalic trigeminal neurons. In the present study we aimed at examining calretinin-immunoreactive structures in the tectum of *Rana esculenta,* in order to identify specific cell populations containing this 29 kDa calcium-binding protein. Ten adult animals from both sexes were used. Vibratome or cryostat sections were cut from the fixed tectum and processed for either immunofluorescence or ABC immunohistochemistry. In some cases the left eye was enucleated from the frogs one month before the immunocytochemical processing. The results show that calretinin is present in optic axons which terminate in the B C and F sublayers of layer 9 of the optic tectum. These axons derive from specific ganglion cell populations of the retina. Local tectal neuron populations in layer 2, 4, 6 and 8 were also labelled. Layer 2, 4 and 6 cells are mostly large pear-shaped neurons while cells in the 8th layer are probably small pear-shaped cells.

Less than 10% of the cells in layers 2, 4, 6 and 8 were found to be immunoreactive for calretinin. After enucleation the stained optic axons in the superficial tectal sublayers disappeared but the local calretinin-immunoreactive cells remained largely unchanged.

The presence of calretinin in large number of optic axons and specific cell populations of nerve cells in the optic tectum indicates that this protein may be one of the major calcium regulating factors which is primarily responsible for excitability of the neurons in the tectum.

This study was supported by an OTKA grant (F 160-10).

THE BINDING OF AN ENDOGENOUS NEUROPEPTIDE MET^5-ENKEPHALIN-ARG^6-PHE^7 TO OPIOID AND SIGMA RECEPTORS IN RAT AND FROG BRAIN

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In previous meetings (MIT 1993 and 1995) we reported the characteristics of the binding of an endogenously occurring heptapeptide (^{3}H)met^5-enkephalin-arg^6-phe^7 (MERF) to opioid receptors in frog and rat brain membrane fractions. It has been confirmed that in both preparations the affinity to the opioid receptor subtypes decreased in the following order kappa$_2$>delta>mu>kappa$_1$.

The one binding site was more probable than the two calculated from the Ligand programme in homologous displacement experiments but using heterologous competitors as opioid ligands only the high affinity site was displaced by most of the applied drugs. In contrary other endogenous opioid peptides like dynorphin, leu-enkephalin and met-enkephalin were effective competitors at both sites.

Among alkaloids the highest affinity was displayed by the benzomorphan (-)-N-allylnormetazocine. The K_i value was in the rat brain fraction 0.25 nM and in the frog brain 0.2nM, whereas the K_i of the (+)-N-allyl-normetazocine was 1870 nM in rat brain and 2600 nM using frog brain membrane fractions. The K_i of haloperidol in the rat brain was 1750 nM.

These data let us to conclude, that the high affinity site is of opioid character, probably a new subtype of MERF-binding opioid receptor and the low affinity site could be one of the sigma-receptor subtypes (sigma$_2$).

ULTRASTRUCTURAL ANALYSIS OF GRAFTED RAT PINEAL ORGAN

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Forty whole pineals from 6-day-old rats were transplanted into an incised bed of the parietal cortex of adult rats, of which 29 survived 4-5 weeks after transplantation. The pinealocytes and capillaries in the grafts were comparable in structure to those in the control. Grafts were demarcated from the host cortical tissue by a double, meninx *plus* gland-capsule sheath through which no nerve ingrowth was seen into the graft from the host brain. On the other hand, sympathetic nerves originating from the cervical ganglia reached the grafted pineal along the perivascular spaces of blood vessels, as is the case *in situ*. On this basis, the present meningeal graft is thought to be a model of the pineal gland surviving without its intracerebral neural control.

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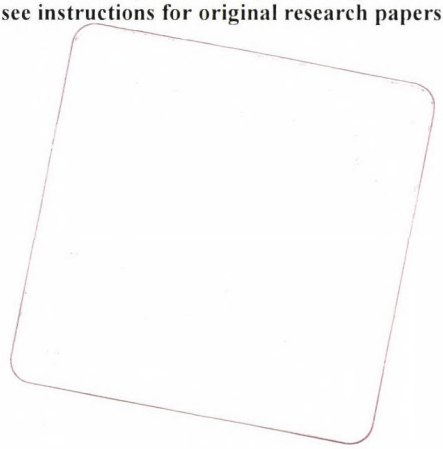
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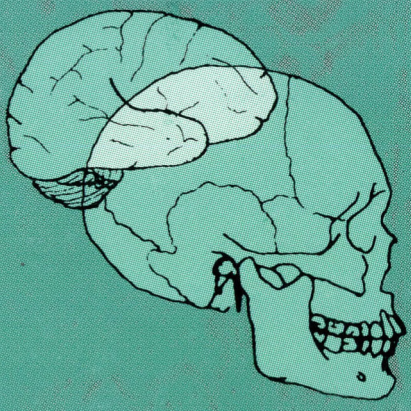
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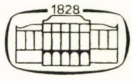
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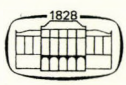
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Research report

AMINO ACID DISTRIBUTION IN IMMATURE RAT BRAIN

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Summary: We compared the levels of amino acids in the free pool in 6 regions (cerebral cortex, olfactory bulb, substantia nigra, globus pallidus, caudate nucleus, and spinal cord) in the newborn rat brain. The amino acid distribution was heterogeneous, with the area of highest concentration containing 2-3 fold as much as the lowest area. These differences were considerably less than those previously found for adult brain. Although some areas often contained high levels of amino acids, and others mostly low levels, the distribution of the various amino acids was highly variable. This heterogeneity of distribution in the newborn brain was different from that in the adult brain. We conclude that there is significant heterogeneity of amino acid distribution, that it is different for each amino acid, and that it undergoes major changes during development.
Keywords: Amino acid level heterogeneity; Regional distribution of amino acids; Developmental changes in brain; Newborn brain regions; Cerebral amino acids

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INTRODUCTION

Recently, in a series of publications, we tried to delineate the heterogeneity of the regional distribution of free amino acids in adult and aging brain. In these experiments we assayed the amino acids in 53 discrete brain areas of 3- and 29-month-old rats microdissected by the punch technique. The difference in concentration between the area of the lowest content and that of the highest content was on average 10-fold, with a minimum of 5-fold, and some such as GABA, 21-fold; arginine, 23-fold; taurine, 34-fold (Banay-Schwartz et al., 1989a,b; 1990a,b). In the aged brain the levels of the majority of the amino acids were decreased. We repeated these studies on human brain, assaying the amino acids for regional heterogeneity and its changes with aging, in 52 discrete areas. The heterogeneity of the amino acid distribution in the human brain was somewhat lower; but the concentration differences between the lowest and highest levels of the various amino acids were still 5-6 fold. The changes with aging were also lower in the human brain (Banay-Schwartz et al., 1992; 1993a,b). Though developmental changes in the cerebral free amino acid pool have been examined in several studies (Agrawal et al., 1966; Lajtha and Toth, 1973), the regional heterogeneity of amino acids in the immature brain has not. In the rat, postnatal changes in amino acid concentrations were measured either in the whole brain (de Guglielmone and Gomez, 1966; Oja and Piha, 1966; Agrawal et al., 1966; Agrawal and Himwich, 1970) or in the cerebral cortex (Vernadakis and Woodbury, 1962). We report here the distribution of amino acids in six selected areas of newborn and three areas of one-week-old rats.

MATERIALS AND METHODS

Sprague-Dawley newborn (3 hours after birth) and one-week-old rats were used (n = 6-12 in each group). The animals were decapitated, and the brains were removed and frozen on dry ice within one minute. Coronal sections (150 μm thick) were cut in a cryostat at -10°C, and brain areas (see Table 1) were removed under a stereomicroscope by micropunch needles with 0.2-0.5 mm inside diameter (Palkovits, 1973). During the microdissection procedure, the sections were kept between -5 and -15°C. Microdissected tissue samples were collected in 0.5-ml Eppendorf tubes, frozen on dry ice, and stored at -70°C until use.

Amino acid determination

The amino acids were assayed using reverse-phase high-pressure liquid chromatography after precolumn derivatization as described before (Banay-Schwartz et al., 1989a; Neidle et al., 1989). The frozen tissue samples were rapidly weighed, homogenized in 3% (w/v) perchloric acid, and left in suspension for 30 min at 0°C. An aliquot of this suspension was used for determination of the protein content; another portion was centrifuged, and the supernatant was neutralized and reacted with naphthylisocyanate for the amino acid assay. The excess unreacted compound was removed by cyclohexane extraction. A Shimadzu automated amino acid analysis system was used, separating the amino acids on a C18 silica column and using a fluorescence detector with norleucine as the internal standard. The sensitivity of the assay was below 5 pmol of amino acid, and variations between parallel assays of the same extract were below 5%. Since small pieces of frozen tissue cannot be weighed accurately because ice forms so rapidly on the tissue, the original tissue weights are questionable. For this reason we express results on the basis of the protein content of the tissue (nmol amino acid per mg protein). Expressing results this way does not reflect the changes in amino acid levels in tissue water during development since the protein content per unit weight of brain is lower in newborn than in adult. Changes in amino acid levels in tissue water during development have been measured previously (Lajtha and Piccoli, 1971; Lajtha and Toth, 1973; Davis and Himwich, 1973; Huether and Lajtha, 1991); and were not the focus of the present study, whose purpose was to examine the regional heterogeneity of amino acid distribution in the immature brain. Our assay of the protein content of the 52 brain areas indicated no significant regional variation (Banay-Schwartz et al., 1992); therefore, expressing the results on the basis of protein would not alter amino acid distribution as compared to expression based on tissue weight.

Protein determination

The aliquot of the tissue homogenate was brought to a volume of 1.0 ml with distilled water and 0.1 ml of 0.45% deoxycholate was added; after 10 min of standing it was centrifuged for 15 min at 3,000 × g. The protein in the pellet was assayed according to Peterson (1977). The pellet was

suspended in 0.4 ml of distilled water and 0.4 ml of reagent A (10% Na_2CO_3, 0.2% K tartrate, 0.1% $CuSO_4$, 0.8 N NaOH, 10% SDS) was added; after 10 min at room temperature 0.2 ml of 6-fold diluted Folin Ciocalteau reagent was added with mixing. After 30 min the sample was read at 750 nm using bovine serum albumin as standard. The usual range of protein to be assayed was 2-30 μg.

RESULTS

The distribution of amino acids measured in newborn brain areas and in spinal cord is shown in Table 1 (for comparison, the previously published results on adult brain are also shown in this table). The distribution of amino acids in one-week-old brain is shown in Table 2. To better visualize the differences in amino acid levels among the various areas of newborn and adult brain, in Table 3, the heterogeneity of distribution is presented as a comparison of the level in the area of the highest concentration to that in the lowest. As can be seen, the ratios in newborn brain vary about 2 to 3 times for most areas; that is, the level in the area of highest concentration is 2 to 3 fold higher than the level in the area of the lowest concentration. The heterogeneity of distribution is lower in the newborn brain than in the adult brain, with the highest to lowest concentration ratio varying between 2 and 3 in the newborn, which is less than that in the adult brain. For 16 of the 18 amino acids compared, the ratio is higher in adults, and it is about the same for 2. There are some similarities in that the olfactory bulb levels are the highest for about half of the amino acids in both newborns and adults, the substantia nigra levels are highest in about 1/3 of newborns and 1/2 of adults, and levels in the cortex are generally low at both ages. Comparison of the concentration ratios in the same areas for newborns and for adults in Table 2 shows only a few similarities, because most ratios are significantly different at the two ages. This indicates that not only the levels but also the regional distribution of amino acids changes during development. The general distribution pattern seems to be complex - an area may contain low levels of some amino acids and high levels of others - and the regional distribution pattern of the various amino acids seems to be different. In general, cortical levels are lower and olfactory lobe levels higher, but some amino acids are at their highest level, others at their lowest, in the substantia nigra or the globus

Table 1. Amino acid levels in newborn and adult rat brain areas

nmol amino acid per mg tissue protein												
	Newborn						Adult					
	Cer. cort.	Olf. bulb	Subst. nigra	Glob. pall.	Caud. nucl.	Spin. cord	Cer. cort.	Olf. bulb	Subst. nigra	Glob. pall.	Caud. nucl.	Spin. cord
Aspartate	18	22	17	38	17	17	36	44	44	12	27	21
Glutamate	49	74	40	83	52	49	73	96	35	23	75	30
Serine	16	25	27	15	24	33	18	22	12	9	17	4.8
Glycine	15	38	25	18	22	38	10	12	23	10	8	27
Glutamine	47	82	38	87	56	89	42	93	45	20	55	20
Taurine	175	210	62	290	210	90	39	200	38	28	70	5.3
Threonine	4.2	14	14	10	15	7.8	6.8	14	11	4.2	7.5	3.7
Alanine	14	12	24	8	21	9.7	8.3	20	9	2.5	9	3.8
GABA	26	35	72	27	38	20	29	106	154	35	29	8.1
Histidine	2.3	1.9	2.3	0.8	1.6	2.0	1.0	2.9	1.3	0.4	-	0.7
Arginine	2.2	3.0	3.5	5.8	4.2	4.1	1.9	3.0	7.3	2.9	-	3.4
Tyrosine	2.2	3.7	2.8	1.6	1.7	3.1	1.1	1.2	1.4	0.4	1.3	1.3
Valine	2.7	5.3	4.3	3.8	3.6	2.5	4.1	6.6	4	1.7	6	5.9
Methionine	0.7	0.8	0.8	1.0	1.1	1.3	1.5	1.1	2.1	1.6	-	0.5
Isoleucine	1.2	2.6	2.6	2.4	2.1	1.4	1.0	1.0	1.3	0.3	0.8	0.5
Leucine	2.8	3.6	3.7	1.6	3.6	3.3	1.7	2.7	3	0.8	1.6	1.3
Phenylal.	1.3	1.9	2.1	3.6	2.2	1.8	1.2	1.3	2.3	0.7	1.4	1.1
Ornithine	0.9	2.8	2.1	1.8	1.4	2.7	-	0.8	0.4	-	-	-
Lysine	2.9	4.4	3.4	3.4	2.9	3.2	2.5	5.8	3.8	1.4	-	3.9

Areas from two animals were pooled for each sample, and the results of assays of 3 such samples (6 for spinal cord) for newborn central nervous system regions are shown. The standard deviations among samples was about 20% for the amino acid at higher levels, and 30% for those below 5 nmol/g protein. This represents animal to animal variations since duplicate assays of the same sample varied less than 5%. For comparison, data published previously on adult brain distribution (Banay-Schwartz *et al.* 1989a,b; Banay-Schwartz et al. 1990a,b) in the same regions are given.

pallidus. The concentration of glutamate and aspartate in the cerebral cortex and the olfactory bulb is higher in the adult than the newborn animals. Taurine is present in all of the investigated brain areas at birth in much higher concentration than in adult rats (Table 1).

Table 2. Amino acid levels in one-week-old rat brain areas

	nmol amino acid per mg tissue protein		
	Cerebral cortex	Olfactory bulb	Spinal cord
Aspartate	28	28	24
Glutamate	64	79	76
Serine	20	26	36
Glycine	19	18	56
Glutamine	36	60	93
Taurine	157	187	83
Threonine	6.4	11	8.8
Alanine	17	14	12
GABA	17	30	23
Histidine	2.2	2.5	1.9
Arginine	2.0	3.6	5.6
Tyrosine	1.3	3.9	2.2
Valine	3.4	3.2	2.1
Methionine	3.8	0.8	0.8
Isoleucine	0.8	1.3	1.5
Leucine	1.7	2.8	3.6
Phenylalanine	1.0	1.5	1.7
Ornithine	1.3	1.8	1.0
Lysine	2.0	3.6	2.4

Areas from two animals were pooled for each sample, and the results of assays of 3 such samples (6 for spinal cord) for one-week-old central nervous system regions are shown. The standard deviations among samples was about the same as for newborn brain areas.

GABA is highly concentrated in the substantia nigra and olfactory bulb of the adult in comparison to those of the newborn and one-week old rats

(Table 1). The concentrations of the other amino acids, with certain variations, changed less during development. The ratio of amino acid levels of the olfactory bulb versus cortex in the one-week-old brain was often different from the value for the newborn brain (Table 2).

Table 3. Comparison of concentrations in the areas measured

	highest: lowest ratio for newborns		same areas for adults	highest: lowest ratio for adults	
Amino acid	ratio	area ratio	ratio	area ratio	ratio
Aspartate	2.2	A	0.27	C	3.7
Glutamate	2.0	A	0.66	G	4.2
Serine	1.8	C	1.3	G	2.4
Glycine	2.5	B	1.2	C	2.3
Glutamine	2.3	A	0.44	G	4.7
Taurine	4.7	A	0.74	G	7.1
Threonine	3.3	B	2.1	G	3.3
Alanine	3.0	C	3.6	G	8.0
GABA	2.8	D	5.3	D	5.3
Histidine	2.9	C	3.3	G	7.3
Arginine	2.6	F	1.5	D	3.8
Tyrosine	2.3	G	3.0	C	3.5
Valine	2.0	B	1.0	G	3.9
Methionine	1.6	E		C	3.5
Isoleucine	2.2	B	0.73	C	4.3
Leucine	2.3	C	3.8	C	3.8
Phenylalanine	2.8	F	0.58	C	3.3
Ornithine	3.1	B			
Lysine	2.3	B	2.3	G	4.1

A = Globus pallidus/substantia nigra; B = Olfactory bulb/cortex; C = Substantia nigra/globus pallidus; D = Substantia nigra/cortex; E = Caudate nucleus/cortex; F = Globus pallidus/cortex; G = Olfactory bulb/globus pallidus.

For comparison, the concentration ratios of highest to lowest area in newborn brain are given in column 2. The areas that this ratio was derived from are given in column 3. Column 4 shows the concentration ratios in the same areas (of column 3) for adult brain. Column 5 shows the areas of highest and lowest level in adult brain, and column 6 shows the concentration ratios for adult areas.

DISCUSSION

Our results show that the distribution of the free amino acid pool is heterogeneous in the immature brain, as had been shown previously for adult brain. From the assay of only six areas, several differences can be seen between the distribution in newborn brain and that in adult. The degree of regional heterogeneity in the newborn tissue is somewhat lower than that in the adult, although even in the few areas assayed the concentration of most amino acids varies 2-3 fold. The pattern is also different - the region of the highest levels for the newborn is usually different from that for the adult. Data presented here on the concentrations of the major amino acids in the various brain areas are in good agreement with previous measurements on the whole rat brain (Oja and Piha, 1966; de Guglielmone and Gomez, 1966; Agrawal et al., 1966; Agrawal and Himwich, 1970) or on the rat cerebral cortex (Vernadakis and Woodbury, 1962). The postnatal increase in glutamate, aspartate, and GABA, and the decrease in taurine and glycine, during development indicate structural and functional differentiation of the central nervous system. The progressive elevation of glutamate and aspartate concentrations in the cerebral cortex may be related to the development of dendrites and axons of the cortical projecting neurons. Our knowledge is not sufficient to indicate the functional significance of the heterogeneous distribution of cerebral amino acids or the influence that the changes in level have. The metabolic turnover - the metabolic degradation of amino acids, and the incorporation of amino acids into proteins - is rapid. This can be illustrated by the rate of use of amino acids for protein synthesis. In the tissue the amino acids are primarily protein-bound - the level of protein-bound essential amino acid is 100-600 times as high as it is in the free pool. The net synthesis of proteins in newborn brain is 1%/h (Dunlop et al., 1978), at which rate the content of the free pool would be used up for protein formation in less than 10 minutes if not rapidly replaced. There is in newborn a 2%/h rate of incorporation, balanced by 1%/h breakdown and 1%/h net synthesis; thus the actual incorporation in less than 5 min equals the free pool content, indicating a very rapid turnover of the free pool. It has to be emphasized that there is no convincing evidence that free amino acid levels influence protein metabolic rates.

The significance of the developmental changes in amino acid levels also has not been elucidated. The increase in the level of nonessential amino acids, including neurotransmitter amino acids, in parallel with increased

neurotransmission activity and the decrease in essential amino acid levels in parallel with decreased protein turnover, during brain development suggest some functional role, but this role could be more in influencing neurotransmitter rather than protein synthesis, since there is no evidence supporting the dependence of protein synthesis rates in vivo on amino acid levels in the free pool. The fact that both phenylalanine and tyrosine are heterogeneously distributed may influence dopamine or norepinephrine synthesis, but this needs to be established. It has been shown in several studies that increasing the level of neurotransmitter precursor amino acids leads to an increase in the level of the neurotransmitter derived from the precursor (Fernstrom, 1983; Fernstrom and Wurtman, 1972; Gibson and Wurtman, 1978), whereas increase of the precursor level had no effect on protein synthesis (Dunlop et al. 1993). An interesting task would be to identify the factors involved in the heterogeneous distribution of amino acids. The transport processes of amino acids are group-specific, and do not distinguish significantly between members of the same transport group, so the large neutral amino acids valine, leucine, isoleucine, and phenylalanine are transported by the same system, although their regional distribution is different. This would indicate that although transport is an important factor, it does not by itself explain the distribution pattern. Factors of influence would most likely be differences in glial neuronal uptake and metabolism, vesicular transport, transport affected by neuronal activity, local blood flow, etc. The regional heterogeneity shown here probably represents an underestimate, and if more areas had been assayed the difference between the highest and lowest level might have been greater, and if smaller areas of specific cells were assayed, differences might be even greater. It is likely that this distribution heterogeneity is of functional importance, but its role remains to be revealed.

REFERENCES

Agrawal, H.C., Davis, J.M. and Himwich, W.A. (1966) Postnatal changes in free amino acid pool of rat brain. J. Neurochem. 13, 607-615.

Agrawal, H.C. and Himwich, W.A. (1970) Amino acids, proteins and monoamines of developing brain. In: Developmental Neurobiology (ed. Himwich, W.A.) pp. 287-310, C.C. Thomas, Springfield.

Banay-Schwartz, M., Kenessey, A., DeGuzman, T., Lajtha, A. and Palkovits, M. (1992a) Protein content of various regions of rat brain and adult and aging human brain. Age 15, 51-54.

Banay-Schwartz, M., Lajtha, A. and Palkovits, M. (1989a) Changes with aging in the levels of amino acids in rat CNS structural elements: I. Glutamate and related amino acids. Neurochem. Res. 14, 555-562.

Banay-Schwartz, M., Lajtha, A. and Palkovits, M. (1989b) Changes with aging in the levels of amino acids in rat CNS structural elements: II. Taurine and small neutral amino acids. Neurochem. Res. 14, 563-570.

Banay-Schwartz, M., Lajtha, A. and Palkovits, M. (1990a) Changes with aging in the levels of amino acids in rat CNS structural elements: III. Large neutral amino acids. J. Neurosci. Res. 26, 209-216.

Banay-Schwartz, M., Lajtha, A. and Palkovits, M. (1990b) Changes with aging in the levels of amino acids in rat CNS structural elements: IV. Methionine and basic amino acids. J. Neurosci. Res. 26, 217-223.

Banay-Schwartz, M., Lajtha, A. and Palkovits, M. (1992b) Regional distribution of glutamate and aspartate in adult and old human brain. Brain Res. 594, 343-346.

Banay-Schwartz, M., Palkovits, M. and Lajtha, A. (1993a) Heterogeneous distribution of functionally important amino acids in brain areas of adult and aging humans. Neurochem. Res. 18, 417-423.

Banay-Schwartz, M., Palkovits, M. and Lajtha, A. (1993b) Levels of amino acids in 52 discrete areas of postmortem brain of adult and aged humans. Amino Acids 5, 273-287.

Davis, J.M. and Himwieh, W.A. (1973) Amino acids and proteins of developing mammalian brain. In: Biochemistry of the Developing Brain (ed. W. Himwich), pp. 55-110. M. Dekker, New York.

De Guglielmone, A.E.R. and Gomez, C.J. (1966) Free amino acids in different areas of rat brain. Acta Physiol. Lat. Amer. 1, 26-31.

Dunlop, D.S., Van Elden, W. and Lajtha, A. (1978) Protein degradation rates in regions of the central nervous system in vivo during development. Biochem. J. 170, 637-642.

Dunlop, D.S., Yang, X. and Lajtha, A. (1993) Elevated plasma phenylalanine and brain protein synthesis rates. Proc. Am. Soc. Neurochem. 24, 113.

Fernstrom, J.D. (1983) The role of precursor availability in the control of monoamine biosynthesis in brain. Physiol. Rev. 63, 484.

Fernstrom, J.D. and Wurtman, R.J. (1972) Brain-serotonin content: physiological regulation by plasma neutral amino acids. Science 178, 414-416.

Gibson, C.J. and Wurtman, R.J. (1978) Physiological control of brain norepinephrine synthesis by brain tyrosine concentration. Life Sci. 22, 1399-1406.

Huether, G. and Lajtha, A. (1991) Changes in free amino acid concentrations in serum, brain, and CSF throughout embryogenesis. Neurochem. Res. 16, 145-150.

Lajtha, A. and Piccoli, F. (1971) Alterations related to the cerebral free amino acid pool during development. In: Cellular Aspects of Neural Growth and Differentiation (ed. D.C. Pease) UCLA Forum Med. Sci. 14, 419-432, University of California Press, Los Angeles.

Lajtha, A. and Toth, J. (1973) Perinatal changes in the free amino acid pool of the brain in mice. Brain Res. 55, 238-241.

Neidle, A., Banay-Schwartz, M., Sacks, S. And Dunlop, D.S. (1989) Amino acid analysis using 1-naphthylisocyanate as a precolumn HPLC derivatization reagent. Anal. Biochem. 180, 291-297.

Oja, S.S. and Piha, R.S. (1966) Changes in the concentration of free amino acids in the rat brain during postnatal development. Life Sci. 5, 867-870.

Palkovits, M. (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. 59, 449-450.

Peterson, G.L. (1977) A simplification of the protein assay method of Lowry et al., which is more generally applicable. Anal. Biochem. 83, 346-356.

Vernadakis, A. and Woodbury, D.M. (1962) Electrolyte and amino acid changes in rat brain during maturation. Amer. J. Physiol. 203, 748-752.

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Research report

SOME DOUBTS ABOUT THE BASIC CONCEPT OF HOLE-BOARD TEST

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Summary: Hole board test is a generally used method for screening the potential anxiolytic character of drugs. The test is based on the assumption, that head-dipping activity of the animals is inversely proportional to their anxiety state. We tested this assumption by measuring the head-dipping activity of animals in environments with different levels of aversive character (illumination). The anxiolytic chlordiazepoxide significantly elevated head-dipping activity in moderately aversive environment, it was not active in non-aversive environment, and it exhibited inhibitory activity in highly aversive environment. When the latency of the first head-dip was measured, we found that the proportion of animals with short latency was significantly increased in moderately and highly aversive environments. It is concluded, that the inverse relation between anxiety state and head-dipping activity is true only in a certain range of anxiety level. In more aversive situations, when the anxiety level of the animals is high, the holes nay represent a possible way to escape from the aversive environment instead of an explorable object. In this case the relation between anxiety state and head-dipping activity is directly and not inversely proportional.
Keywords: anxiety level, hole-board, rat, escape reaction

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INTRODUCTION

Hole-board test, as many of the currently used models of anxiety relies on the animal's exploratoly behaviour. The hole board apparatus is a box with a number of holes (usually four) in the floor through which an animal can poke its head. The method was introduced by Boissier and Simon (1964) as a suitable tool for separating the locomotor activity from the exploratory behaviour of the mice. The authors concluded that the hole-poking reflects directed exploration (investigation), and it was validated by File and Wardill (1975).

The use of hole-board method as a test for anxiolytic activity has relied on the hypotheses that the behaviour of animals exposed to a novel situation resulted from a competition between an exploratory tendency (motivated by curiosity or boredom) and a withdrawal tendency (motivated by fear) (Montgomery, 1955). According to the hypotheses elevation in anxiety state results a decreased head-dipping activity. Anxiolytic drugs, such as benzodiazepines increase head-dipping activity (Nolan and Parkes, 1973) presumably by reducing the anxiety state of the animals, thus releasing the natural tendency of explotation (Crawley, 1985). However, it was described (Welker, 1959) that locomotor activation may reflect an increase in escape behaviors. Rats display hyperactivity when exposed to an inescapable novel situation (Mogenson and Nielsen, 1984), so fear may induce not only a passive withdrawal, but an active escape reaction, as well. Moreover, this may include exploratory elements, too. Certain drugs that appear to lack anxiolytic effects, such as RO 15-1788 (flumazenil), also increase head-dipping activity (File et al., 1982), while decreased exploration can also be observed following treatment with sedatives (Lister, 1990), so head-dipping activity does not exclusively reflect changes in the level of anxiety. A further disadvantage of the model is that reference anxiolytic drugs show inconsistent effects; the extent of the inconsistency is unusual even in anxiety models. Decrease in exploration of rats are observed following the administration of even low doses of benzodiazepin acutely (File, 1981). In mice, decrease in head-dipping activity can also be observed following moderate doses of benzodiazepines (Lister, 1987a) or ethanol (Lister, 1987b). Head-dipping activity depends on among others the time of the day, or on the sensation of hunger (File, 1973).

Despite that hole-board test is a simple and fast method, easy to automatize, due to the above-mentioned problems it has not gained too much popularity. The controversions in the literature and the following observations led us to question the reliability of this method as anxiolytic

screening test: a: In some of our experiments reference anxiolytics in non-sedative doses tended to decrease the head-dipping activity of the animals in this test. b: The anxiogenic pentetrazole and m-chlorophenylpiperazine (mCPP) in some (low) doses elevated the number of nose-pokes (unpublished observation). c: Some animals when tested in aversive environment (in intensive light) just after placing them into the hole-board apparatus jumped to the hole and sticked their heads into the hole. The aim of our study was to investigate the influence of the aversivity of the environment on the behaviour of the animals in the hole-board apparatus, and its interaction with drug effects. We performed experiments measuring the hole-board activity of the animals in three test situations differing in their level of aversiveness.

METHOD

Effect of the level of illumination on hole-board activity

160-200 g male Wistar rats (Charles River) were fasted for 24 hours prior to the experiment. The animals were treated orally one hour before testing with 0.4% methylcellulose (control group) or chlordiazepoxide in the dose of 5 mg/kg (volume 5 ml/kg). All procedures were carried out in a quiet, air-conditioned laboratory between 09:00 and 13:00 h at ambient temperature of 23 ~2 °C. Head-dipping activity was measured using automated test chambers (Digiscan, RXYZCM16, OMNITECH Electronics Inc., Columbus, Ohio). Six test chambers were connected to the monitor (Digiscan Analyser, OMNITECH Electronics Inc., Columbus, Ohio), so the activity of six animals was measured simultaneously. The floor of each box contained 4 equally spaced holes (3.5 cm in diameter) in which the rat could dip its head. Interruptions of 16 infrared beams located 1.0 cm below the floor level were automatically recorded by the Digiscan analyser and than transmitted to a computer and analysed by OASIS software. We used three types of illumination conditions: dark (7 lux), light (200 lux) and bright (1000 lux). The light sources were: 7 W linear fluorescent lamp at 3 m high, directed to the ceiling (7 lux); normal neon lighting (200 lux); 23 W compact fluorescent lamps at 30 cm high above each box (1000 lux). The hole-board testing involved placing the rat in the center of the floor and allowing it to explore for 3 minutes measured as three-one minute intervals. At the end of each session any boluses were removed and the box was thoroughly wiped. Head-dipping activity was recorded as the total number of beam interruptions.

Statistical significance was evaluated with two-way ANOVA (treatment and illumination) followed by Duncan-test.

Effect of the level of illumination on the time elapsed until the first nose-poke

160-200 g male Wistar rats were kept in a sound-attenuated, dimly lit room for 6 hours. After this resting period they were carried individually to the hole-board apparatus in a dark container, the illumination was set and the experimental session started. The time elapsed until the first nose-poke was measured. Three types of lighting conditions were used: dark (7 lux), light (200 lux) and bright (1000 lux). The light sources were the same described above. At the end of each session any boluses were removed and the box was thoroughly wiped. Data were normalized, statistical significance was calculated with Kruskal-Wallis ANOVA. For further analyses three subgroups in each group were created and they were compared with chi-square test.

RESULTS

Effect of the level of illumination on hole-board activity

The effect of chlordiazepoxide on head-dipping activity in various lighting conditions can be seen in Fig. 1. Statistical investigation of the data revealed significant interaction between treatment and lighting conditions ($F(2,128)=5.253$; $p=0.0064$). In dark environment animals displayed a considerable amount of head-dips which was not affected chlordiazepoxide treatment. In light environment the basal head-dipping activity of the animals was low and chlordiazepoxide significantly elevated it. In bright environment the head-dipping activity of the saline-treated animals was high, and it was significantly antagonized by chlordiazepoxide. (Fig. 1.)

Effect of the level of illumniation on the time elapsed until the first nose-poke

The data was lognormally distributed, so it was normalized before any statistical examination. (Fig. 2). The variance of the data significantly differed according to Bartlett-test ($p<0.001$), so Kruskal-Wallis ANOVA was

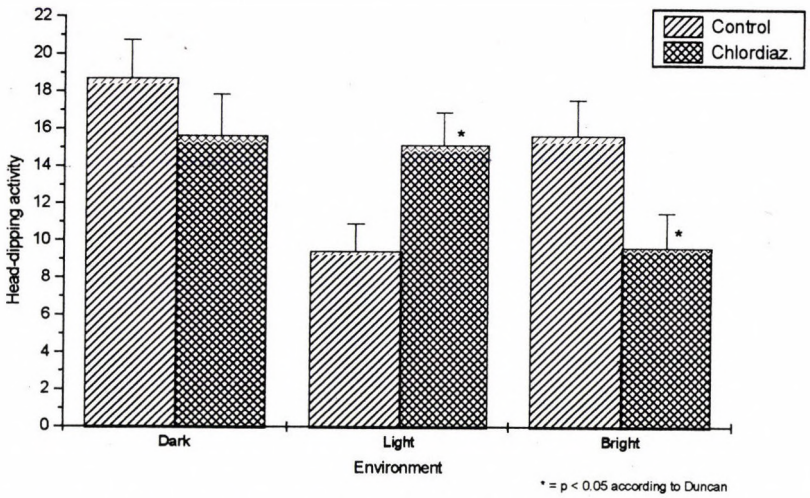


Fig. 1. Effect of chlordiazepoxide on head-dipping activity of rats in hole-board test in different lighting conditions

chosen for statistical evaluation. There was no significant difference between the mean nose-poke latencies of the groups tested in differently lit environment ($H(2,240)=1.197$; $p=0.5497$) (Table 1). However, we observed that some animals when tested in brightly lit environment just after placing them into the hole-board apparatus jumped to the hole and sticked their heads into the hole. Similar behaviour was never observed in dark and very rarely in light environment.

Table 1. Head-dipping latency values in differently aversive environment

Environment	Latency (mean ± S.D.)	Ig latency (mean ± S.D.)
Dark	41.06±19.86	1.565±0.208
Light	49.09±59.02	1.402±0.592
Bright	48.03±51.63	1.454±0.507

For further analyses of the data three sub-groups were created in each group: "Fast animals", whose head-dipping latency was lower than the mean latency in the given group minus twice the standard deviation in the dark group. "Normal animals", whose head-dipping latency was within the range of mean latency in the given group plus-minus twice the standard deviation

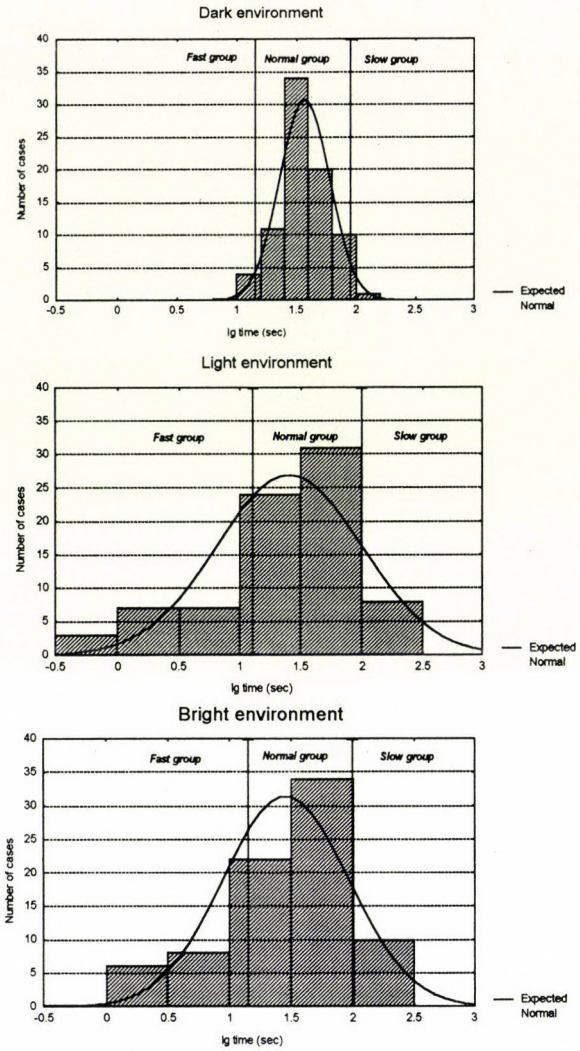


Fig. 2. Distribution of latency time of first head-dipping of rats in hole-board testing differently lit environments

in the dark group - "Slow animals", whose head-dipping latency was higher than the mean latency in the given group plus twice the standard deviation in the dark group (Table 2).

Table 2. The ratio of animals in sub groups created from head-dipping latency values

Environment	Fast animals	Normal animals	Slow animals	Total
Dark	3	76	1	80
Light	18	54	8	80
Very light	16	54	10	80

The propotion of the "slow animals" increased in the light and bright environment compared to the dark group, although this change failed to reach the level of significance according to chi-square test (χ^2=7.66, df.=5, p = 0.176). There was a significant elevation (χ^2=12.75, df.=5, p = 0.026) in the proportion of "fast animals" in the light and bright groups.

DISCUSSION

Anti-anxiety drugs increase exploratory behaviour in several paradigms as it was demonstrated in the open-field test (Christmas and Maxwell, 1970), hole-board test (Nolan and Parkes, 1973), light-dark transition test (Crawley, 1981) and plus maze test (Lister, 1987c). The theoretical explanation for this effect is a drug-induced inhibition of certain fearful responses to a strange or aversive environment, thus releasing and enhancing the natural tendency of the animal to explore any new habitat (Crawley, 1985). It also follows from this argumentation that as the animal's level of anxiety increaes, its exploratory activity decreases giving place to the manifestation of fear motivated behaviour. High level of anxiety results in decreased head-dipping activity, low level of anxiety means high head-dipping activity: it is the basic assumption of the hole-board paradigm, but contradictory results were obtained by several authors. We tested head-dipping activity in the hole-board apparatus as a measure of directed exploration in three groups of animals with different levels of anxiety. The anxiety level of the animals was influenced by illumination. Nocturnal rodents, such as rats and mice generally prefer dimly lit environment, and it is assumed that increasing the level of illumination elevates the anxiety state of the animals (Costall, et al., 1989, Onaivi and Martin, 1989). In our first experiment we found that when the animals were tested in the dimly lit arena the head-dipping activity of the rats was high in the control group, and an anxiolytic drug could not elevate it probably because of a ceiling effect. In this relatively little aversive environment the rats were supposingly in low general anxiety state resulting in nearly maximal exploration which could not be increased further. In the light environment the exploratory activity of the control animals was lower

reflecting somewhat more elevated anxiety level, and chlordiazepoxide showed the expected anxiolytic activity, namely it increased the number of head-dips. In the highly illuminated (aversive) "bright environment" the control activity was again higher than in the less stressful "light environment"; moreover, inverse effect of the drug was detected. File (1985) interpreted the inverse activity of low dose benzodiazepine as the result of the sedative action of the drug. An alternative possible explanation for this observation may be that in very aversive environments the hole means not an explorable place for the animal, but rather a possible way to escape from a fearful environment. When the anxiety level of the animals is too high (they are stressed for example), the fearfulness may results in intense escape attempts manifested by high head-dipping activity. We hypothesize that the anxiety level of the animal forms a continuum along of which different types of behaviours appear depending on the intensity of anxiety. In low anxiety states exploratory behaviour is diplayed to a great extent. As the level of anxiety increases exploratory activity decreases. Further increase in anxiety gives rise to active escape reactions which can reach the state of panic-like activity (Fig. 3).

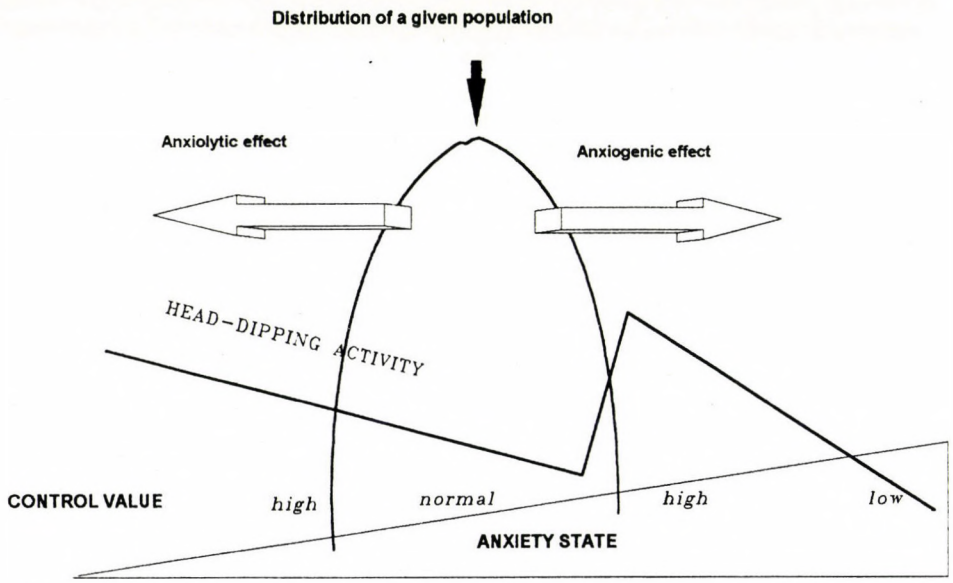


Fig. 3. Hypothetical relationship between anxiety state and head-dipping activity of rats in hole-board apparatus

At the top of the scale anxiety is so high that it even inhibits the active escape reaction and freezing becomes the dominant response of the animal to novel environment. When the animals are emotionally in this state, one can measure inverse drug effect, since decrease in anxiety results in decrease of active behaviour. When the experimental group consists of animals with different level of anxiety an anxiolytic compound can exert various (even opposite) effects in the individual animals. This strongly elevates the uncertainity of the results since the overall effect of the drug depends on the proportion of the animals with low, medium and high anxiety levels in the given sample. Hole-board test is especially sensitive to this effect, because opposite changes in the level of anxiety may result in the same effect, i.e. higher head-dipping activity. This means that head-dipping activity sometimes directly correlates with exploratory activity (and negatively with anxiety), sometimes inversely does it and yet in other cases it shows no correlation at all. When the animal group is not homogenous in respect of anxiety level (and usually it is the case), there are animals that react normally, inversely or do not react at all to anxiolytic treatment. The type of reaction chosen depends probably on the basal, individually characteristic anxiety state of the animal.

Our second experiment measuring the latency of the first head-dip in differently illuminated environments provided further evidence for our hypothesis. We found a non-significant elevation in the proprtion of animals with high latency values ("slow animals"), and a significant elevation in the ratio of animals with low latency values ("fast animals") in aversive conditions (light and bright environment). This finding suggests that the animals can react in two ways as their anxiety level is increased: the first is a slower, more cautious approach to and exploration of the hole, the other is an escape-like reaction presumably form a brightly lit environment to a safe, dark place. This assumption can also explain the fact why the mean latency of the first head-dip did not changed significantly over the levels of illumination. Most of the animal models of anxiety disorders have some common undesirable features, such as the large variance of the data or the inverted U-shaped and rather varying dose-response curve (Gyertyan, 1992). These properties may account for the relative uncertainity of the effectiveness of reference drugs in anxiolytic tests. As we shown above, hole-board test inherently involves an additional factor which renders this test very unreliable even among the anxiolytic methods. Lister stated in his article (Lister, 1990) that "it seems premature to accept the hole-board as a valid model of anxiety". According to our opinion, although alteration in anxiety level induces changes in nose-poke activity, the direction of change

in an individual animal depends on a series of uncontrolled factors, so hole-board test in this form is not useful for screening anxiolytic or anxiogenic activity of drugs.

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REFERENCES

Boissier, J.-R. and Simon, P. (1964) Dissociation de deux composantes dans le comportement d'investigation de la souris. Arch.Int.Pharmacodyn. 147 (3-4), 372-387

Christmas, A.J. and Maxwell, D.R. (1970) A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behavior in mice and rats. Neuropharm. 9, 17-29

Costall, B., Jones, B.J., Kelly, M.E., Naylor ,R.J. and Tomkins, D.M. (1989) Exploration of mice in a black and white test box: validation as a model of anxiety. Pharm.Biochem.Behav. 32, 777-785

Crawley, J.N. (1981) Neuropharmacological specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmac. Biochem.Behav. 85, 695-699

Crawley, J.N. (1985) Exploratory behavior models on anxiety in mice. Neurosci.Biobehav.Rev. 9, 37-44

File, S.E. (1973) Effect of chlorpromazine on exploration and habituation in the rat. Br.J.Pharmac. 49, 303-310

File, S.E. (1981) Rapid development of tolerance to the sedative effects of lorazepam and triazolam in the rat. Psychopharm. 73, 240-245

File, S.E. (1985) What can be learned from the effects of benzodiazepines on exploratory behavior? Neurosci.Biobehav.Rev. 9, 45-54

File, S.E. and Wardill, A.G. (1975) The reliability of the hole-board apparatus. Psychopharm. 44, 47-51

File, S.E., Lister, R.G. and Nutt, D.J. (1982) Anxiogenic actions of benzodiazepine antagonists. Neurosci.Lett. 32, 165-168

Gyertyán, I. (1992) Animal Models of anxiety: A critical review. Acta Physiol.Hung. 79 (4), 369-379

Lister, R.G. (1987a) Interactions of Ro 15-4513 with diazepam, sodium pentabarbital and ethanol in a hole-board test. Pharmac. Biochem.Behav. 28, 75-79

Lister, R.G. (1987b) The effects of ethanol on exploration in DBA/2 and C57BlJ6 mice. Alcohol 4, 17-19

Lister, R.G. (1987c) The use of a plus-maze to measure anxiety in the mouse. Psychopharm. 92, 180-186

Lister, R.G. (1990) Ethologically-based animal models of anxiety disorders. Pharmac.Ther. 46, 321-340

Mogenson, G.J. and Nielsen, M. (1984) Neuropharmacological evidence to suggest that the nucleus accumbens and subpallidal region contribute to exploratory locomotion. Behav.Neural.Biol. 42, 52-60

Montgomery, K.C. (1955) The relation between fear induced by novel stimulation and exploratory behaviour. J.Comp.Physiol.Pychol. 48, 254-260

Nolan, N.A. and Parkes, M.W. (1973) The effects of benzodiazepines on the behavior of mice on a hole board. Psychopharm. 29, 277-288

Onaivi, E.S., Martin, B.R. (1989) Neuropharmacological and physiological validation of a computer-controlled two-compartment black and white box for the assessment of anxiety. Prog.Neuro-Psychopharmacol & Biol.Psychiat. 13, 963-976

Welker, W. (1959) Escape, exploratory, and food-seeking responses of rats in a novel situation. J.Comp.Physiol.Psychol. 52, 106-111

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Research report

TAU PROTEINS BIND TO KINESIN AND MODULATE ITS ACTIVATION BY MICROTUBULES

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Summary: Microtubule-associated tau proteins are likely candidates to interfere with axonal transport of membranous organelles. We studied that tau proteins influenced the enzyme activity of kinesin, known to drive anterograd transport along microtubules. An *in vitro* reconstituted system was applied; microtubules were assembled from purified tubulin with or without tau proteins. Both types of reconstituted microtubules stimulated MgATPase activity of purified kinesin in a concentration dependent, saturable manner. The extent of maximal stimulation by tau-coated microtubules was lower than that of microtubules without tau proteins. Analysis of kinetic data, on the other hand, suggests that tau-coated microtubules apparently bind kinesin with higher affinity then microtubules not associated with tau proteins. Tau proteins, similarly to tubulin dimers, seem to bind to the heavy chain of kinesin. These data support the notion that tau proteins could act as regulators of kinesin-driven processes.
Keywords: kinesin, ATPase, tau proteins, microtubule-associated proteins, regulation, organelle motility complex

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INTRODUCTION

Microtubules serve as roadways and anchoring points in the course of directed intracellular traffic of membrane-bounded organelles and soluble cell constituents. Transport along microtubules is driven by kinesin and cytoplasmic dynein (Endow, 1991; Vale, 1990), molecular motor proteins sharing the ability of interacting both with membranous organelles and with microtubules. Polarity of the microtubules determines the direction of the movement; kinesin transports its load towards the growing end (anterograde direction), while dynein moves towards the opposite direction. *In situ,* kinesin and dynein appear to associate to membranous organelles but not to microtubules (Hollenbeck, 1989; Pfister et al., 1989; Hirokawa et al., 1990; Lin and Collins, 1992). Reconstitution studies (Yu et al., 1992; Lacey and Haimo, 1992) and immunochemical analysis of purified subcellular organelles (Leopold et al., 1992; Jellali et al., 1994) demonstrate stable association between the motor proteins and membranes *in vitro* as well. Recent results indicate that organelle-specific proteins might act as regulated receptors for motor proteins (for a review, cf. Burkhardt, 1996).

Native brain kinesin is an $\alpha_2\beta_2$ heterotetramer forming a pair of globular heads and a fan-shaped tail, connected by a stalk (Kuznetsov et al., 1988). The heads carry sites for both ATP sensitive microtubule binding and ATPase activity (Yang et al, 1990), while the tail is suggested to participate in the binding to the membranous organelles (Hirokawa et al., 1989).

Functional characterization of kinesin was based mainly on studies in *in vitro* assay systems using taxol - stabilized microtubules free of MAPs (microtubule-associated proteins). These microtubules are known to stimulate kinesin ATPase activity (Kuznetsov and Gelfand, 1986), and they are able to glide on kinesin-covered glass surfaces (Vale et al., 1985; Vale, 1990). However, *in situ* the surface of microtubules is coated by MAPs; axonal microtubules are typically coated by tau proteins (Papasozomenos and Binder, 1987), while in the dendrites MAP2 is the most abundant among the microtubule associated proteins (Bernhardt and Matus, 1984). Besides binding to microtubules, MAP2 and tau proteins are both able to bind to the membranes of intracellular organelles as well as shown by our previous studies on brain mitochondria (Jancsik et al., 1989). Further studies with microtubules reconstituted from tubulin and MAP2 or tau led to the suggestion that, depending on their organelle localization, these MAPs could play either a crosslinking or a spacing role (Jung et al., 1993).

It is of importance to understand whether or not the presence of MAPs influence the functioning of kinesin.

Gliding of microtubules on kinesin- (or dynein-) coated glass surface is inhibited by surprisingly low levels of MAP2, whereas very high concentration of tau proteins should be applied to affect gliding (Paschal et al., 1989; Heins et al., 1992; Lopez and Sheetz, 1993). Steric inhibition by MAP2 has been suggested to diminish the motility of organelles *in vitro* and *in vivo* as well. Competition, cosedimentation and motility studies point toward the possibility that motor proteins, tau and MAP2 all compete for the same binding domains on microtubules. Taking into account these data, we decided to re-evaluate the possible role of tau proteins, by analysing its effect on the microtubule-stimulated ATPase activity of kinesin.

In this paper we provide evidences for direct interaction of tau proteins with the heavy chain of kinesin. We show that the presence of tau proteins affect the stimulation of the MgATPase activity of kinesin by microtubules. The possible mechanism of this effect as well as functional implications of the above findings will be discussed.

MATERIALS AND METHODS

Materials

Adenosine 5'-[γ-^{32}P]-triphosphate (^{32}P-ATP) was purchased from ICN Biochemicals, Inc.; specific activity : 167 TBq/mmol, Rotiszint 2211 was a product of Roth (Germany). Taxol was kindly provided by M. Suffness, Natural Products Branch, Division of Cancer Treatment (NCI, Bethesda, MD).

Antibodies. Monoclonal anti-α-tubulin (Tu-01) and polyclonal anti-tau serum were generous gifts of dr. V. Viklicky and dr. J. Baudier, respectively; polyclonal antibody against the head portion of the kinesin heavy chain was kindly provided by dr. V. Gelfand. Alkaline phosphatase conjugated goat-anti-mouse and goat-anti-rat second antibodies as well as the Chemiluminescent Substrate Kit were from Bio-Rad.

Protein purification

Kinesin was isolated from bovine brain as described by Kuznetsov and Gelfand (1986). Purified enzyme preparations were stored at + 4 °C; at these conditions ATPase activity was stable for at least 5 days. Prior to activity measurement the purified kinesin preparation was dialysed against IME buffer (Imidazol, 50 mM; $MgCl_2$, 3 mM; EDTA, 0.1 mM; EGTA, 0.1 mM; β -mercapto-ethanol, 1 mM; pH 6.7).

Purified tubulin and tau proteins were prepared from bovine brain. In the first step, microtubules were prepared by two cycles of polymerization-depolymerization (reassembly buffer: 0.1 M 2-[N-morpholino] ethane sulfonic acid, 1 mM $MgCl_2$ and 1 mM EGTA, pH 6.8) according to Shelansky and Gaskin (1973). Dissociation of microtubules was induced by treatment with high salt concentration to induce their dissociation, followed by ion exchange chromatography on DEAE-Sephadex (Vallee, 1986). Two main fractions were collected, which contained pure tubulin (DEAE-tubulin) and the mixture of MAPs, respectively. Further separation of the MAPs mixture on Sepharose-CL 4B (Vallee, 1986) yielded pure tau proteins.

MAPs-free taxol-stabilized microtubules (Mt) were prepared by dialysing purified DEAE-tubulin (protein concentration ≥ 2 mg/ml) against IME buffer for two hours at room temperature. Dialysed tubulin was then incubated with 20 μM taxol for 30 min at 37 °C.

Tau-coated taxol-stabilized microtubules were obtained by incubation of taxol-stabilized MAPs-free microtubules with purified tau proteins for 10 minutes at room temperature. Molar ratios of tau/tubulin dimer were calculated from the molar concentrations of the two components.

Both MAPs-free and tau-coated taxol stabilized microtubules were prepared immediately prior to use.

Protein concentrations

Protein concentrations of kinesin and microtubule solutions were determined by the Bradford assay (Bradford, 1986), with bovine serum albumin as standard. Purified tau proteins were quantified on the basis of OD_{278} value, by considering $\varepsilon_{278(1mg/ml)}=0.29$ (Cleveland et al., 1977). Molar concentrations were calculated by taking into account the following molecular masses: tau proteins (average), 58 kDa; kinesin, 372 kDa. Molar concentrations of tubulin

heterodimers (molecular mass: 110 kDa) were calculated from the protein concentration of the microtubule solutions.

ATPase assay

For enzyme activity measurement 10 µl purified kinesin solution (20-40 µg/ml) and 5 µl ^{32}P-ATP solution of appropriate concentration (cf. Figs) were incubated for 10 min at 37 °C in the presence of 10 µl taxol-stabilized microtubule suspension. Taxol-stabilized microtubules were applied either MAPs-free or tau-coated, as indicated in the legends to the figures. Reaction was stopped by adding 25 µl ice-cold 2% SDS.The amount of $^{32}P_i$ formed was determined as follows (Seals et al., 1978). First, 25 µl phosphate reagent (0.02 M silicotungstic acid, 4 N sulfuric acid and 4% ammonium molybdate) was added to each reaction mixture. The phosphate-molybdate complex formed was extracted with 500 µl organic solvent mixture (xylene : isobutanol = 35 : 65). 250 µl aliquot of the organic phase was mixed with 2 ml Rotiszint 2211 and the radioactivity was measured in a Packard Tri-Carb 4000 scintillation counter.

Immunoblot and overlay

Reactivity of the antibodies used was tested on immunoblots. Purified protein preparations (cf. 2.2.) were subjected to electrophoresis on 7.5 % lithium - dodecyl sulfate - polyacrylamide gel (LiDS-PAGE) (Laemmli, 1970), then electroblotted onto nitrocellulose membrane (Towbin et al., 1979). The membrane was cut and incubated overnight at + 5 °C with the corresponding antibody. Alkaline phosphatase conjugated goat-anti-mouse or goat-anti-rat secondary antibodies were applied with the chemiluminescent substrate AMPPD. Emitted light was detected by using Hyperfilm MP (Amersham) in an X-ray exposure cassette with two intensifying screens.

By using the above conditions, anti-kinesin reacted with the heavy chain of kinesin, Tu-01 selectively marked α-tubulin, and the polyclonal anti-tau labeled all the five bands present in our purified tau protein preparation

In the overlay experiments kinesin (3µg/well) was subjected to electrophoresis and electroblotting, as described above. The membrane was then incubated overnight at + 5 °C with DEAE tubulin (100 µg/ml) or tau proteins (100 µg/ml) in IME buffer. Unbound tubulin and tau proteins were removed by washing. Tubulin and tau proteins bound to the solid-phase

kinesin were detected by using anti-α-tubulin or anti-tau antibodies, respectively. Visualization of the primary antibodies was done as described above.

RESULTS

Purified kinesin preparations generally exhibit low MgATPase activity without microtubules, while this activity is strongly stimulated by the presence of microtubules. Under standard assay conditions our kinesin preparations hydrolyzed ≤ 0.05 μmol MgATP /mg protein/min. In the presence of saturating concentrations (≥ 1 mg/ml) of taxol-stabilized MAPs-free microtubules the hydrolysis rate was between 0.8 and 1.5 μmol MgATP/mg protein/min, corresponding to a stimulation of 25-50 fold.

Tau proteins did not cause any measurable effect on the non-stimulated MgATPase activity of the purified kinesin preparations (results not shown), while their presence significantly modified the microtubule-stimulated enzyme activity. Tau proteins, even at low levels, diminished the extent of activation by microtubules, but stimulation was not completely abolished up to 2 molecules of tau added per tubulin heterodimer (Fig. 1).

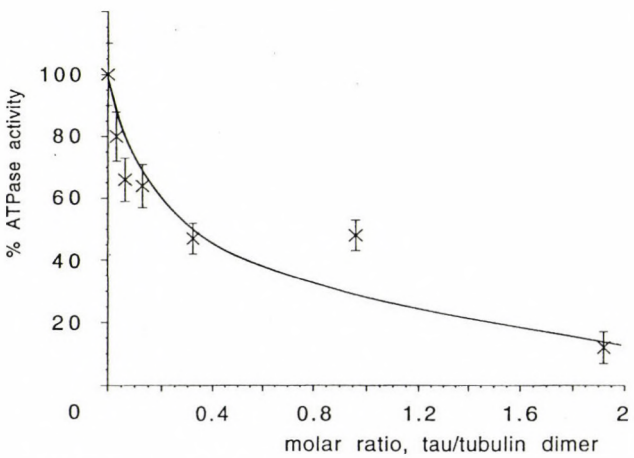


Fig. 1. Effect of tau proteins on the stimulation of kinesin ATPase by microtubules. Activities were measured at saturating MgATP concentration (1 mM); microtubule concentration was 0.3 mg/ml. MgATPase activity in the presence of MAPs-free microtubules was taken as 100%

Tubulin concentration dependence of the stimulation by MAPs-free and tau-coated microtubules is shown in Fig. 2. Tau proteins diminished the level of the maximal stimulated activity at a tau/tubulin dimer ratio as low as 1/25; this effect was somewhat more pronounced at higher tau protein levels.

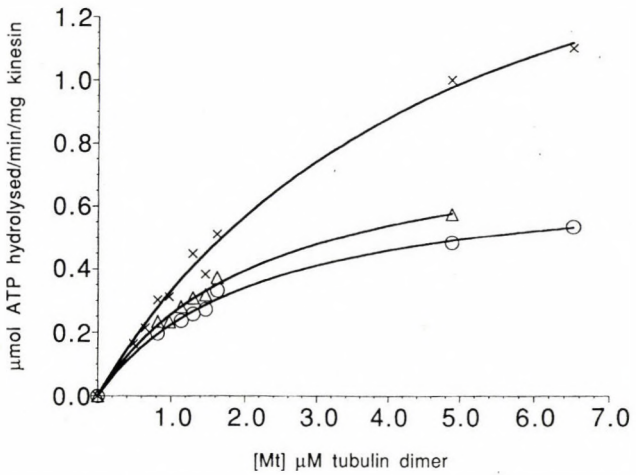


Fig. 2. Concentration dependence of the stimulation of kinesin ATPase by microtubules coated with tau proteins. ATPase activity of kinesin was measured at saturating MgATP concentration in the presence of increasing concentrations of MAPs-free (X-X) or tau-coated Mt; molar ratio of tubulin dimers to tau proteins: 25 (-) or 5 (O-O). Data of one of three independent experiments are shown. Similar results were obtained by testing several kinesin preparations

Analysis of the above data by double-reciprocal plot of MgATPase activity *versus* tubulin concentration revealed that the concentration of microtubules necessary for half-maximal activation (K_{app}) was lower for tau-coated microtubules as compared to MAPs free microtubules (Table 1). Figs. 1, 2 and Table 1 show that tau-coated microtubules are specific and saturable activators of kinesin ATPase; the extent of stimulation and the microtubule concentration needed for half-maximal activation of MgATP hydrolysis by kinesin is affected by the presence of tau proteins on the surface of microtubules.

The above kinetic data could be explained by supposing direct binding of kinesin to tau proteins. Kinetic studies alone, however, may have controversial aspects for detecting protein-protein interactions. Therefore, we

applied the blot overlay technique (Rozdzial et al., 1990; Rendon et al., 1987) to study if a direct interaction existed between kinesin and tau proteins.

Table 1 . Effect of tau proteins on the characteristics of the stimulation of kinesin ATPase by microtubules

tub/tau ratio*	$V_{m,app}$ (μM/min/mg)	K_{app} (μM dimer)
MAPs-free	1.9 ± 0.5	4.9 ± 0.5
25	0.8 ± 0.1	2.2 ± 0.3
5	0.7 ± 0.1	2.1 ± 0.3

Experiments were performed at saturating MgATP concentration (1 mM). Double-reciprocal plot of ATPase activity *versus* tubulin concentration was applied to determine the values of $V_{m,app}$ (ATPase activity at saturating microtubule concentrations) and K_{app} (concentration of microtubules inducing half-maximal activation). Fitting of the data to the straight line was performed using linear regression. Standard deviations are given in the Table, $p < 0.05$.
(*) In the case of taxol stabilized microtubules with tau protein added, molar ratio of tubulin heterodimers to tau proteins is marked; MAPs-free: taxol stabilized microtubules without added tau proteins.

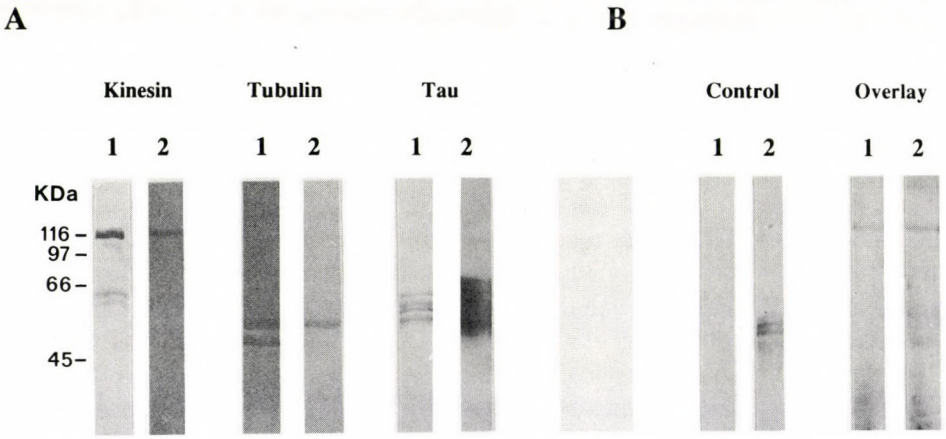


Fig. 3. Binding of tubulin and tau proteins to solid phase kinesin.
A: Characterization of the purified proteins and the antibodies. Lanes (1) Coomassie blue LiDS-PAGE of purified kinesin, tubulin and tau proteins. Lanes (2) corresponding immunoblots allowed to react with anti-kinesin, anti-α-tubulin and anti-tau antibodies, respectively.
B: Overlay experiment. Control: electroblotted kinesin was allowed to react with anti-α-tubulin (1) or anti-tau (2), to check crossreactivity of the antibodies. Overlay: electroblotted kinesin incubated with tubulin (1) or tau proteins (2), followed by reaction with anti-α-tubulin and anti-tau, respectively, as described in Methods.

Electrophoretically separated protein components of the purified kinesin preparation were electroblotted onto nitrocellulose membrane and incubated with purified DEAE-tubulin or tau proteins. Immunochemical techniques revealed α-tubulin or tau - immunoreactivity at 116 kDa on the overlayed strips (Fig. 3 B, Overlay), indicating therefore the binding of depolymerized tubulin (tubulin dimers) and tau proteins to the heavy chain of kinesin.

DISCUSSION

When evaluating the possible role of microtubule-associated proteins in intracellular motility on the basis of *in vitro* microtubule gliding studies, inhibition by MAP2 has been found, whereas tau proteins either did not inhibit microtubule gliding (Lopez and Sheetz, 1993), or a slight effect was observed at high concentrations (Heins et al., 1991). For interpreting microtubule gliding assay results special steric constraints originating from the observation technique have to be considered. What is more important, the mechanism of the entire mechanochemical cycle is to be taken into account. Due to this complexity different experimental approaches are also needed in searching for the possible regulators of kinesin-driven intracellular movements. It is known that microtubule-kinesin interaction manifests itself by a marked stimulation of kinesin's ATPase activity, accompanied by a considerable enhancement of the affinity of the enzyme towards its substrate (Kuznetsov and Gelfand, 1986). Thus, it is reasonable to expect that comparison of stimulation by MAPs-coated and MAPs-free microtubules would provide essential informations about the supposed role of microtubule-associated proteins.

Considering tau proteins as the most likely candidates to interfere with axonal transport of membranous organelles, we compared the stimulation of kinesin by tau-coated and microtubules free of MAPs. Low levels of tau proteins (molar ratio of 1/25, i.e. one molecule of tau per 25 tubulin dimers) diminished considerably the extent of the stimulation, while complete inhibition was not observed even at a molar ratio of 2/1 (Fig. 1). It is worth noting, that these data do not exclude the possibility that under extreme conditions tau proteins may suspend completely the stimulation of kinesin ATPase by microtubules. Interestingly, microtubule concentration needed for half-maximal stimulation of kinesin was lower in the case of tau-coated

microtubules then in the case of those free of MAPs (Fig. 2, Table 1). In other words, tau-coated microtubules apparently bind kinesin with higher affinity then MAPs-free microtubules. Tau proteins did not affect significantly $K_{m,app}$ values. Steric inhibition (shielding) alone would hardly account for these observations, whereas binding of kinesin to tau proteins could produce the effects described. This notion was strongly supported by protein overlay results, showing that not only tubulin dimers but also tau proteins bound to the heavy chain of kinesin (Fig. 3).

The above results raise the possibility that in the axons, where microtubules are coated with tau proteins, kinesin can bind either to the surface of microtubule or to the tau proteins, likely to their sidearms emerging from the surface. Furthermore, it is conceivable that the extent of stimulation of kinesin and/or the affinity of the microtubules towards kinesin might be modified by alterations in the abundance or in the conformation of tau proteins (known to occur e.g. upon modification of their phosphorylation state).

In conclusion, the findings described above let us to presume that tau proteins, as well as other MAPs could be constituents of the "organelle translocation complex" (Sheetz et al., 1989) or "motility complex" (cf. Burkhardt, 1996). If so, one might speculate that kinesin-driven processes could be regulated by extra- and intra-cellular signals *via* alteration of the abundance, localization or microtubule-binding characteristics of MAPs.

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REFERENCES

Bernhardt, R. and Matus, A. (1984) Light and electron microscopic studies of the distribution of microtubule-associated protein 2 in rat brain: difference between the dendritic and axonal cytoskeleton. J. Comp. Neurol. 226. 203-221.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of protein utilising the principle of protein-dye binding. Anal. Biochem. 72. 248-254.

Burkhardt, J.K. (1996) In search for membrane receptors for microtubule-based motors - is kinectin a kinesin receptor? Trends in Cell Biol. 6. 127-131.

Cleveland, D.W., Hwo, S.Y. and Kirschner, M. W. (1977) Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. J. Mol. Biol. 116. 227-247.

Endow, S.A. (1991) The emerging kinesin family of microtubule motor proteins. Trends in Biochem. Sci. 16. 221-225.

Heins, S., Song, Y.H., Wille, H., Mandelkow, E. and Mandelkow, E.M. (1991) Effect of MAP2, MAP2c and tau on kinesin-dependent microtubule motility. J. Cell Sci. Suppl. 14. 121-124.

Hirokawa, N. (1996) Organelle transport along microtubules - the role of KIFs. Trends in Cell Biol. 6. 135-141.

Hirokawa, N., Pfister, K.K., Yorifuji, H., Wagner, M.C., Brady, S.T. and Bloom, G.S. (1989) Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. Cell 56. 867-878.

Hirokawa, N., Sato-Yoshitake, R., Yoshida, T. and Kawashima, T. (1990) Brain dynein (MAP1C) localizes on both anterogradely and retrogradely transported membranous organelles. J. Cell Biol. 111. 1027-1037.

Hollenbeck, P. J. (1989) The distribution, abundance and subcellular localization of kinesin. J. Cell Biol. 108. 2335-2342.

Jancsik, V., Filliol, D., Felter, S. and Rendon, A. (1989) Binding of microtubule associated proteins (MAPs) to rat brain mitochondria: a comparative study on the binding of MAP2, its microtubule-binding and projection domains, and tau proteins. Cell Motil. Cytoskel. 14. 372-381.

Jellali, A., Metz-Boutique, M. H., Surgucheva, I., Jancsik, V., Schwartz, C., Filliol, D., Gelfand, V. and Rendon, A. (1994) Structural and biochemical properties of kinesin heavy chain associated with rat brain mitochondria. Cell Motil. Cytoskel. 28. 79-93.

Jung, D., Filliol, D., Miehe, M. and Rendon, A. (1993) Interaction of brain mitochondria with microtubules reconstituted from brain tubulin and MAP2 or tau. Cell Motil. Cytoskel. 24. 245-255.

Kuznetsov, S.A. and Gelfand, V.I. (1986) Bovine brain kinesin is a microtubule-activated ATPase. Proc. Natl. Acad. Sci. USA 83. 8530-8534.

Kuznetsov, S.A., Vaisberg, E.A., Shanina, N.A., Magretova, N.N., Chernak, V.Y. and Gelfand, V.I. (1988) The quaternary structure of bovine brain kinesin. EMBO J. 7. 353-356.

Lacey, M.L. and Haimo, L.T. (1992) Cytoplasmic dynein is a vesicle protein. J. Biol. Chem. 267. 4793-4796.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 277. 680-685.

Leopold, P.L., McDowall, A.W., Pfister, K.K., Bloom, G.S. and Brady, S.T. (1992) Association of kinesin with characterized membrane-bounded organelles. Cell Motil. Cytoskel. 23, 19-33

Lin, S.H.X. and Collins, C.A. (1990) Immunolocalization of cytoplasmic dynein to lysosomes in cultured cells. J. Cell Sci. 101. 125-137.

Lopez, L.A. and Sheetz, M.P. (1993) Steric inhibition of cytoplasmic dynein and kinesin motility by MAP2. Cell Motil. Cytoskel. 24. 1-16.

Papasozomenos, S.C. and Binder, L.I. (1987) Phosphorylation determines two distinct species of tau in the central nervous system. Cell Motil. Cytoskel. 8. 210-226

Paschal, B.M., Obar, R.A. and Vallee, R.B. (1989) Interaction of brain cytoplasmic dynein and MAP2 with a common sequence at the C terminus of tubulin. Nature 342. 569-572.

Pfister, K.K., Wagner, M.C., Stenoien, D.L., Brady, S.T. and Bloom, G.S. (1989) Monoclonal antibodies to kinesin heavy and light chains stain vesicle-like structures, but not microtubules, in cultured cells. J. Cell Biol. 108. 1453-1463.

Rendon, A., Filliol, D. and Jancsik, V. (1987) Microtubule-associated proteins bind to 30 kDa and 60 kDa proteins of rat brain mitochondral: visualisation by ligand blotting. Biochem. Biophys. Res. Commun. 149. 776-783.

Rozdzial, M.M., Neigbors, B. W. and McIntosh, J.R. (1990) Eur. J. Cell Biol. 52. 27-35.

Seals, J.R., McDonald, J.M., Burns, D. and Jarett, L. (1978) A sensitive and precise isotopic assay of ATPase activity. Anal. Biochem. 90. 785-795.

Sheetz, M.D., Steuer, E.R. and Schroer, T.A. (1989) The mechanism and regulation of fast axonal transport. Trends Neur. Sci. 12, 474-478

Shelanski, M.L., Gaskin, F. and Cantor, C.R. (1973) Assembly of microtubules in the absence of added nucleotides. Proc. Natl. Acad. Sci. USA 70. 765-768.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76. 4350-4354.

Vale, R.D. (1990) Microtubule-based motor proteins. Curr. Op. Cell Biol. 2. 15-22.

Vale, R.D., Reese, T.S. and Sheetz, M.P. (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42. 39-50.

Vallee, R.B. (1986) Reversible assembly purification of microtubules without assembly-promoting agents and further purification of tubulin, microtubule-associated proteins and MAP fragments. In: Methods in Enzymology, Vol. 134, pp 89-104. Academic Press, Inc., New York.

Yang, J.T., Saxton, W.M., Stewart, R.J., Raff, E.C. and Goldstein, L.S.B. (1990) Evidence that the head of kinesin is sufficient for force generation. Science 249. 42-47.

Yu, H., Toyoshima, I., Steuer, E.R. and Sheetz, M.P. (1992) Kinesin and cytoplasmic dynein binding to brain microsomes. J. Biol. Chem. 267. 20457-20464

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Research report

OPPOSITE SHORT-TERM CHANGES INDUCED BY AN ORGANOPHOSPHATE IN CORTICAL AND HIPPOCAMPAL EVOKED ACTIVITY

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Summary: Organophosphates are the most widely used pesticides throughout the world. The considerable amount brought out to the environment poses a risk on the whole population. As organophosphates are neurotoxic substances and their residues can persist in the environment for several weeks, their influence on the nervous system of humans and animals is of principal interest. In the present study, we investigated the alterations induced by dichlorvos, a common pesticide substance, in parameters of somatosensory evoked potentials and hippocampal evoked population spikes of rats. The changes of the cortical vs. hippocampal evoked responses were opposite and only hippocampal effects could be directly explained through an increased cholinergic activity.
Keywords: organophosphate, cholinergic system, cortical evoked potential, hippocampal population spike, rat

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INTRODUCTION

Organophosphates (OPs), multi-substituted organic esters of phosphoric acid (WHO, Geneva, 1986), are well known synthetic neurotoxic substances and are primarily used as insecticides. Their main mode of action is an irreversible inhibition of the acetylcholinesterase enzyme (AChE). As, however, the OP's toxicity is not confined to insects or arthropods, using them poses a risk to other living species including man. Most of the early reports on OP toxicity, first of all in human cases, dealt mainly with changes in the acetylcholine (ACh) metabolism (see Kaloyanova's review, 1975). Metcalf and Holmes (1969) described prolonged alterations of the central nervous system function in humans after OP exposure. Changes in the EEG were later confirmed (Duffy et al., 1979).

Dichlorvos (DDVP) is a highly effective and toxic OP (WHO, Geneva, 1989). Due to its anticholinesterase potency, it induces an accumulation of ACh in the brain (Stavinoha et al., 1976). The changes of cortical electrical activity caused by dichlorvos (and related OPs) are, however, not always accompanied by corresponding changes in ACh levels or AChE activity (Dési, 1983) or they outlast them (Gralewicz et al., 1991).

It has been reported by the same authors (Gralewicz et al., 1989) that, parallel with the OP-induced changes in EEG (described also in Nagymajtényi et al., 1988 and Dési et al., 1994), a characteristic increase in hippocampal theta activity, possibly due to an increased cholinergic input from the septal area (Szerb et al. 1977; Wainer et al., 1985) can be seen; even in (e.g. urethane) anaesthesia (Monmaur et al., 1993). This, considering the central role of hippocampus in memory and behavioral functions, may give some explanation for OP-induced changes of those (Schulz et al., 1990). Previous research of our laboratory (Dési et al., 1991; Nagymajtényi et al., 1994) has shown that, beside EEG changes, cortical evoked potentials also undergo characteristic changes on OP treatment.

In the present experiments, our aim was to compare the effects of acute dichlorvos treatment on the typical forms of cortical vs. hippocampal evoked activity i.e. the somatosensory evoked potential (Schlag, 1973) and the hippocampal population spike (Andersen et al., 1971). We wanted to see whether there is any correlation between the dichlorvos-induced alterations of the two and whether these can be explained by the AChE inhibition.

METHODS

The experiments were done on male Wistar rats of about 300 g body weight. Following urethane anaesthesia (1000 mg/kg b.w., i.p.) the animal's head was fixed in a stereotaxic frame and the skull above the left hemisphere was opened. Wounds were sprayed with lidocaine. Following surgery the animal, with its body covered in a warm cloth, was put aside for at least 30 min for recovery. Somatosensory cortical evoked potential was recorded over the barrel field with a silver surface electrode. Electrical stimuli were delivered to the contralateral whiskery skin by a pair of needles. For recording hippocampal activity, a bipolar stimulating electrode was positioned to the perforant path (AP: 6, L: 4.5, V: 4 mm stereotaxic coordinates) while the recording glass microelectrode was put in the CA1 region (AP: -3.5, L: 2.5, V: 2 - 4 mm (taking bregma as reference point, according to the rat brain atlas of Paxinos and Watson, 1982). Parameters for electrical skin stimulation were 3 V/ 0.2 ms, 1 Hz; for perforant path stimulation: 1 mA, 0.5 ms, 0.1 Hz. Low frequency hippocampal stimulation was chosen to avoid any facilitation. Fifty evoked responses were averaged using the "pCLAMP" (Axon Instruments, Inc, Foster city, CA, USA) computer software. Latency, duration and amplitude of the evoked responses were measured later, off line, using the same software. Relative changes and principal statistical parameters of those for the whole group (i.e., 6 animals each) were calculated. In case of evoked potentials, start latency (from the stimulus artifact), total duration and peak-to-peak amplitude was measured between specific points as shown in Fig. 1A. For population spikes, the measurements were similar.

After taking 3 - 5 control records (one in 30 minutes), 1/5 LD_{50} (16 mg/kg b.w.) DDVP was applied per os by gavage, via a plastic tube lead through the oesophagus into the stomach during preparation.

Per os LD_{50} for DDVP was 80 mg/kg b.w. as determined in preliminary experiments (Dési et al., 1991). Records were taken again every 30 minutes up to 120 minutes from administration. This experimental sequence meant that every animal served as its own control. Beyond that, a saline-treated control group was recorded for 2.5 hours to prove that there were no spontaneous fluctuations interfering with the measurement.

Acetylcholinesterase activity in the brain was determined in separate groups of animals (also anaesthetized), one group receiving the same OP treatment as above and another serving as control. The method of Ellman et al. (1961) was used.

RESULTS

Cortical evoked potential

Somatosensory cortical evoked potentials had a clear biphasic shape which was sometimes distorted by the stimulus artifact (Fig. lA). Punctum maximum of the response was searched by moving the recording electrode on the cortical surface. DDVP administration induced a steady increase in the latency and duration of the evoked responses. In a typical experiment, the latency changed from 2.91 ms (control) to 4.05 ms (at 120 min) and its duration from 2.55 ms (control) to 3.14 ms (120 min). Amplitude of the response, however, was diminished, e.g. from 2126 μV (control) to 1525 μV (120 min). Time courses of the relative changes of the evoked response are demonstrated in Fig. 2. Averaged changes in the latency, duration and amplitude of the evoked responses after treatment and in controls are given in Table 1.

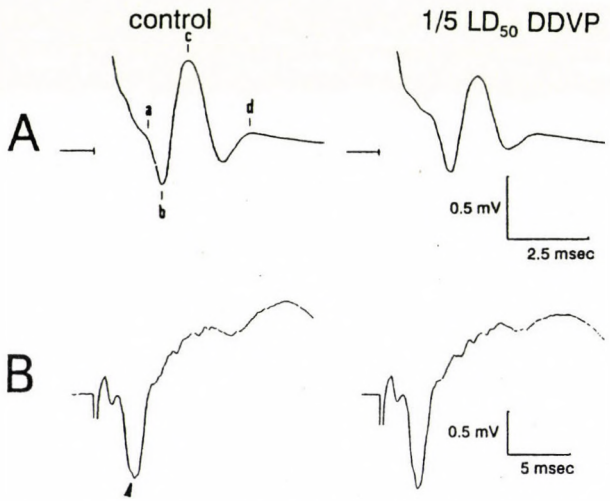


Fig. 1. Samples of somatosensory evoked potentials (A; average of 50) and hippocampal population spikes (B: average of 50). Left: control, right DDVP treated (60 and 65 min, respectively). Upward deflection means positivity. Note different calibration for A and B. Measurements on the evoked potential (A): latency, between stimulus artifact and a; duration, between a and d; amplitude, between b and c. A solid arrow points to the peak of the population spike (B), latency and amplitude of which was measured between the stimulus artifact and the peak

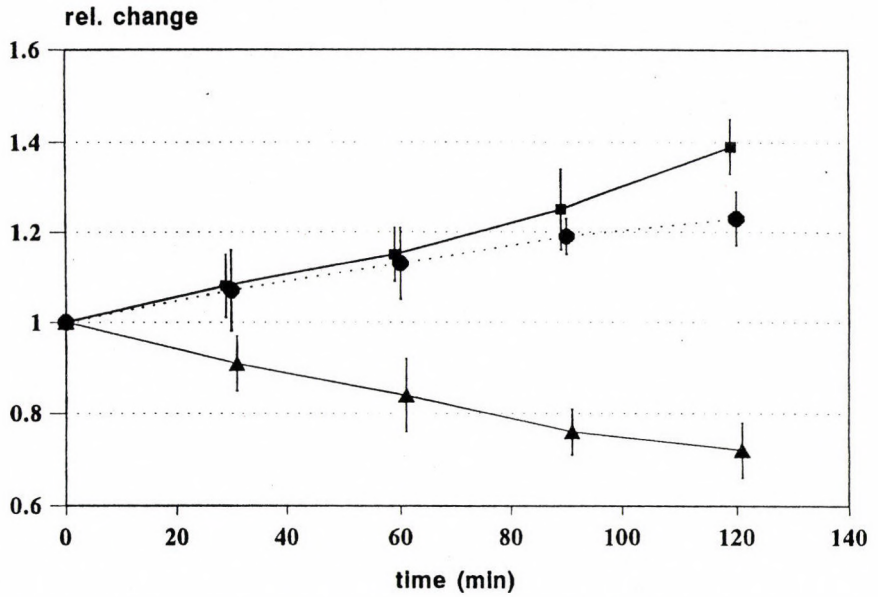


Fig. 2. Time course of the relative changes in parameters (squares: latency, circles: duration, triangles: amplitude) of the somatosensory evoked potential. DDVP was given (per os) at 0 min

Hippocampal population spike

This type of evoked activity can be described as a brief negative surge superposed on the field potential (Fig. lB). It is a result of the synchronous discharge of the pyramidal neurons. In some of our experiments, we saw the population spike in an inverted form, but, based on shape and latency, its identity was undoubted.

The changes in the population spike caused by DDVP treatment were opposite to those seen on cortical evoked potentials and the features most affected were also dissimilar. There was no significant alteration in latency and duration of the population spikes. Their amplitude, however,- increased, e.g. from 810 μV (control) to 1130 μV (at 120 min). Figure 3 shows the time course of the changes in the population spikes' parameters. Averaged relative changes are given in Table 1.

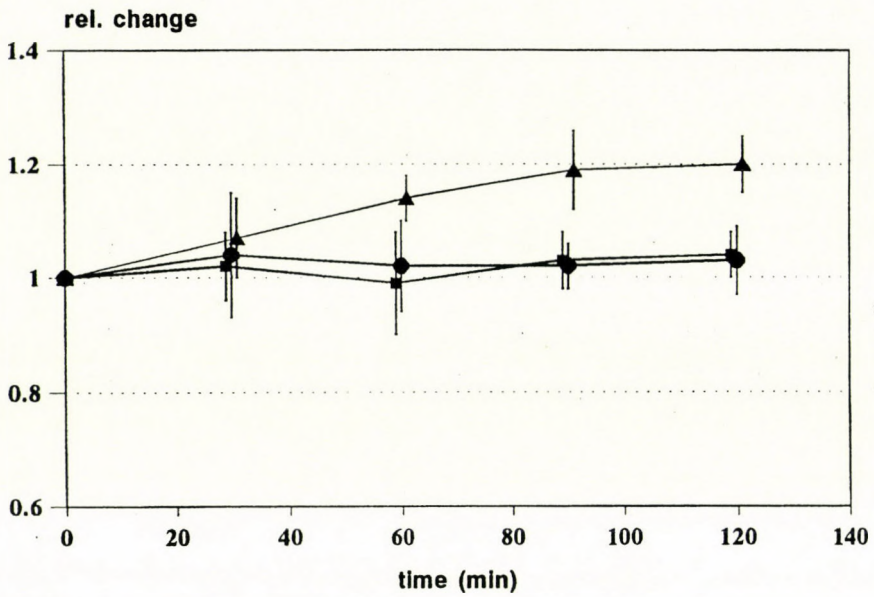


Fig. 3. Time course of the relative changes in parameters (squares: latency, circles: duration, triangles: amplitude) of population spikes. DDVP was given (per os) at 0 min

Acetylcholinesterase activity

In an untreated group (n=6) of animals, averaged (mean ± s.d.) specific AChE activity was 0.039 ± 0.003 mg substrate/μg protein × minute. In the DDVP-treated group it was 0.033 ± 0.004, resulting in a relative activity of 85%.

Table 1. Averaged (mean ± s.d., n=6) final relative changes (in % of control) of the parameters of the somatosensory evoked potential and the hippocampal population spike in the DDVP-treated and the control (saline-treated) groups.
Changes marked with * were significant ($p < 0.05$) using the paired t-test.

		Amplitude	Latency	Duration
Somatosensory evoked potential	DDVP	72.6*±4.4	139.2*±5.4	123.1*±3.7
	control	97.5±5.2	103.1±4.3	102.5±4.4
Hippocampal population spike	DDVP	120.4*+3.1	104.2±5.7	102.8±6.1
	control	101.6±6.2	95,4±3,9	103.1±4.4

DISCUSSION

Organophosphate-induced changes in the brain electrical activity have been well known. Most observations dealt with the spontaneous activity - first of all that of the cortex (Duffy et al., 1979; Duffy and Burchfiel, 1980; Philippens et al., 1992) and in lesser extent of the hippocampus (Gralewicz et al., 1989, 1991). Evoked activity - as shown above - also undergoes certain alterations on organophosphate action. The changes of evoked cortical activity, seen in the present experiments, are in concordance with those found in earlier works of our group (Dési et al., 1991; Nagymajtényi et al., 1994). It is still uncertain at which stages in the sensory pathway these changes happen and what is their nature. Considering the massive modulatory cholinergic innervation of the cortex (Johnston et al., 1981; Wainer et al., 1993) and the known influence of ACh on sensory evoked potentials (Rasmusson, 1993) the OP-induced changes could be ascribed to their anticholinesterase action (Koelle, 1981). Certain reports, however, indicate that "pharmacological" anticholinesterases, like physostigmine, do not fully mimic the acute neurotoxic action of OPs (Tomas and Gralewicz, 1992) and that anticholinergic drugs, like atropine, do not prevent or reverse that (Dési et al., 1991). Further, AChE can be present at the synapses in high excess (Koelle, 1992). Thus, only a massive blockage can be expected to show an effect, while in our experiment the brain AChE activity went down only to 85% of the control. And, where a clear cholinergic influence on sensory evoked activity was seen (Donoghue and Caroll, 1987; Rasmusson and Dykes, 1988) it was always facilitatory. Taken together, these data suggest that the decrease of the somatosensory evoked potential seen by us can be due to a non-cholinergic action of the OP.

The effect of the OP treatment on the hippocampal population spike, on the contrary, corresponds well with an increased cholinergic activity. Septo-hippocampal cholinergic fibres, ending mainly on the pyramidal neurons (Wainer et al., 1985) facilitate transmission (Krnjevic and Ropert, 1982). When, by means of the OP-induced AChE blockage, cholinergic influence is increased, a given stimulus can elicit a stronger response - as it was seen by us. A similar efffect of locally applied physostigmine had been published (Krnjevic and Ropert, 1982).

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REFERENCES

Andersen, P., Bliss, T.V.P. and Skrede, K.K. (1971) Unit analysis of hippocampal population spikes. Exp. Brain Res. 13, 208-221.

Dési, I. (1983) Neurotoxicological investigation of pesticides in animal experiments. Neurobehav. Toxicol. Teratol. 5, 503-515.

Dési, I., Nagymajtényi, L., Lorencz, R. and Molnar, Z. (1991) The effects of organophosphorous compounds on the central nervous system of rats. Arch. Toxicol. Suppl. 14, 33-37.

Dési, I., Nagymajtényi, L. and Schulz, H. (1994) EEG changes caused by dimethoate in three generations. NeuroToxicol. 15, 731-734.

Donoghue J.P. and Caroll, K.L. (1987) Cholinergic modulation of sensory responses in rat primary somatic sensory cortex. Brain Res. 408, 367-371.

Duffy, F.H., Burchfiel, J.L., Bartels, P.H., Gaon, M. and Sim, M. (1979) Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol. Appl. Pharmacol. 47, 161-176.

Duffy, F.H. and Burchfiel, J.L. (1980) Long term effects of the organophosphate sarin on EEGs in monkeys and humans. Neurotoxicology 1, 667-689.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholineterase activity. Biochem. Pharmacol. 7, 88-95.

Gralewicz, S., Tomas, T. and Socko, R. (1989) Effects of single exposure to chlorphenvinphos, an organophosphate insecticide, on electrical activity (EEG) of the rat brain. Polish J. Occup. Med. 2, 309-320.

Gralewicz, S. Tomas, T., Gorny, R., Kowalczyk, W. and Socko, R. (1991) Changes in brain bioelectrical activity (EEG) after repetitive exposure to an organophosphate anticholinesterase. II. Rat. Polish J. Occup.-Med. Env. Health 4, 183-196.

Johnston, M.V., McKinney, M. and Coyle, J.T. (1981) Neocortical cholinergic innervation: A description of extrinsic and intrinsic components in the rat. Exp. Brain Res. 43, 159-172.

Kaloyanova, F. (1975) Cholinesterase activity as a biochemical indicator for monitoring exposure to certain pesticides. In: Proceedings of the International Conference on Environmental Sensing and Assessment, Vol. 1, Las Vegas, Nevada.

Koelle, G.B. (1981) Organophosphate poisoning - an overview. Fund. Appl. Toxicol. 1, 129-138.

Koelle, G.B. (1992) Pharmacology and toxicology of organophosphates. In: Clinical and Experimental Toxicology of Organophosphates and Carbamates (eds Ballantyne, B. and Marrs, T.C.) pp. 35-39. Butterworth-Heinemann, Oxford.

Krnjevic, K. and Ropert, N. (1982) Electrophysiological and pharmacological characteristics of facilitation of hippocampal population spikes by stimulation of the medial septum. Neuroscience 7, 2165-2183.

Metcalf, D.R. and Holmes, J.K. (1969) EEG, psychological and neurological alterations in humans with organophosphorus exposure. Ann. N.Y. Acad. Sci. 160, 357-365.

Monmaur, P., Ayadi, K. and Breton, P. (1993) Hippocampal EEG responses induced by carbachol and atropine infusions into the septum and the hippocampus in the urethane-anaesthetized rat. Brain Res. 631, 317-324.

Nagymajtényi, L., Dési, I. and Lorencz, R. (1988) Neurophysiological markers as early signs of organophosphate neurotoxicity. Neurotox. Teratol. 10, 429-434.

Nagymajtényi, L., Dési I. and Schulz, H. (1994) Changes of brain evoked potentials caused by dimethoate treatment in three generations of rats. NeuroToxicology 15, 741-744.

Paxinos, G. and Watson, C. (1982) The rat brain is stereotaxic coordinates. Academic Press, New York.

Philippens, I.H., Melchers, B.P., de Groot, D.M. and Wolthuis, O.L. (1992) Behavioral performance, brain histology and EEG sequelae after immediate combined atropine/diazepam treatment of soman-intoxicated rats. Pharmacol. Biochem. Behav. 42, 711-719.

Rasmusson, D.D. (1993) Cholinergic modulation of sensory information. Progr. Brain Res. 98, 357-364.

Rasmusson, D.D. and Dykes, R.W. (1988) Long-term enhancement of evoked potentials in cat somatosensory cortex produced by co-activation of the basal forebrain and cutaneous receptors. Exp. Brain Res. 70, 276-286.

Schlag, J. (1973) Generation of brain evoked potentials. In: Bioelectric Recording Techniques (eds Thompson, R.F. and Patterson, M.M.) Part A, pp. 273-284. Academic Press, New York.

Schulz, H., Dési, I. and Nagymajtényi, L. (1990) Behavioral effects of subchronic intoxication with parathion-methyl in male Wistar rats. Neurotox. Teratol. 12, 125-127.

Stavinoha, W.B., Modak A.T. and Weintraub, S.T. (1976) Rate of accumulation of acetylcholine in discrete regions of the rat brain after dichlorvos treatment. J. Neurochem. 27, 1375-1378.

Szerb, J.C., Hadhazi, P. and Dudar, J.D. (1977) Release of [^{3}H]acetylcholine from rat hippocampal slices: Effect of septal lesion and of graded concentrations of muscarinic agonists and antagonists. Brain Res. 128, 285-291

Tomas, T. and Gralewicz, S. (1992) A comparison of changes in spontaneous (EEG) and evoked brain activity induced by chlorphenvinphos and physostigmine in rats and rabbits. Polish J. Occup. Med. Env. Health 5, 55-69.

Wainer, B.H., Levey, A.J., Rye, D.B., Mesulam, M-M. and Mufson, E.J. (1985) Cholinergic and non-cholinergic septohippocampal pathways. Neurosci. Lett. 54, 45-52.

Wainer, B.H., Steininger, T.L., Roback, J.D., Burke-Watson, M.A., Mufson, E.J. and Kordower, J. (1993) Ascending cholinergic pathways: functional organization and implications for disease models. Progr. Brain Res. 98, 9-30.

WHO (1986) Organophosphorus Insecticides: a General Introduction. Organization, Geneva.

WHO (1989) Dichlorvos. Environmental Health Criteria 79. World Health Organization, Geneva.

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Research report

QUANTITATIVE ANALYSIS OF GYRIFICATION OF CEREBRAL CORTEX IN DOGS

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Summary: The degree of gyrification of the cerebral cortex was studied in a population of various breeds of dogs. The aim of the study was to demonstrate whether or not variations in brain weight, body size or body weight affect gyrification within a species. Results suggest that in spite of significant correlations between all these parameters the degree of gyrification is essentially determined by the size of the brain. This parameter shows neither sex-dependence nor asymmetry between the two hemispheres. The examination of frontal serial sections through dogs' brains has also revealed that in agreement with data on the primate brain the association cortical regions show an extraordinarily high gyrification. Accordingly, gyification is dependent from the overall growth of the brain in particular from that of the association areas growing most intensively in mammals. Measurement of the gyrification index is therefore, thought to be a suitable method to express in quantitative terms developmental trends of cortical organization.
Keywords: cerebral cortex, gyrification, dog

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INTRODUCTION

One of the most conspicuos macroscopic features of the mammalian cerebral cortex in many species is its division by sulci and gyri. Accordingly, *lissencephalic* (even cortical surface without gyri and sulci) and *gyrencephalic* (folded cortical surface segmented by gyri and sulci) brains are to be distinguished. It appears, however, that large animals possess usually gyrencephalic brains, while small animals have lissencephalic brains.

The pattern of gyri and sulci and their variations were extensively studied particularly in subhuman primates and in man (Connolly, 1950, Eberstaller, 1884a, b, c, d, Falk,1978, Hershkovitz, 1979, Jensen, 1870, Ono et al., 1990, Prothero and Sundsten, 1984, Pubols and Pubols, 1979, Retzius, 1896, Spitzka, 1907, Turner, 1890). It has been shown that some sulci may represent important borderlines between functionally distinct cerebral cortical regions (Ariens-Kappers et al., 1960, Brodmann, 1909, 1913, Welker, 1990, Welker and Campos, 1963). This way for example, in several mammals the marked cental sulcus forms a border between the somatosensory and motor cortical areas, or the calcarine sulcus marks the border between the upper and lower visual fields. The depth of the central sulcus has been used as an estimate of the size of the primary motor cortex in man (White et al., 1994).

Valuable as these descriptive, purely qualitative analyses may be for a number of purposes, they do not yield any information as to the extent of sulcus and gyrus formation (gyrification) within or between species , or concerning the reasons of the gyrification process. These questions can be addressed only by quantitative and experimental methods (Armstrong and Curtis, 1989, Armstrong et al., 1991a, b, 1993, 1994, Barron, 1950, Evrard et al., 1978, Goldman and Galkin, 1978, Goldman-Rakic and Rakic, 1984, Richman et al., 1975, Todd, 1982, 1986, Welker, 1990, Zilles et al., 1988, 1989).

In the past, therefore, repeated efforts were made to measure the "free cortical surface", i.e. the area which is not infolded in the sulci and the surface buried in the sulci. This was thought to give a measure of gyrification by comparing the two surface areas (Anton 1903, Aresu, 1914, Elias and Schwartz, 1969, 1971, Henneberg, 1910, Leboucq, 1929, Schlenska, 1969, Wagner, 1864). Nevertheless, a determination of surface fully eliminating the systematic errors inherent in the morphometric methods applied so far (Anton, 1903, Elias and Schwartz, 1969, 1971, Hennig, 1957, Kraus and Ditto, 1927, Kraus et al., 1928, Schlenska, 1969, Tramer, 1916), is not available due to the fact that surface determinations in serial sections are leading to systematic errors depending from the form of hemispheres and

gyri (Bok, 1939, Heitmann, 1993, Kretschmann and Wingert, 1967, Popoff, 1929, Stephan, 1960, 1961). Under these circumstances systematic errors of surface measurement cannot be avoided in serial sections whithout 3D reconstruction and triangulation procedures (for review and critical evaluation see Heitmann, 1993). Moreover, these errors cannot be predicted with an accuracy sufficient for a reasonable correction procedure. Although the method of surface determination with a thin foil (Henneberg 1910, Wagner, 1864) may avoid the systematic errors of morphometric investigations, it is extremely time-consuming so that only a few brains were studied. These do not generate a statistically evaluable wealth of representative data. Physico-chemical procedures (Aresu, 1914, Leboucq 1929) have also serious practical limitations. Thus the determination of the cortical surface cannot be regarded as a trivial methodical problem the more so since for the measurement of this parameter no error-free procedure is as yet available to provide the basic data for the assessment of gyrification.

In the present study therefore, we have chosen an entirely different approach to measure the degree of gyrification in the cerebral cortex. Instead of measuring surface areas we determine in every section of the telencephalic series the proportion between the total length of cortex contour (contour length of free cortical surface + contour length of cortex surface turned into the sulci) and the net free cortical surface contour length. This ratio of length values which can be determined without systematic error is called *gyrification index* (GI, Zilles et al., 1988). In the lissencephalic brains GI = 1, in the gyrencephalic brains it increases with the depth of sulci. With this method the degree of gyrification can be determined for each single section through the cortex and thereby it is possible to study whether some functionally circumscribed cortical regions are distinguished by specifically high gyrification indices. In subhuman primates and man for example, particularly high gyrification indices characterize the cortical association areas (Armstrong et al., 1991a, b, Moser, 1988, Zilles et al., 1988, 1989). For dogs whose brain was analyzed in the present study, similar data are not available.

The question of the degree of cerebral cortical gyrification has been found important from the aspects of brain evolution (Ariens-Kappers, et al., 1960, Armstrong and Curtis, 1989, Armstrong et al., 1991a,b, 1993, Brodmann, 1913, Buxhoeveden and Armstrong, 1991, Clark, 1945, Connolly, 1950, Elias and Schwartz, 1969, 1971, Falk, 1978, 1980, 1983a, b, 1985, Falk et al., 1989, Hershkovitz, 1979, Hofman, 1989, Holloway, 1968, 1981, 1984, 1988, Leboucq, 1929, Moser, 1988, Prothero and Sundsten, 1984, Pubols and Pubols, 1979, von Bonin, 1941, Welker and

Campos, 1963, Zilles et al., 1989), brain ontogenesis (Armstrong et al., 1994, Barron, 1950, Chi et al., 1977, Cunningham, 1980, Dan et al., 1986, Dorovini-Zis and Dolman, 1977, Omran, 1992, Smart and McSherry 1986a, b), neuropathological syndromes (Evrard et al., 1978, Tramer, 1916) and the left-right asymmetry of the hemispheres (Falk et al., 1986, Geschwind and Levitsky, 1968, White et al., 1994).

The formation of sulci is generally explained as a result of evolutionary growth in size of the cerebral cortex with a parallel limitation of growth in brain volume caused by factors such as reasonable birth weight and optimization of the neurocranium volume. The higher the gyrification the larger cortical surface - which means more cortical cell columns - can be accomodated within a given hemisphere-volume. Comparison of different species of primates has revealed that the degree of cortical gyrification increases with body and brain size. However, within the species *Homo sapiens sapiens* there was no correlation observed between gyrification and body size or brain weight (Zilles et al., 1988). This contradiction between interspecies (different primates) and intraspecies (human) comparisons can still not be regarded as a general rule relevant to a possible correlation between body and brain size on one hand, and gyrification on the other, because if compared with investigations in various primate species the relevance of human studies is diminished by the relatively low variations in human body size between individuals as opposed to the wide variability in their brain morphology.

The aim of this work was therefore, to study the influence of body and brain size on the gyrification of the cerebral cortex in one single species, the dog whose breeds differ markedly in body mass and brain size. We sought answer to the question whether in an intraspecies comparison the gyrification varies dependently or independently from body and/or brain size.

MATERIALS AND METHODS

The brains of 20 dogs (12 male and 8 female) belonging to 12 different breeds were investigated (for details see Table 1). In this population body weight varied within a range of 15.2-fold, shoulder (brisket) height 3-fold and brain weight (Table 2), 2.4-fold. These variation ranges of general body and brain sizes are thus much wider than those of man and permit a more conclusive determination of correlations between gyrification, and body and brain sizes.

Brains were removed from the skull and immediately weighed (Table 2), then fixed for some weeks in 4% formaldehyde solution. After dehydration in graded ethanol brains were embedded through chloroform in paraffin. Paraffin-embedded brains were serially cut in the frontal plane with a Tetrander large-section microtome (section thickness: 250 mm). Each section was mounted on a glass slide (49 - 88 section/brain), deparaffinated and coverslipped with DePeX.

From every second section a contour drawing of the cerebral cortex was made on a sheet of paper under a drawing microscope. With the aid of a digitizer and a computer programme, in each drawing two different contour length values were defined (Fig. 1):

(1) the length of the total outer contour of the cerebral cortex including the parts folded into the sulci (= C_{tot}),

(2) the outer contour length of the free cortical surface without the parts turned into the sulci (=C_{out})



Fig. 1. Drawing of the outer cortex contour of a frontal section from the brain of a Hungarian pointer. The continuous line represents the total contour length, the dashed line indicates the contour length of the free surface

Gyrification index (=GI) was calculated in each single section according to the formula [1]

[1]
$$GI = \frac{C_{tot}}{C_{out}}$$

Since the gyrification index expresses a relative value, shrinkage due to paraffin embedding could be neglected: after a sufficiently long fixation period both contour segments become equally shrunken.

The position of each single section in the brain could be defined by the serial technique. As a result, the GI values of all sections could be ordered into a rostro-caudal sequence. To compare brains of different absolute sizes, the rostro-caudal distance (X-axis) was measured and taken as 100%. Mean GI's were calculated separately in the right and left hemispheres to point out eventual interhemispheric asymmetries. In addition, another mean GI was calculated for the entire brain from the values of both hemispheres.

Calculations of correlations: simple and multiple linear regressions between body weight, shoulder height, brain weight and gyrification indices were carried out using the P-STAT statistical package.

For histological demonstration, one brain was cut into 20 mm serial sections and stained with Nissl's stain as modified by Merker (1983).

RESULTS

Correlation between brain weight, body weight and shoulder (brisket) height

In all dog breeds studied brain weights showed a significant correlation with shoulder height ($r = 0.751$, $P = 0.001$, $n = 17$, Fig. 2) and with body weight ($r = 0.714$, $P = 0.001$, $n = 20$, Fig. 3). Poodles, spitzes, terriers, Dobermanns and Hungarian pointers have higher brain weights as could be predicted on the basis of the actual shoulder height, while in other breeds brain weights were found lower than expected. Accordingly, different races of dogs exhibited different degree of encephalization when viewed from the aspect of body size. A similar although less marked pattern is obtained by the comparison of regression curves and breed to demonstrate the brain weight / body weight relationship. There was only one of the terriers studied in which due to an abnormally high body weight this relationship fell under the regression curve, while the value in beagles was found slightly above the

regression curve. One should, however, consider that body weight is much more dependent on different nutritional conditions and/or eventual diseases than shoulder height.

Therefore, body weight seems to be a less reliable measure of body size than shoulder height.

Table 1. Breed, gender, age and general body mass of the dogs studied

Breed	Prot. - No.	Sex	Age (years)	Body weigh (g)	Shoulder height (cm)
Dachshund	H20u	female	6	7000	30
Dachshund	H37	male	12	4600	30
Dachshund	H29	male	15	6200	35
Pekin	H34	male	13	6200	25
German sheep-dog	H12	female	11	32000	58
German sheep-dog	H4	male	12	38000	63
German sheep-dog	H43	female	14	28000	58
Dobermann	H44	male	2	27000	69
Foxterrier	H44u	male	5	11000	40
Mongrel	H22	female	4	4100	-
Mongrel	H32	female	11	8300	-
Mongrel	H17	male	14	3800	-
Hungarian pointer	H23u	male	9	20000	45
Spitz	H38	male	8	11200	28
Terrier	H14	male	11	9000	50
Terrier	H6	male	11	12000	50
Terrier	H9	male	8	18000	50
Poodle	H11	female	11	3500	45
Beagle	H18	female	12	5800	25
Pinscher	H20	female	10	2500	25

Owing to the fact that all the parameters measured were in correlation with each other, the simultaneous dependence of brain weight from both body size indicator values could be calculated using multiple linear regression. Then the brain weight could be expressed relative to shoulder height as the measure of body size (partial correlation coefficient $r = 0.409$), whereas between brain weight and body weight an only 0.253 value of partial correlation coefficient existed. Thus the multiple linear regression between brain weight and body weight *plus* shoulder height is as follows:

Brain weight = 0.55 . body weight + 0.71 . shoulder height +37.5

This result supports the earlier statement that in dogs kept with all probability under widely different nutritional conditions, the shoulder height appears to be a more reliable indicator of body size then body weight. Moreover, our results suggest that body size and brain size are correlation even within one species.

Table 2. Brain weights and gyrification indices of the right and left hemispheres and the mean GI's for both hemispheres as calculated from 20 dogs

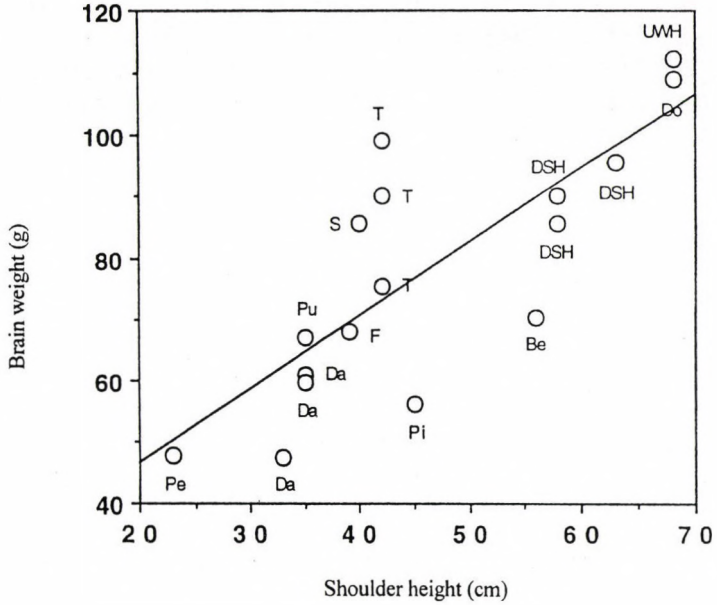
Prot. No	Brain weight (g)	Gyrification index (GI)					
		right hemisphere		left hemisphere		both hemispheres	
		GI	SEM	GI	SEM	GI	SEM
H20u	47.4	1.59	0.037	1.63	0.044	1.61	0.033
H37	59.7	1.56	0.039	1.54	0.043	1.55	0.039
H29	61.0	1.65	0.034	1.64	0.031	1.65	0.028
H34	47.8	1.67	0.040	1.64	0.041	1.66	0.038
H12	85.7	1.75	0.110	1.72	0.100	1.73	0.100
H4	95.5	1.64	0.041	1.68	0.030	1.66	0.034
H43	90.1	1.67	0.037	1.68	0.047	1.68	0.039
H44	109.0	1.67	0.034	1.67	0.038	1.67	0.033
H44u	68.1	1.58	0.038	1.68	0.038	1.63	0.036
H22	55.0	1.60	0.050	1.64	0.047	1.62	0.046
H32	61.2	1.59	0.042	1.61	0.033	1.60	0.034
H17	59.3	1.56	0.034	1.61	0.040	1.59	0.034
H23u	112.2	1.82	0.043	1.84	0.059	1.83	0.042
H38	85.8	1.61	0.033	1.63	0.042	1.62	0.030
H14	90.1	1.80	0.040	1.75	0.039	1.77	0.035
H6	99.2	1.69	0.031	1.70	0.037	1.69	0.030
H9	75.3	1.60	0.046	1.66	0.032	1.63	0.032
H11	66.8	1.57	0.036	1.58	0.041	1.58	0.034
H18	70.2	1.59	0.037	1.56	0.035	1.58	0.032
H20	56.2	1.54	0.034	1.54	0.045	1.54	0.037

Gyrification index (GI) and lateralization

In all dog breeds measured during this study a principally comparable sulcus pattern was observed between the breeds and between the left and right hemispheres (Figs 5, 6).

Figs 2, 3. →

Correlation between brain weight and body weight (Fig. 3) and shoulder (brisket) height (Fig. 2) in different breeds of dogs. Be: beagle, Da: dachshund, Do: Dobermann, DSH: German shepherd, F: foxterrier, M: mongrel, Pe: Peking, Pi: Pinscher, Pu: poodle, S: spitz, T: terrier, UWH: Hungarian pointer


120
100
80
60
40
20
30
40
50
60
70
Brain weight (g)
Shoulder height (cm)
UWH
Do
T
T
S
DSH
DSH
DSH
T
Pu
F
Be
Da
Da
Pi
Pe
Da

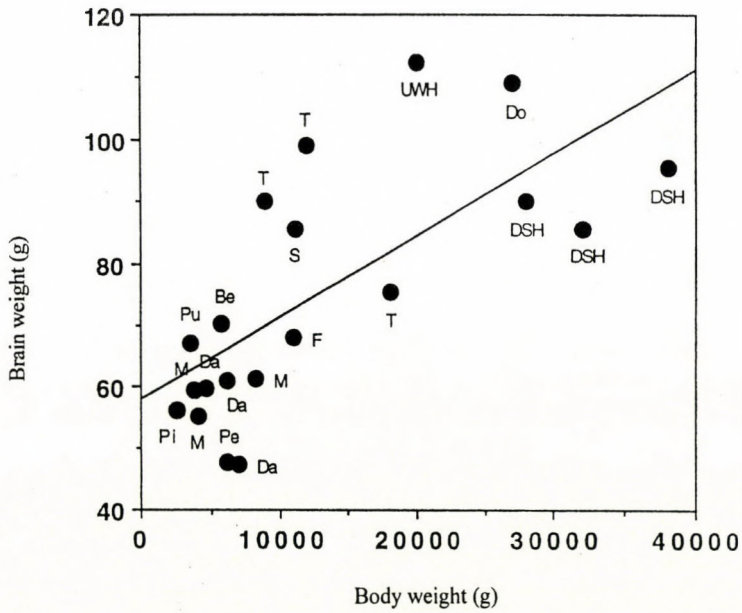

120
100
80
60
40
0
10000
20000
30000
40000
Brain weight (g)
Body weight (g)
UWH
Do
T
T
S
DSH
DSH
DSH
T
Be
Pu
F
M
Da
M
Da
Pi
M
Pe
Da

Figure 7 and Table 2 show the mean gyrification indices separately for the right and left hemispheres. Since particularly in man, a number of cortical structures (for example the shape of the lateral fissure or the depth of central sulcus) exhibit a clear-cut interhemispheric asymmetry (lateralization), we checked whether mean GI's of right and left hemispheres differ significantly. Using the Wilcoxon matched Pair-Test, no significant difference could be detected, hence in dogs hemispheric asymmetry of gyrification is not to be reckoned with. Because of the lack of significant differences between right and left hemispheres, in the foregoing description only the average value of GI's of both hemispheres will be analyzed.

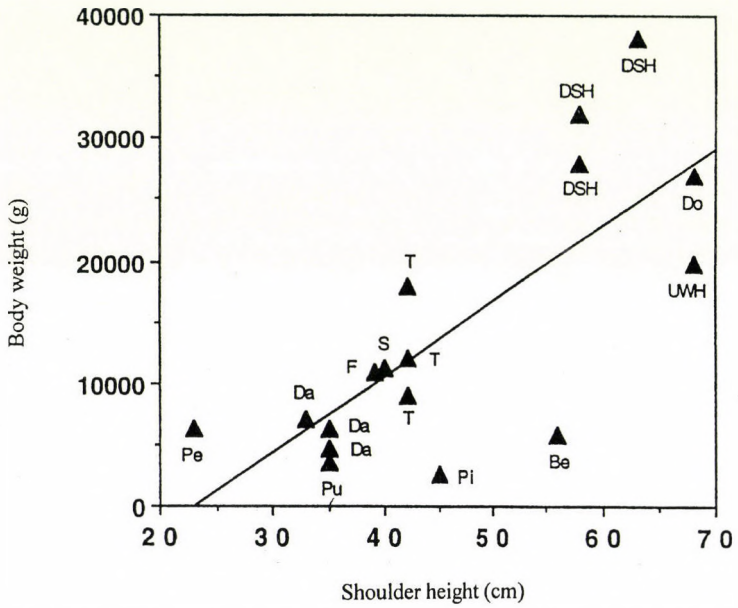


Fig. 4. Correlation between shoulder height and body weight in different breeds. Be: beagle, Da: dachshund, Do: Dobermann, DSH: German shepherd, F: foxterrier, M: mongrel, Pe: Peking, Pi: Pinscher, Pu: poodle, S: spitz, T: terrier, UWH: Hungarian pointer

The mean GI of both hemispheres calculated from the whole population studied amounts to 1.62 ...0.15. As it appears from Fig. 8, no dog breed exceeds or remains under the range defined by the standard error of

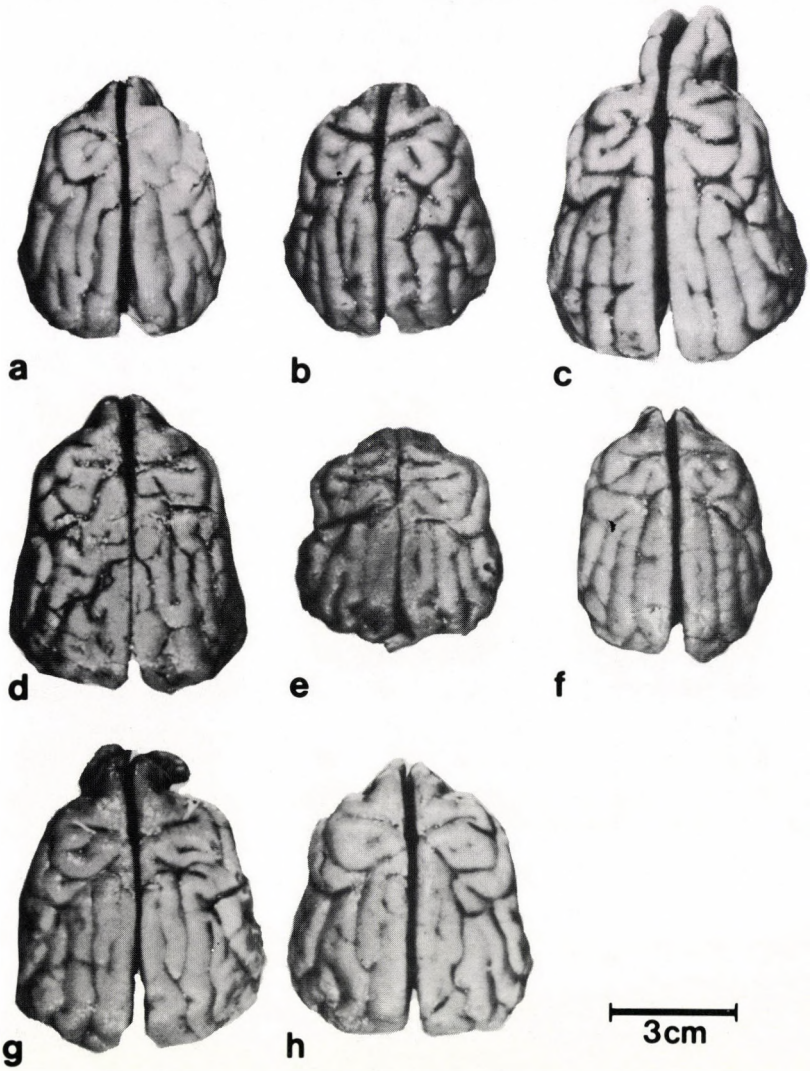


Fig. 5. Dorsal view of the forebrain in beagle (a), dachshund (b), dobermann (c), German shepherd (d), Peking (e), Pinscher (f), spitz (g) and terrier (h)

the mean. This allows to postulate that the basic organizatorial principles of the cortex which lead to the gyrification are similar in all dog breeds. Deviations of the mean GI's within one breed can therefore be interpreted as the expression of dependence of GI's from breed-specific factors such as brain weight, shoulder height (as a measure of body size) and body weight.

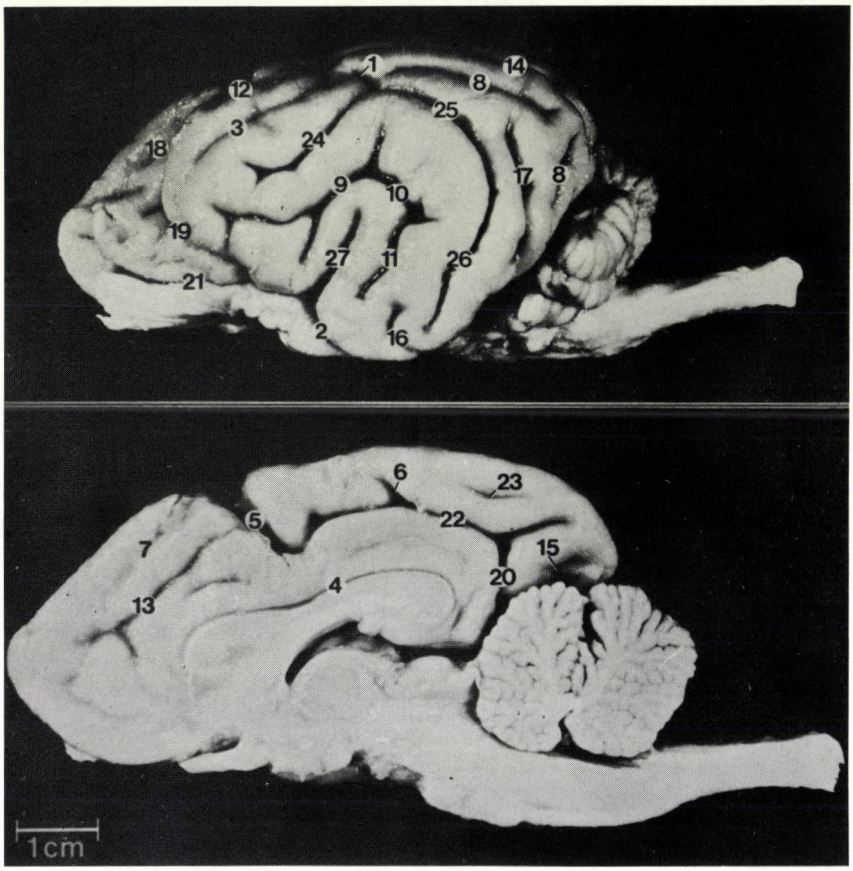


Fig. 6. Sulcus pattern of a Hovart-mongrel from lateral and medial. 1: dorsal process of suprasylvian sulcus, 2: caudal rhinal sulcus, 3: coronal sulcus, 4: sulcus corporis callosi, and 12: sulcus cruciatus, 6: sulcus cruciatus minor, 7: ectogenual sulcus, 8: ectolateral sulcus, 9-11: ectosylvian sulcus, 13: genual sulcus, 14: lateral sulcus, 15 and 17: occipitotemporal sulcus, 16: sulcus posticus, 18: precruciate sulcus, 19: presylvian sulcus, 20: retrosplenial sulcus, 21: nasal rhinal sulcus, 22: splenial sulcus, 23: suprasplenial sulcus, 24-26: suprasylvian sulcus, 27: lateral (Sylvian) fissure

Gyrification (GI) and sexual dimorphism

In order to see if gender has an influence on GI, the following studies were carried out using the Mann-Whitney U-test to demonstrate possible differences between males (n = 12) and bitches (n = 8). Mean GI values of both sexes do not differ significantly (P = 0.11). The mean GI's were in males and bitches 1.66 0,02 and 1.62 0.02, respectively. The slightly higher GI value in males is most probably due to the fact that in this population a somewhat higher brain weight was measured than in females (mean brain weight for males and females: 80.3 36.2 and 66.6 5.3) and that GI and brain weight are strongly in correlation (see subchapter 4).

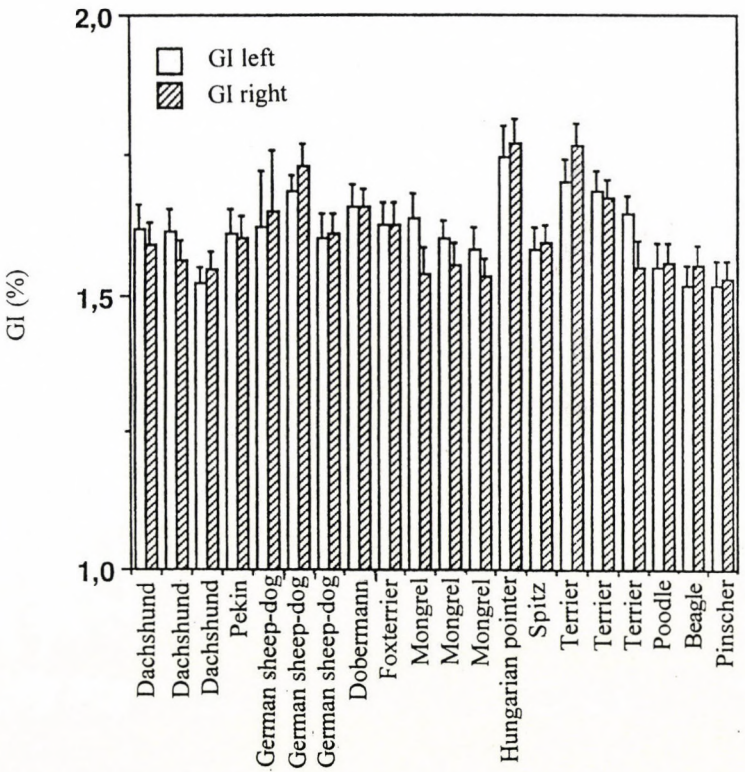


Fig. 7. Mean gyrification indices of the right and left hemispheres. DSH: German shepherd

Relationship between gyrification index and brain weight, body weight and shoulder height

Similar to calculations with brain weight, the correlations between GI and other parameters were each determined by the model of linear regression.

Between mean GI and body weight a significant correlation of r = 0.526 (P = 0.017, n = 20) existed. The same applied for the relation between GI and shoulder height (Fig. 10, r = 0.518, P = 0.033, n = 17). Nevertheless, there was a conspicuously higher and more significant correlation between GI and brain weight (Fig. 11, r = 0.707, P = 0.001, n = 20).

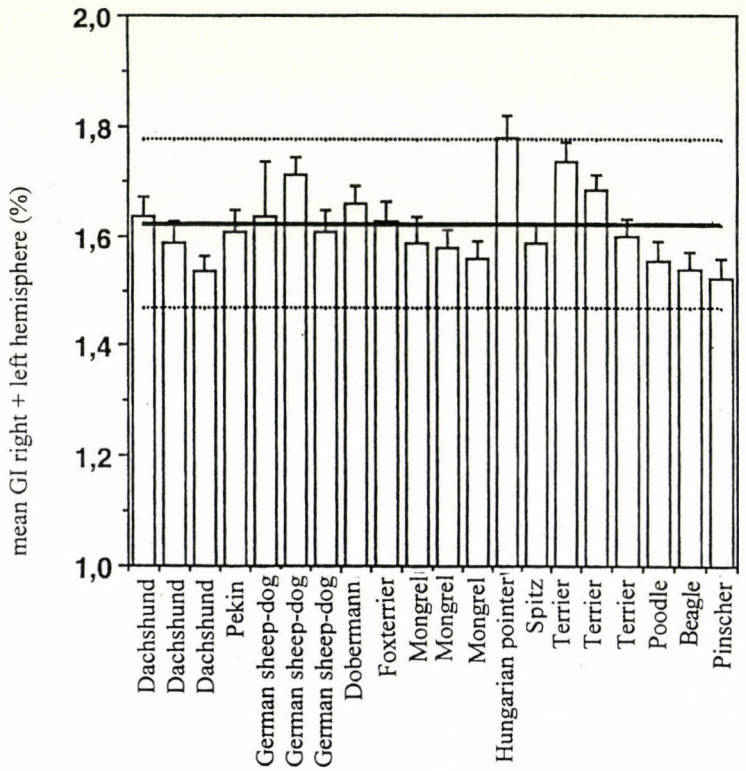


Fig. 8. Mean gyrification indices (means for both hemispheres) in different breeds. DSH: German shepherd. Continuous line marks the mean value for all dogs measured, dotted lines indicates standard error of the mean

Since findings indicated that all parameters studied were in correlation with each other and measurement errors could be regarded as negligible, here too, the relationship of all parameters had to be calculated with multiple linear regression. On this basis GI appeared to be determined almost exclusively by the brain weight (partial correlation coefficient r = 0.540). Between GI and body weight the partial correlation coefficient r amounted to 0.077, while between GI and shoulder height this value was 0.069. The multiple linear regression between GI and brain weight + body weight + shoulder height is as follows:

GI = 0.0007 . body weight + 0.0005 . shoulder height + 0.003 . brain weight + 1.463.

This result shows unequivocally that within the complex network of interrelations between gyrification, shoulder height, body and brain weight, it is the brain weight which should be regarded as a determinant factor of gyrification.

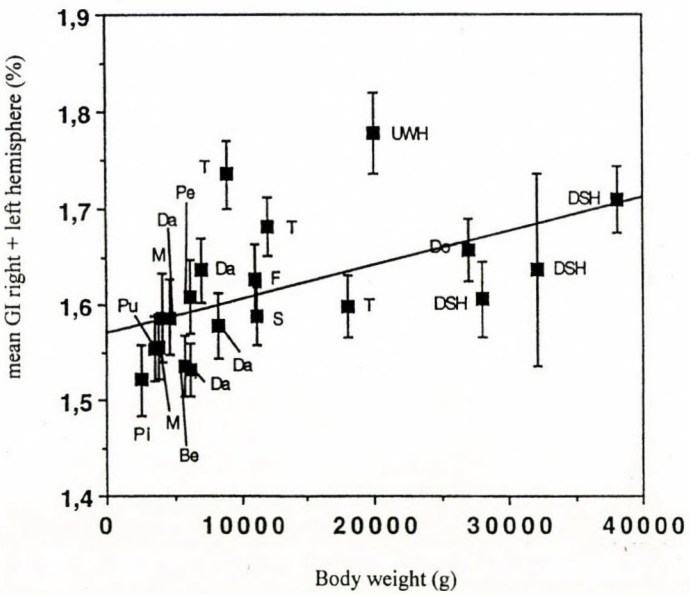
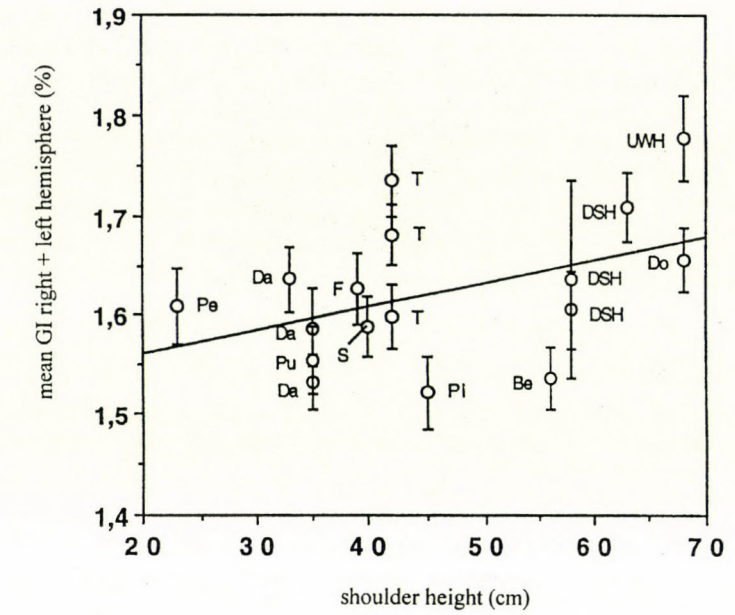


Fig. 9. Correlation between GI and body weight in different breeds of dogs. Be: beagle, Da: dachshund, Do: Dobermann, DSH: German shepherd, F: foxterrier, M: mongrel, Pe: Peking, Pi: Pinscher, Pu: poodle, S: spitz, T: terrier, UWH: Hungarian pointer


mean GI right + left hemisphere (%)
1,9
1,8
1,7
1,6
1,5
1,4
20
30
40
50
60
70
shoulder height (cm)
UWH
T
T
DSH
Do
DSH
DSH
Da
F
Pe
Da
T
S
Pu
Da
Pl
Be

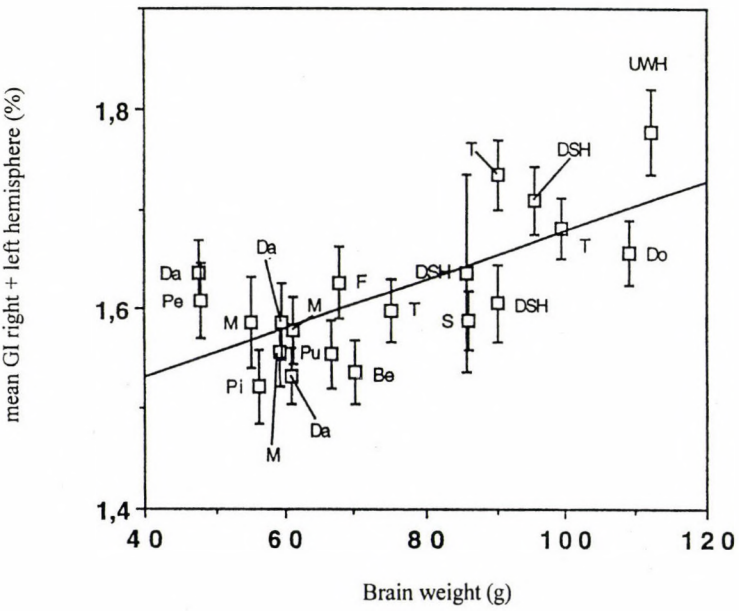

mean GI right + left hemisphere (%)
1,8
1,6
1,4
40
60
80
100
120
Brain weight (g)
UWH
T
DSH
T
Do
Da
Da
F
DSH
Pe
M
M
T
S
DSH
Pu
Pi
Be
Da
M

Fig. 10. Correlation between GI and brisket height in different breeds. Be: beagle, Da: dachshund, Do: Dobermann, DSH: German shepherd, F: foxterrier, M: mongrel, Pe: Peking, Pi: pinscher, Pu: poodle, S: spitz, T: terrier, UWH: Hungarian pointer
←

Fig. 11. Correlation between GI and brain weight in different breeds. Be: beagle, Da: dachshund, Do: Dobermann, DSH: German shepherd, F: foxterrier, M: mongrel, Pe: Peking, Pi: Pinscher, Pu: poodle, S: spitz, T: terrier, UWH: Hungarian pointer
←

Gyrification index and cortical topography

The GI's of single section levels of a brain could be demonstrated in a position-correspondingly reconstructed rostro-caudal sequence. This way the large cortical areas, e.g. frontal, occipital, could be compared as to their degree of gyrification. In addition, this kind of demonstration enabled regional and/or breed-specific aspects of gyrification to be studied comparing the rostro-caudal distribution patterns.

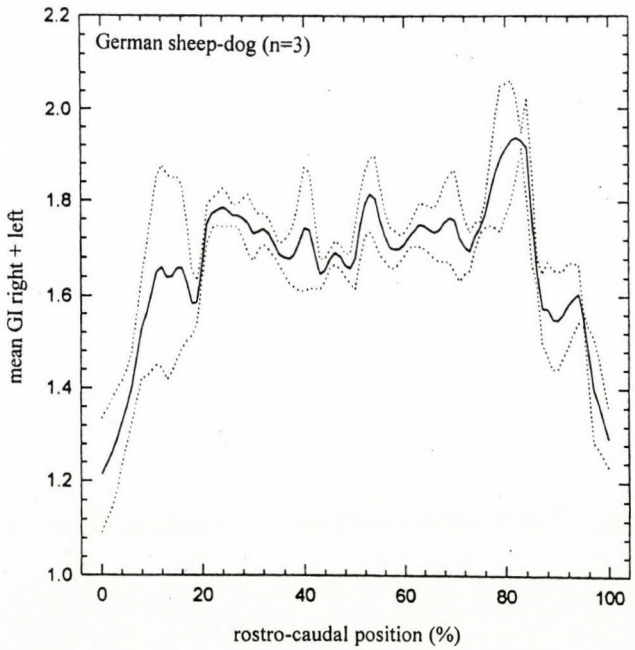


Fig. 12. Rostro caudal distribution of GI in German shepherd. The mean curve with scatter was plotted from measurements of three brains

Figure 12 shows the rostro-caudal sequence of mean GI's with deviation in three German shepherds, in Fig. 13 in three dachshunds and in Fig. 14 in three terriers. In Fig. 15 a direct comparison of mean value curves is given relevant to the above three breeds. The GI profile curves of terriers and Dachshunds show absolute peaks for the frontal cortex, about the end of the prefrontal cortex, at the level of the olfactory cortex and at the beginning of the sensorimotor cortex. This 'frontal type' of distribution could also be observed in beagle, Dobermann, foxterrier, Peking, Pinscher, poodle and in two of the the three mongrels studied. In German shepherds, on the contrary, absolute maxima occurred in the region of the caudal association cortex, i.e. in the transitional area between parietal, temporal and occipital cortices. This 'caudal type' of distribution was to be observed also in spitz, Hungarian pointer and in one of the mongrels studied.

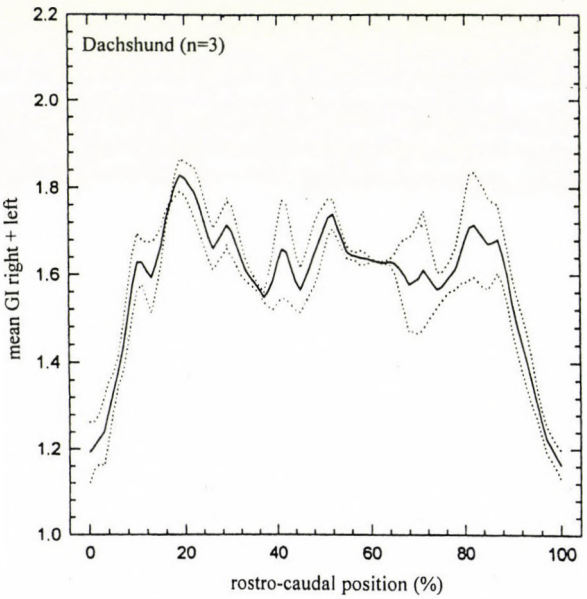
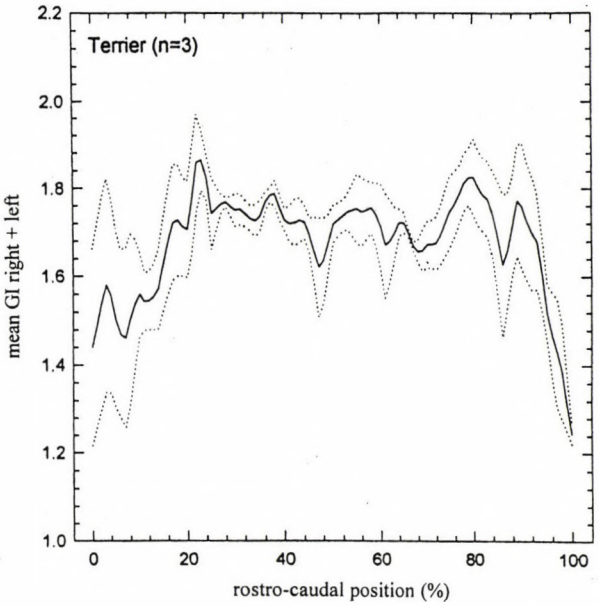


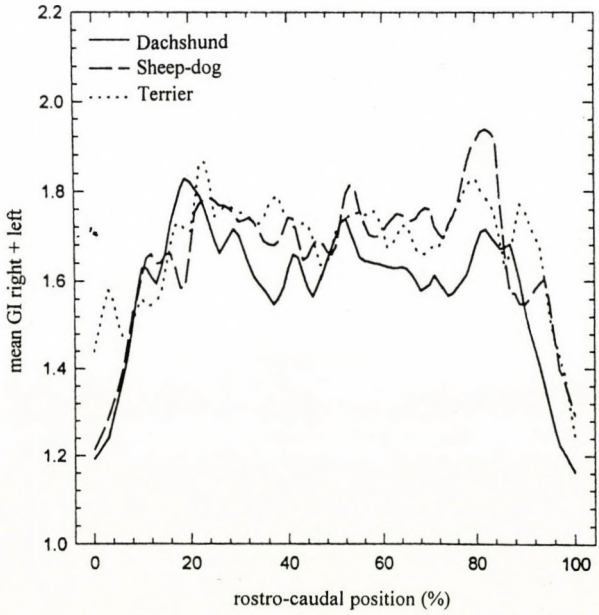
Fig. 13. Rostro caudal distribution of GI in dachshund. The mean curve with scatter was plotted from measurements of three brains

→→

Fig. 14. Rostro caudal distribution of GI in terrier. The mean curve with scatter was plotted from measurements of three brains.

Fig. 15. Comparison of rostro-caudal GI's on the basis of superimposed mean curves of Figs 11-13, in dachshund, German shepherd and terrier


Terrier (n=3)
mean GI right + left
rostro-caudal position (%)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
20
40
60
80
100


Dachshund
Sheep-dog
Terrier
mean GI right + left
rostro-caudal position (%)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
20
40
60
80
100

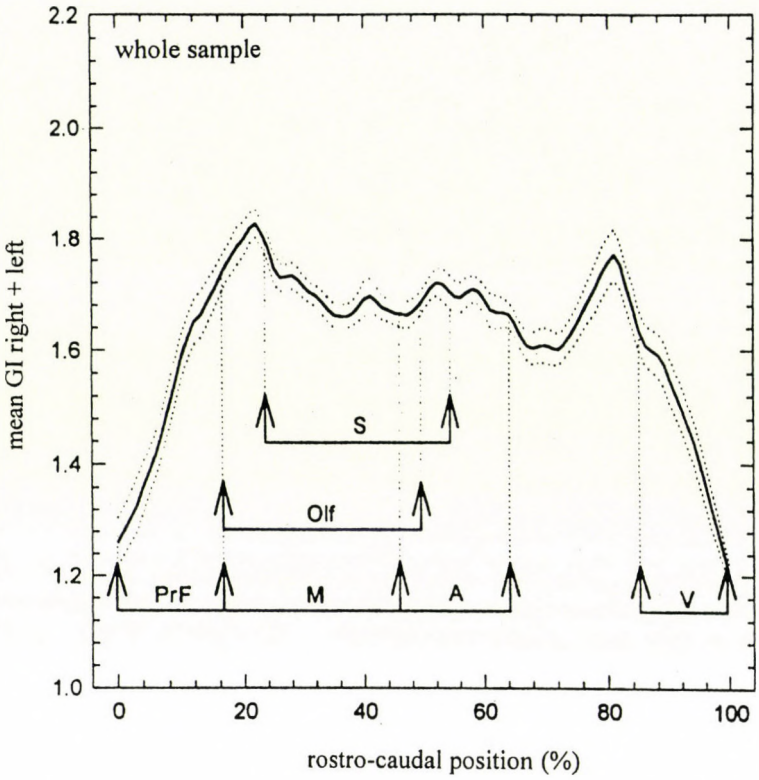


Fig. 16. GI profile curve after the summation of GI profiles of all breeds studied. Mean values are indicated by a heavy, continuous line. Above and underneath it, the dotted lines show the scatter area (..... standard error of the mean). A: extent of the primary auditory cortex, M: extent of the primary motor cortex, Olf: extent of the primary olfactory cortex, PrF: extent of prefrontal cortex, S: extent of primary somatosensory cortex, V: extent of the primary visual cortex

DISCUSSION

The aim of the present work was to study the degree of gyrification in different dog breeds which differ substantially in their body and brain sizes. Efforts were made to define the parameters which show a correlation with the gyrification index of the different breeds.

Sample and method

This type of analysis of GI was undertaken to show intraspecies differences because the analysis of GI in a similar vein in humans did not reveal a correlation between GI, brain weight, body weight and body length (Zilles et al., 1988). On the other hand, the comparative analysis of different primate species has disclosed a good correlation existing between the above parameters (Zilles et al., 1989). This has raised the question that if gyrification correlates only in interspecies terms with general body measures and brain weight, while in intraspecies comparison it is widely variable, can it be interpreted as the expression of individual cortex morphology? The answer to this question may also provide a contribution to the causal analysis of the phenomenon of gyrification. It seemed therefore, of importance to compare results obtained in human with findings relevant to another mammal, since it cannot be excluded that the lack of any correlation is unique to the human race due to its relatively narrow range of variations in brain weight and body mass (Zilles et al., 1988). However, a wide variation range might be decisive in analyses correlating parameters such as body weight, shoulder height and brain weight with GI.

Discussing the methods employed the question emerges whether the number of sections taken from one brain was sufficient to allow a representative determination of the mean GI? In a previous study (Zilles et al., 1988) it has been shown that if out of a full series of sections through a brain every 6th section was measured, the error of the mean GI was under 4%. In accordance with this finding, in the present study every 6th section was mounted for measuring so as to ensure a determination of mean GI with high accuracy.

GI and hemispheric asymmetry.

Several studies of human brains have shown that brain regions and sulci of the two hemispheres may be asymmetric as far as their size, length and depth are concerned (Falk et al., 1986, Geschwind and Levitsky, 1968, White et al., 1994). These structural asymmetries are due to the lateralization of certain functions (for example language with left hemispheric dominance). In this study an index was worked out to express the degree of gyrification which is relevant to the entire brain and not to a circumscribed area only. With this overall index no regional interhemispheric asymmetry occurred, on the whole both hemispheres appeared equally gyrified. This finding, however, does not contradict data about asymmetries that were detected in

all cases by comparing circumscribed hemispheric regions. For instance in the cortical representation area of the human hand the left central sulcus was found deeper than its right counterpart, but outside the 'hand area' this sulcus was, on balance, deeper on the right than on the left side (White et al., 1994). Obviously, the measurement of the depth of the whole sulcus would certainly have masked the existing asymmetry. The fact that in this study an equal degree of gyrification was observed for both hemispheres proves that GI is not significantly modified by ontogenetically developing functional specializations that lead to the various asymmetries but follows most likely a genetically programmed, bilaterally equivalent organizatory principle.

Sex differences

Because in this work a mixed population of males and females were used, it had to be clarified whether or not the values of body size, brain weight and GI reflect a sexual dimorphism.

In our population the male animals belonged overwhelmingly to bigger breeds with higher brain weights as compared to the females. This uneven representation of breeds in the male and female populations is the result of the selection of animals primarily for the purpose of demonstrating the dependence of gyrification indices from brain and body weights rather than to point out sex differences. As to this latter aspect the measurements of Ebinger (1980) are worth of notion concerning brain and body weight relations in wolves and dogs. This study was based on the investigation of 510 dogs of different breeds out of which 245 were males and 239 females. Data indicated that sex differences, although statistically significant, are rather low. Accordingly, females had a mean brain weight 2.68% lower than males. Even in poodles where differences were found the largest, the brain weight of bitches was only 5.8% lower than in males. Therefore, the conclusion appears justified that sex-associated differences in dogs' brain weight are not significant. Indeed, in our studies GI's did not indicate any sex differences in dogs. This is in accordance with an earlier study of Zilles et al. (1988), in which GI was determined in a large population of human brains. Thus the mixed population used by us to investigate the dependence of GI from brain weight and body size yielded representative summarizing results valid for either sex.

Gyrification index, body and brain size

Data presented have shown that in spite of the dependence of brain weight from body size and the simultaneous correlation between GI and brain weight, it can be stated that GI is almost entirely determined by brain weight or volume. This means that the former view mentioned when gyrification index was explained, that the larger the species the higher its gyrification is, should be revised when seeking a causal correlation. It is not the body size but the brain size which is responsible for the degree of gyrification. Of course the finding of larger brains in larger species often coincides with a higher degree of gyrification and a bigger body. Taking this new aspect into consideration an explanation can be offered for the virtually paradoxic case of a Cercopithecus, *Miopithecus talapoin* that has in spite of a small body size a relatively intensely gyrified brain (Zilles et al., 1989). In this kind of primate a secondary regression of body size during evolution has been assumed (Stephan et al., 1981) which was not proportionately followed by the brain weight. As brain weight is higher than allometry (relationship between body size and brain weight in primates) would predict it, the gyrification index should also be higher then expected on the basis of body size. Our data can therefore, highlight the reason of the relatively high gyrification index of *Miopithecus talapoin.* A further implication is that the brain size - gyrification index relationship is a general organizatory principle in mammals.

Gyrification and topography of cerebral cortex

Our regional analysis of gyrification has revealed another organizational principle of gyrencephalic brains: the cortical gyrification is particularly high in regions whose differentiation results in the formation of specific cortical areas. In man and subhuman primates these are mainly the regions in which the prefontal and parieto-temporo-occipital association areas are to be found (Armstrong and Curtis, 1989, Armstrong et al., 1991a,b, 1993, 1994, Zilles et al., 1988, 1989). In dogs GI was found especially high in the transitional region between the prefrontal and frontal cortices and between the temporal and occipital cortices. In this work we identified a 'frontal type' of gyrification in which in addition to the frontal absolute maximum a second, only slightly lower temporo-occipital peak occurred. On the contrary, in the 'caudal type' a similar secondary peak of the GI-profile curve was observed in the frontal region. Although in the dog the absolute and relative size of the association cortex is lower than in primates, the GI peaks occur neatly at the

same localizations. The evolutionary relatively new but particularly intensively developed association areas of the mammalian cortex are thus the places where gyrification appears to be the strongest in all species studied so far. It may be assumed that this is a general organizational principle for the formation of sulci in mammals. To corroborate this claim further studies are required in other gyrencephalic mammals such as felidae, ungulates and in mammals with extremely gyrified brains such as whales and dolphins. Nevertheless, in whales and dolphins the regions of association cortex have not been defined as yet. This is why our present study and the works referred to in it constitute for the time being the only data which enable to analyze the assignment of intense gyrification to cortical association areas.

REFERENCES

Anton G (1903) Gehirnvermessung mittels des Kompensations-Polar Planimeters. Wiener Klin Wochenschr 16: 1263-167

Aresu M (1914) La superficie cerebrale nell uomo. Arch ital Anat Embriol 12: 380

Ariens-Kappers CU, Huber GC, Crosby EC (1960) The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. Hafner: New York 1960

Armstrong E, Curtis M (1989) The development of cortical folding in human and rhesus brains. Soc Neurosci Abstr 15: 73

Armstrong E, Curtis M, Buxhoeveden DP, Fregoe C, Zilles K, Casanova MF, McCarthy WF (1991 a) Cortical gyrificytion in the rhesus monkey: A test of the mechanical folding hypothesis. Cerebral Cortex 1: 426-432

Armstrong E, Zilles K, Curtis M, Schleicher A (1991 b) Cortical folding, the lunate sulcus and the evolution of the human brain. J Hum Evol 20: 341-348

Armstrong E, Zilles K, Schleicher A (1993) Cortical folding and the evolution of the human brain. J Human Evol 25: 387-392

Armstrong E, Zilles K, Omran H, Schleicher A (1994 in press) The Ontogeny of human gyrification. Cerebral Cortex

Barron BH (1950) An experimental analysis of some factors involved in the development of the fissure pattern of the cerebral cortex. Exp Zool 113: 553-579

Bok ST (1939) A method of measuring the surface of a body from a series of parallel sections. Acta Neerl Morphol 2: 280-292

Brodmann K (1909) Vergleichende Lokalisationslehre der Großhirnrinde. Barth: Leipzig

Brodmann K (1913) Neue Forschungsergebnisse der Grosshirnrindenanatomie mit besonderer Berücksichtigung anthropologischer Fragen. Verh Ges Dtsch Naturforsch Ärzte 85: 20 0-240

Buxhoeveden D, Armstrong E (1991) Relative amounts of grey and white matter in rhesus and human brains. Am J Phys Anthropol 83: 1 77

Chi JG, Dooling EC, Gilles FH (1977) Gyral development of the human brain. Ann Neurol 1: 86-93

Clark WE, Le Gros (1945) Deformation patterns in the cerebral cortex. In: Essays on Growth and Form (Clark WE, Le Gros, Medawar P, Eds), Oxford University Press: London, pp 1-22

Connolly CJ (1950) External Morphology of the Primate Brain. Thomas: Springfield

Cunningham DJ (1890) On cerebral anatomy. Br Med J 2: 277-283

Dan R, Jequier SO, Gorman AM (1986) Normal and abnormal gyral development of the premature infant on transfontanelle ultrasound. Ann Radiol 298: 674-680

Dorovini-Zis K, Dolman CL (1977) Gestational development of brain. Arch Pathol Med 101: 193-195

Eberstaller O (1884a) Zur Oberflächen-Anatomie der Grosshirn-Hemisphären. Vorläufige Mitteilung. Wiener Med Blätter 7: 479-482

Eberstaller O (1884b) Zur Oberflächen-Anatomie der Grosshirn-Hemisphären II. Der Hinterhauptlappen. Wiener Med Blätter 7: 543-550

Eberstaller O (1884c) Zur Oberflächen-Anatomie der Grosshirn-Hemisphären III. Wiener Med Blätter 7: 580-582

Eberstaller O (1884d) Zur Oberflächen-Anatomie der GrosshirnHemisphären. IV. Das untere Scheitelläppchen. Wiener Med Blätter 7: 610-615 and 644-646

Ebinger P (1980) Zur Hirn-Körpergewichtsbeziehung bei Wölfen und Haushunden sowie Haushundrassen. Z Säugetierkunde 45: 148-153

Elias H, Schwartz D (1969) Surface areas of the cerebral cortex of mammals determined by stereological methods. Science 166: 1011-1013

Elias H, Schwartz D (1971) Cerebral-cortical surface areas, volumes, lengths of gyri and their interdependence in mammals, including man. Z Säugetierkunde 36: 147-163

Evrard P, Caviness VS, Pratsvin J, Lyon G (1978) Mechanism of arrest of neuronal migration in Zellweger malformation: Hypothesis based upon cytoarchitectonic analysis. Acta Neuropathol (Berl) 41: 109-117

Falk D (1978) External neuroanatomy of Old World monkeys (Cercopithecoidea). Contrib Primatol 15: 525-539

Falk D (1980) A reanalysis of the South African Australopithecine natural endocasts. Am J phys Anthrop 53: 525-539

Falk D (1983a) Cerebral cortices of east african early hominids. Science 221: 1072-1074

Falk D (1983b) The Taung endocast: A reply to Holloway. Am J phys Anthrop 60: 479-489

Falk D (1985) Apples, oranges, and the lunate sulcus. Am J phys Anthrop 60: 479-489

Falk D, Cheverud J, Vannier MW, Conroy GC (1986) Advanced computer graphics technology reveals cortical asymmetry in endocasts of rhesus monkeys. Folia Primatol 46: 98-103

Falk D, Hildebrot, C, Vannier MW (1989) Reassessment of the Taung early hominid from a neurological perspective. J hum Evol 19: 485-492

Geschwind N, Levitsky W (1968) Human brain: Left-right asymmetries in temporal speach regions. Science 161: 167-168

Goldman PS, Galkin TW (1978) Prenatal removal of frontal association cortex in the fetal rhesus monkey: Anatomical and functional consequences in postnatal life. Brain Res 152: 451-485

Goldman-Rakic PS, Rakic P (1984) Experimental modification of gyral patterns. In: Cerebral Dominance: The Biological Foundation (Geschwind N, Galaburda A, Eds), Harvard University Press: Cambridge, pp 179-192

Heitmann KU (1993) Quantitative Untersuchungen zur Gyrifizierung der menschlichen Hirnrinde. Med-Diss, Universität Köln

Henneberg R (1910) Messung der Oberfläche der Großhirnrinde. J Psychol Neurol 17: 144-158

Hennig A (1957) Volum- und Oberflächenmessung in der Mikroskopie. Verh Anat Ges 54: 252-265

Hershkovitz P (1979) Cerebral fissural patterns in platyrrhine monkeys. Folia Primatol 13: 213-240

Hofman MA (1989) On the evolution and geometry of the brain in mammals. Prog Neurobiol 32: 137-158

Holloway, RL (1968) The evolution of the primate brain: Some aspects of quantitative relations. Brain Res 7: 121-172

Holloway, RL (1981) Revisiting the South African Taung Australopithecine endocast: The position of the lunate sulcus as determined by the stereoplotting technique. Am J Phys Anthrop 56: 43-58

Holloway, RL (1984) The Taung endocast and the lunate sulcus: A rejection of the hypothesis of its anterior position. Am J phys Anthrop 64: 285-287

Holloway, RL (1988) Some additional morphological and metrical observations on Pan brain casts and their relevance to the Taung endocast. Am J phys Anthrop 77: 22-33

Jensen J (1870) Die Furchen und Windungen der menschlichen Großhirnhemisphären. Allg Z Psychiat 27: 473-515

Kraus WM und Ditto MW (1927) A method of measuring the cerebral and cerebellar cortical surface. Arch Neurol Psychiat 17: 193-197

Kraus WM, Davison C, Weil A (1928) The measurement of cerebral and cerebellar surfaces: III. Problems encountered in measuring the cerebral cortical surface in man. Arch Neurol Psychisat 19: 454-477

Kretschmann H-J, Wingert F (1967) Über die Fehlergröße der Oberflächenbestimmung nach der Schnittserienmethode. Z wiss Mikrosk 68: 93-114

Leboucq G (1929) Le rapport entre le poids et la surface de I hemisphere cerebral chez 1 homme et les signes. Acad Roy Belgique, Classe des Sciences Memoi 9: 3-56

Merker B (1983) Silver staining of cell bodies by means of physical development. J Neurosci Meth 9: 235-241

Moser KH (1988) Der Gyrifizierungsindex in einer aufsteigenden Primatenreihe. Med-Diss, Universität Köln

Omran H (1992) Ontogenese der Gyrifizierung des Cortex cerebri des Menschen. Med-Diss, Universität Köln

Ono M, Kubik S, Abernathey CD (1990) Atlas of the Cerebral Sulci. Thieme Verlag: Stuttgart

Popoff I (1929) Über einige Größenverhältnisse der Affenhirne. J Psych Neurol 38: 82-90

Prothero JW, Sundsten JW (1984) Folding of the cerebral cortex in mammals. A scaling model. Brain Behav Evol 24: 152-167

Pubols BH, Pubols LM (1979) Variations in the fissural pattern of the cerebral neocortex of the spider monkey (Ateles). Brain Behav Evol 16: 241-252

Retzius G (1896) Das Menschenhirn. Studien in der makroskopischen Morphologie. Norstedt: Stockholm

Richman DP, Stewart RM, Hutchison JW, Caviness SV (1975) Mechanical model of brain convolutional development. Science 189: 18-21

Schlenska G (1969) Messungen der Oberfläche und der Volumenanteile des Gehirnes menschlicher Erwachsener mit neuen Methoden. Z Anat Entwickl-Gesch 128: 47-59

Smart IHM, McSherry GM (1986 a) Gyrus formation in the cerebral cortex of the ferret. I. Description of the external changes. J Anat 146: 141-152

Smart IHM, McSherry GM (1986 b) Gyrus formation in the cerebral cortex of the ferret. Description of the internal histological changes. J Anat 147: 27-43

Spitzka EA (1907) Study of the brains of 6 eminent scientists and scholars belonging to the American Anthropometric Society, together with a description of the skull of Professor ED Coppe. Trans Am Philos Soc 21: 175-308

Stephan H (1960) Methodische Studien über den quantitativen Vergleich architektonischer Struktureinheiten des Gehirns. Z Wiss Zool 164: 143-172

Stephan H (1961) Vergleichend anatomische Untersuchungen an Insektivorengehirnen. V. Die quantitative Zusammensetzung der Oberflächen des Allocortex. Acta Anat 44: 12-59

Stephan H, Frahm H, Baron G (1981) New and revised data on volumes of brain structures in insectivores and primates. Folia Primatol 35: 1-29

Todd PH (1982) A geometric model for the cortical folding pattern of simple folded brains. J Theor Biol 97: 529-538

Todd PH (1986) Intrinsic geometry of biological surface growth. In: Lecture Notes in Biomathematics, Vol 67. Springer Verlag: New York

Tramer M (1916) Über Messung und Entwicklung der Rindenoberfläche des menschlichen Großhirns mit Beitrag zur Kenntnis der Microcephalia vera. Arb Hirnanat Inst Zürich 10

Turner W (1890) The convolutions of the brain: A study in comparative anatomy. J Anat Physiol 25: 105-153

Von Bonin G (1941) Side lights on cerebral evolution: Brain size of lower vertebrates and degree of cortical folding. J Gen Psychol 25: 273-282

Wagner H (1864) Maßbestimmungen der Oberfläche des großen Gehirns. Inaug Diss, Göttingen

Welker W (1990) Why does cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In: Cerebral Cortex, Vol 8B (Jones EG, Peters A, Eds). Plenum: New York, pp 3-136

Welker Wl, Campos GB (1963) Physiological significance of sulci in somatosensory cerebral cortex in mammals of the family Procynidae. J Comp Neurol 120: 19-36

White LE, Lucas G, Richards A, Purves D (1994) Cerebral asymmetry and handedness. Nature 368: 197-198

Zilles K, Armstrong E, Schleicher A, Kretschmann H-J (1988) The human pattern of gyrification in the cerebral cortex. Anat Embryol 179: 173-179

Zilles K, Armstrong E, Moser KH, Schleicher A, Stephan H (1989) Gyrification in the cerebral cortex of primates. Brain Behav Evol 34: 143-150

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Short announcement

6th Meeting of the International Neurotoxicology Association
29 June - 4 July, 1997
Szeged, Hungary

GENERAL INFORMATION

The International Neurotoxicology Association (INA) is an organization of scientists working in the field of nervous system toxicology. Fields of interest include all disciplines related to the study of the adverse effects of drugs and chemicals on the nervous system including neurochemistry, physiology, neuropathology and behavior. Every two years, INA organizes a meeting in a quiet, secluded place to encourage personal contact and the exchange of ideas among scientists from different disciplines and to facilitate the introduction of novices in the field to new colleagues and areas of interest. INA will hold its sixth bi-annual meeting Szeged, a medium-size university town in southeast Hungary.

Being the third biggest town in Hungary, Szeged is the cultural, educational and commercial centre of the region. Szeged has two universities: the Szent-Györgyi Albert Medical University (named after the Nobel price winner Hungarian scientist) and the József Attila University, and several colleges. These institutions, together with the Biological Research Centre, represent a considerable research potential in many fields of science, medicine and arts.

SCIENTIFIC PROGRAM

The program will consist of symposia with invited speakers, workshops, students' courses, a debate session, poster sessions and the "Jacob Hooisma Lecture" by Hugh Tilson (USEPA Health Effects Research Lab., Research Triangle Park, NC). The official language of the whole congress is English. There will be a competition and prizing for students presenting a poster.

Akadémiai Kiadó, Budapest

Up to now, the following topics have been proposed:

- topics, concerning the basic fields of interest of INA
- connections of neurotoxicology and immunology, neuro-immunology
- neuro-pathophysiology
- in vitro neurotoxicology
- role of metallothionins in neurotoxicity and neuroprotection
- neurotoxicology of aluminium and cadmium
- genetic polymorphism in the neurotoxicology, genetic susceptibility
- special neurotoxicological problems in Central and East Europe

Registration fees:

Participants	250 USD
Participants from East Europe*	80 USD
Students**	70 USD
Students from East Europe**	30 USD
Accompanying persons	125 USD

* valid only for participants from the previous socialist countries
** for regular and Ph.D. students only with a proper certificate from their institutions

FURTHER INFORMATION

Prospective participants are kindly requested to contact Prof. Illés Dési at this address:

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ABSTRACTS

from the

Annual Meeting

of the

Hungarian Biochemistry Society
Drug Biochemistry Section

May 29–31
1996

Balatonőszöd

Organizer:
P. Arányi

ROLE OF THE TRANSPORT PROCESSES OF ENDOPLASMIC RETICULUM IN THE REGULATION OF GLUCURONIDATION

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Transport processes of endoplasmic reticulum (ER) are of great importance regarding the function and the regulation of the intraluminal pathways of vital significance. The aim of the present study was to investigate the transport of glucuronides synthesised by UDP-glucuronosyltransferases (UDPGT) in the lumen of ER. UDPGTs are integral proteins of ER. Their active site is intraluminal, therefore a transporter should be supposed both for UDP-glucuronic acid (UDPGA) synthesised in the cytosol and for the aglycone glucuronide produced intraluminally, being both compounds charged and water soluble. However, the microsomal transport of UDPGA and especially that of glucuronides have been hardly characterised. UDPGA is thought to be transported by an antiport jointly with UMP generated in the lumen of ER. Glucuronide transport has not been investigated yet in details, but early results indicate that the majority of glucuronides appears in the cytosol following their synthesis.

The transport of glucuronides synthesised in the lumenal compartment of ER by UDPGT isozymes was studied in rat liver microsomal vesicles. Microsomal vesicles were loaded with p-nitrophenol glucuronide, phenolphthalein glucuronide or UDPGA. It was shown that (i) a spontaneous efflux of p-nitrophenol glucuronide occurred but a sustained intravesicular level of phenolphthalein glucuronide was observed; (ii) addition of UDPGA to the vesicles caused a prompt stimulation of the release of intravesicular glucuronides; (iii) glucuronides also released UDPGA from UDPGA loaded microsomal vesicles. Trans-stimulation of UDPGA entry by loading of microsomal vesicles with p-nitrophenol glucuronide, phenolphthalein glucuronide, UDPGA or UDP-Nacetylglucosamine almost completely abolished the latency of UDPGT, although mannose 6-phosphatase latency remained unaltered. The loading compounds themselves did not stimulate UDPGT activity. This study demonstrates that glucuronides synthesised in the lumen of the endoplasmic reticulum can leave by an antiport, which, in turn, transports UDPGA into the lumen.

THE ROLE OF 3-D PROTEIN STRUCTURE DETERMINATIONS IN DRUG DESIGN

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An overview of the major steps of the protein crystallographic analysis will be illustrated by a few examples elaborated recently by our group. Structure and antagonist binding of calmoduline (CaM) have been investigated by several groups and methods, but only few high resolution crystal structures are known. Therefore we initiated the protein crystallographic analysis of the complex of the generally used CaM antagonist reference compound trifluoro-perazine (TFP) with calmoduline, as well as complexes formed by our own antagonists and CaM. Our most important findings are summarised below.

A solution containing the 1:1:1 ratio mixture of the CaM:TFP:KAR2 molecules (where KAR2 is a bis-indole type CaM antagonist demonstrated to act and bind in a different manner than TFP) yielded crystals isostructural with those gained from the solution containing CaM:TFP=1:40. These latter were shown to contain 4 TFP molecules/CaM. In our case we found only two partially occupied TFP sites in the carboxy terminal domain, while the presence of KAR2 could not be shown. Later it was not detected by CD measurements either from the solution prepared by redissolving the crystals. The experiment also demonstrated that the binding of an antagonist molecule on both domains of CaM is not needed for the protein to assume the globular conformation as opposed to earlier suggestions. From the CaM/KAR2 binary complex we also managed to grow crystals which seem to have twice as long c axis as that in the CaM/TFP complex, while the other two axes remain similar. A diphenylalkyl-amine type molecule of Chinoin Pharmaceuticals (CSA-829) yields isostructural crystals to those of the TFP complex described above. This molecule brings around the same type of structural change as TFP!

The Chinoin molecule 3IE229 (a diphenyl-alkyl-diamino-alkane type compound) also crystallises with CaM, but to our surprise this crystal contains at least new intermolecular contacts. However, it is also highly likely that the intramolecular association between the two CaM domains has also changed in this complex, since molecular replacement using the TFP model did not supplied the solution of the crystal structure. The solution of this structure by the method of isomorphous replacement is in progress. All of the crystals described above contained CaM in its Ca^{2+} complexed form. However, we also managed to collect data on a CaM-KAR2 complex in the absence of Ca^{2+}. No structure of this kind is known thus far.

RELATION OF DRUG METABOLISM TO ANTIOXIDANT DEFENCE IN THE LIVER

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Cofactor supply plays an important role in the regulation of the mixed-function oxidation (phase I) and glucuronidation (phase II) - the two quantitatively most important processes of biotransformation in the liver. Under conditions when biotransformation is shifted towards hydroxylation the production of an antioxidative agent such as ascorbic acid would be advantageous. Under those circumstances glucuronidation and ascorbate synthesis seem to protect against toxic challenges. Both glucuronidation and ascorbate production require UDP-glucuronic acid as a substrate. It has been reported that glycogenolysis - and not gluconeogenesis - is the source of UDP-glucuronic acid for glucuronidation. In animals with the exception of primates, guinea pig and some other species ascorbate is produced in the uronic acid pathway in the liver or kidney from UDP-glucuronic acid. Ascorbic acid synthesis was stimulated by glucagon, dibutyryl cyclic AMP, as well as phenylephrine, vasopressin or okadaic acid in hepatocytes prepared from fed mice. However, no such effect was observed in glycogen depleted cells from starved animals, either in the presence or absence of glucose. The rate of ascorbate synthesis showed close correlation with the glucose release by hepatocytes. In mice the injection of glucagon increased plasma ascorbate concentration fifteenfold, and caused a sixfold elevation of the ascorbate content of the liver. These results suggested that hepatic ascorbate synthesis is dependent on glycogenolysis. In vivo studies have shown that glutathione deficiency induces ascorbate synthesis in mouse liver, therefore relationship among glutathione deficiency, glycogen metabolism and ascorbate synthesis was investigated in isolated murine hepatocytes. Glutathione deficiency caused by various agents increased ascorbate synthesis with a stimulation of glycogen breakdown. Increased ascorbate synthesis from UDP-glucose or gulonolactone could not be further affected by glutathione depletion. Fructose prevented the stimulated glycogenolysis and ascorbate synthesis caused by glutathione consumption. Reduction of oxidised glutathione by dithiothreitol decreased the elevated glycogenolysis and ascorbate synthesis in diamide or menadione treated hepatocytes. Our results suggest that a change in GSH/GSSG ratio seems to be a sufficient precondition of altering glycogenolysis and a consequent ascorbate synthesis.

CARDIOVASCULAR EFFECTS OF HETEROCYCLIC COMPOUNDS CONTAINING NITROGEN AS HETERO ATOM

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Digitalis glycosides have been known as beneficial agents for most patients with congestive heart failure. However, their clinical use is troubled by the narrow ratio of therapeutic to toxic doses. The development of safe and effective positive inotropic drugs is required. During the last decade advances in the understanding of cellular and subcellular control of cardiac contractility resulted in new classes of potent positive inotropic drugs including adrenoceptor agonists, adenylate cyclase activators, phosphodiesterase inhibitors, sarcolemmal calcium channel agonists or calcium sensitisers.

In the current literature a number of bipyridine, diazinone, pyridazonine and quinazoline derivatives have been described as cardiotonic agents. On the base of structure-activity studies of these compounds benzimidazole, benztriazole, triazolo-pyridine and triazolo-quinoline derivatives were synthesised. Cardiovascular effects were measured using various cardiac preparations from guinea pigs in isolated organ experiments, rat hearts in Langendorff experiments and anaesthetised opened-chest rats in haemodynamic experiments. Toxicological and platelet aggregation tests also were performed.

At the present state of our studies the most promising molecule - a triazolo-quinoline derivative - posses good positive inotropic, lusitropic and excellent vasodilator effects. It is neither an a- or ß-adrenoceptor agonist, nor a phosphodiesterase inhibitor and it does not increase sarcolemmal calcium influx but its calcium sensitising potential can be supposed. Recently calcium sensitisation has been extensively discussed as a new mechanism to improve the heart contractility in decompensation without increasing the oxygen demand. Our compound does not increase the cytosolic free calcium concentration therefore, it should be devoid of any arrhytmogenic effect. Clarifying the exact acting mechanism of our lead molecule deserves further investigation.

CA^{2+} DEPENDENT REGULATION OF GLUCURONIDATION

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UDP-glucuronosyltransferase (UDPGT) isozymes are integral membrane proteins of the liver endoplasmic reticulum. They catalyze glucuronidation, the most important phase II biotransformation reaction. Formation of UDP-glucuronic acid, a substrate of the UDPGTs, depends on glycogenolysis. Surprisingly there are some agents (e.g. epinephrine, vasopressin, endotoxin and prostaglandins), which stimulate glycogen breakdown but inhibit glucuronidation. Since they were known to mobilize calcium from intracellular stores, and UDPGTs require bivalent cations, mobilization of intrareticular Ca^{2+} was supposed to be responsible for the phenomenon.

The Ca^{2+}-chelator EGTA, the Ca^{2+}-ionophor ionomycin and A23 187 or the Ca^{2+}-ATPase inhibitor thapsigargin effectively deplete intrareticular calcium stores. Administration of these agents caused a significant decrease of p-nitrophenol glucuronidation in isolated hepatocytes. Moreover, this effect of EGTA was reversible with Ca^{2+} addition. When the Ca^{2+} concentration of the incubation medium was increased p-nitrophenol UDPGT activity in intact microsomal vesicles was not affected, however in permeabilized microsomes it was significantly stimulated. Glucuronidation in isolated microsomes was enhanced by Ca^{2+} accumulation in an ATP-generating medium. These data suggest that changes in intrareticular Ca^{2+} concentration may affect UDPGT activity. Vasopressin and epinephrine are known to mobilize calcium from intracellular stores through inositoltrisphosphate formation. Since they inhibited p-nitrophenol glucuronidation in isolated mouse hepatocytes it was concluded that intrareticular calcium plays important role in regulation of glucuronidation.

Nutritional state can also affect glucuronidation in a calcium dependent way. Acyl-CoAs cause Ca^{2+} mobilization from endoplasmic reticulum, which is the mechanism of the inhibitory effect of fatty acids on glucuronidation.

COMPUTER AIDED MODELING AND DESIGN OF SOME COMPOUNDS

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In EGIS Pharmaceuticals, the research of the new original compounds is organized into projects. A considerable amount of new compounds are synthesized and tested by biochemical and pharmacological methods.
The chemical and biological data are handled with computers. The first step of the process is the registration into ISIS/Base databases, the common starting point of the subsequent studies. The 3D structure of the molecules is established by the program OSIRIS[1] and a conformation analysis is carried out as well. With the aid of the CASSANDRA[1] package structure-activity relations are produced within each project by a 2D pattern recognition method and upon this base activity estimations are possible. On the compounds having a common skeleton, Free-Wilson-Hansch analysis is made by the software DrugIdea. Last but not least, 3D pharmacophore modeling helps the design of new compounds using the program CINDERELLA[1].
The application of these methods made the drug development more effective, the costs of the synthesis and testing of inactive compounds could be reduced.

[1]Developed by I. Kövesdi and A Dancsó, available from the authors.

MODERN METHODS IN THREE-DIMENSIONAL DRUG DESIGN

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The most efficient drug design strategies, currently in use or in testing at Chinoin, are reviewed. The methods are discussed in the two general categories of lead optimisation and finding new lead compounds. Both approaches are inherently aimed at enhancing the drug-receptor interaction.

It was found that for the molecules of interest conformational analysis always had to precede lead optimisation. Search methods based on molecular mechanics and quantum chemistry were found most satisfactory, while molecular dynamics often turned out to sample the conformational space inadequately. In most situations cluster or family analysis was necessary to reduce the number of cases to be dealt with.

Best results in lead optimisation were attained with methods of three-dimensional QSAR, using the PLS method of data reduction. This does not only provide the regression equations to predict the activity of new compounds but also visually marks the spatial volumes where the introduction of further substituents would lead to an improvement or deterioration of drug action.

New lead compounds can be found by different methods. Of these the search for the pharmacophore group and a subsequent flexible 3-D database search for molecules containing this group appears to be the most universally applicable technique. The 'de novo' method, based on the simulated enzyme structure from 3-D QSAR has also been found to provide useful tips as to the possible improvement for the lead (i.e. optimisation) but in order to use it for identifying new leads the knowledge of the enzyme structure would be essential. A promising new development is the planned application of structure-based design using the receptor structure from protein crystallography.

NEW MACROMOLECULE CONJUGATES CONTAINING GNRH PEPTIDE HORMONE ANALOGS WITH SELECTIVE ANTITUMOR ACTIVITY. 4. EFFECT OF NEW GnRH ANALOGS AND THEIR CONJUGATES ON TUMOR GROWTH *IN VIVO*

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We have studied the antitumor activity of 3 novel GnRH analogs (human MI-1544 and chicken MI-1892 antagonist, and sea lamprey GnRH-III) and their conjugates. MI-1544 and its conjugate had strong, whereas MI-1892 and its conjugate had slight, castration effect in rats. The *in vivo* experiments were carried out on the estrogen receptor (ER)-positive MCF-7, the ER-negative MDA-MB-231 human breast cancer xenografts in immunosuppressed CBA-Ca HRIJ-T6 female mice and on the MXT mouse mammary tumor in BDFl hybrid mice. GnRH analogs and their conjugates containing 50-100 mg active subtances/mouse were injected daily for 5-6 weeks subcutaneously. The treatment of xenograft-bearing animals was started on day 24-28 following transplantation, while that of MXT tumor-bearing mice began one day after tumor transplantation. A 25% inhibition of tumor growth was found on the xenograft models treated with MI-1544 or MI-1892. After their conjugation the antitumor activity was largely increased (40-50% inhibition of tumor growth). Their tumor inhibitory effect was stronger in MXT mouse tumor model (~70%) than in the xenograft models. The free antagonist analog MI-1544 resulted in a 40% inhibition of tumor volume. GnRH-III analog was ineffective on growth of MDA-MB-23 1 xenograft, whereas the P-X-GnRH-III conjugate resulted in a 80% decrease of tumor volume. Some of the conjugate-treated mice were tumor-free after nine weeks' treatment. The GnRH antagonists and their conjugates had neither toxic, nor immunosuppressive effect as opposed to some known GnRH analogs.
Our results indicated that GnRH analogs and the conjugates having significant antitumor activity against estrogen-dependent and independent tumors might become important therapeutic agents for the hormonal and/or combined therapy of breast cancer.

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REDUCED TOXIC SIDE EFFECTS AND INCREASED ANTITUMOUR EFFICACY OF DAUNOMYCIN BY CONJUGATION TO AN AMPHOTERIC BRANCHED POLYPEPTIDE

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Recently the interest in the use of macromolecules as polymeric carrier polypeptides for cytostatic agents has increased. Daunomycin (Dau) has been coupled covalently to the synthetic branched polypeptide with a polylysine backbone using its acid-labile cisaconityl derivative (cAD). In our preliminary studies we have experienced that the amphoteric variation of polypeptide containing glutamic acid at the side chain terminal position (EAK) has a favourable blood kinetics and biodistribution.

The aim of this study is to compare the toxicity, immunomodulatory and antitumour activity of EAK-cAD. Modulation of systemic toxicity of Dau by the linkage to the polymeric polypeptide in BDF1 mice was studied on the basis of body weight, lifespan, bone marrow analysis and haematologic parameters. More than three-fold of lethal Dau-dose could be prevented when the drug was attached to the carrier polypeptide.

The dose- and time-dependent modulatory effect of free drug and EAK-cAD conjugate on the humoral and cellular immune response to sheep red blood cell antigens in mice was studied using the haemolytic plaque forming cell- and rosette forming cell assays. EAK-cAD was generally capable to compensate the immunosuppressive effect of free Dau or restore the immune capacity of mice damaged by cytostatic agent. The antitumour activity of EAK-cAD conjugate and the free Dau was compared and studied on BDF1 mice bearing i.p. L1210 and P388 leukaemia. The maximum increase in survival time of free drug treated L1210 tumour bearing mice (i.p., 4 mg/kg dose) was 152% while EAK-cAD conjugate (i.p. at doses corresponding to the 2-10 mg/kg Dau content) - produced a significant increase in the life span and the number of long-term (> 60 days) survivors. In contrast the conjugate, EAK polymeric carrier applied in a mixture with free drug could not influence the systemic toxicity and therapeutic efficacy of Dau against L1210 leukaemia. Our experimental data and observations suggest that coupling plays an important role in the lower drug toxicity and higher tumour inhibitory effect of amphoteric branched polypeptide-bound Dau.

EAK-cAD conjugate as a novel toxicity modulator is promising antitumour agent in cancer chemotherapy.

AN IN VITRO COMPARATIVE STUDY OF ANTIOXIDANTS WITH VARIOUS CHEMICAL STRUCTURES

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The aims of this study were (1) to determine structure-activity relationship of various known antioxidants with regard to their lipid peroxidation (LPO) inhibitory activity, (2) to assess their most important structural element(s) responsible for antioxidant effect, (3) to locate their site of action in the LPO process (i.e. in the initiation, propagation and termination). The known antioxidants were phenolic or polyphenolic antioxidants, chromanes, flavonoids, thiols, dihydroquinolins, benzoquinones, polyalcoholic agents, reducing compounds, molecules with purine and pyrimido-pyrimidine skeleton. The inhibitory effect of the above substances on LPO induced by enzymic (i.e. by NADPH in cerebral microsomes) and non-enzymic (i.e. by Fe2+ in brain homogenate) mechanisms has been investigated. The free radical (superoxide anion, hydroxyl radical, alkoxy-, peroxy radical) scavenging activity of the compounds has been determined. The interaction of antioxidants with reactive oxygen intermedier H_2O_2 has also been studied and comparison of their iron-chelator properties has been made.

The order of LPO inhibitory potency was: dihydroquinoline > phenolic antioxidants, quinones > polyphenolic antioxidants, flavonoids > chromanes.

The order of free radical scavenging activity: flavonoids, polyphenolic antioxidants > thiols, uric acid, thioctic acid, trolox > DMTU, mannitol.

Our data suggest that prerequisites for antioxidant effect and/or free radical scavenging activity are: (1) one or more hydroxy group(s) in aromatic ring, (2) many alcoholic hydroxy groups, (3) free sulphydryl group, (4) electron or proton donating property (5) electron carrier function.

POLYMERIC DRUG DESIGN: PREPARATION AND CHARACTERISATION OF DAUNOMYCIN - BRANCHED POLYPEPTIDE CONJUGATES WITH POLY-[L-LYSINE]

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In the past decade, biodegradable water soluble polymeric branched polypeptides with the general formula poly[Lys-(X_1-DL-Ala_m)] (XAK), poly[Lys-(X_1)] (XK) and poly[Lys-(DL-Ala_m-X_1] (AXK) where i<1, m 3 and X stands for an optically active amino acid were introduced by our laboratory [1]. Polymeric anticancer drug derivatives of these synthetic macromolecules were prepared with a GnRH antagonist [2], methotrexate [3], p-borono-Phe [4] and daunomycin (Dau) [5]. First the acid labile derivative of Dau, cis-aconityl daunomycin (cAD) was synthesised by reacting with cis-aconitic anhydride under alkaline conditions. As demonstrated by HPLC this procedure resulted in a 1:1 mixture of the a and ß isomers. cAD isomers were coupled to the a-amino groups of XAK or AXK type polymers using carbodiimide method. The conjugated Dau content was determined by UV spectroscopy and amino acid acid analysis. Preliminary studies have shown, that the biodistribution in BALB/c mice and *in vitro* cytotoxic properties of the constructs were dependent on the charge and side chain structure of the polymeric polypeptide. Based on prolonged half life in the blood after i. v. injection and favourable *in vitro* cytotoxicity, an amphoteric poly-peptide derivative of cAD, [cAD]-EAK has been selected for studying the effect of covalent attachement of cAD to polymers on the *in vivo* cytotoxicity, antitumor activity and immunostimulatory potential of the free Dau.

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References

1. Hudecz, F.: Anti-Cancer Drugs 6(2): 171-193 (1995). 2. Vincze, B. et al.: J. Cancer Res. Clin. Oncol. 120: 578-584 (1994). 3. Mezö, G. et al.: J. Bioactive and Compatible Polymers (1996) (in press). 4. Hudecz, F. et al.: Bioconjugate Chem. 4: 25-33 (1993). 5. Hudecz, F. et al.: Bioconjugate Chem. 3: 49-57 (1992).

IMPORTANCE OF MULTIPARAMETRIC AGGREGATION INDEX IN DIAGNOSTIC AND DRUG MONITORING

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The methodology of defining the multiparametric aggregation index (MAI) is rewieved and its practicability is evaluated by analysing the data of patients groups with different forms of vascular diseases. This index may be calculated according to the method of Gazzaniga P.P. and Ferroni P. / Journal of Clinical Laboratory Analysis 6. 257-263. 1992/. Determination of multiparametric aggregation index by Gazzaniga:

$$MAI: = \frac{Mx\%/100}{Tp+Ts/3} \quad ADP + \frac{Mx\%/100}{Tp+Ts/5} \quad Epinephrine$$

Tp: Primer aggregation thresholds for ADP and Epinephrine
Ts: Secondary aggregation thresholds for ADP and Epinephrine
Mx%/100: Hundredth of maximum percentages of light transmittance induced by ADP and Epinephrine
The method is suitable for detecting changes in platelet aggregability by a multiparametric analysis based on six parameters. A higher MAI value indicates increased in vitro platelet aggregability.
90 patients were assigned to three study groups cerebrovascular, cardiovascular and peripheral arterial occlusive diseases. The effect of low dose aspirin "Astrix" therapy with 100 mg/day is reflected by a significant reduction in MAI values. In this work has been studied platelet aggregability by low dose of collagen, disaggregation, levels of thromboxane$_{B2}$ and 6-keto-prostaglandin F$_{1\alpha}$-formation. Astrix therapy markedly and significantly reduced hyperaggregability, increased disaggregation and reduced the initially high serum thromboxane$_{B2}$ level without changing 6-keto-prostaglandin F$_{1\alpha}$-formation.
On basis of data currently available this method MAI is considered to be of diagnostic value suitable for monitoring treatment with anti-platelet products and evaluating individual sensitivity.

POLYUNSATURATED FATTY ACIDS FROM MICROALGAE

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Five strains of microalgae - two isolated from freshwater (green and bluegreen algae), three isolated from seawater (green, red and diatom algae) - have been examined in the point of view of polyunsaturated fatty acid synthesis in the Institute for Drug Research.
EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid) and AA (arachidonic acid) content of the different cultures were defined by thin-layer and gas chromatography after setting the appropriate lipid isolation and trans-esterification steps.
Productive strains were chosen and we increased EPA production with these by making culture-medium, light and temperature optimising experiments. We changed the nitrate and phosphate concentration, which is important from the point of view of multiplication of algae, changed sodium-chloride concentration, which is a limiting factor at the seawater strains, and changed light intensity and temperature, which are key factors in the synthesis of polyunsaturated fatty acids.
Highest EPA content, as a percentage of biomass and total fatty acids, was produced by the diatom strain (2.3-4.8 % w/w in biomass, 22.0-33.6 % w/w in fatty acids) and the red algae (1.1-3.8 % w/w in biomass, 16.4-32.7 % w/w in fatty acids). The DHA was low or negligible in every strain. The AA was negligible too, but exception very high AA content was produced by the red algae strain (1.5-2.8 % w/w in biomass, 21.6-28.8 % in fatty acids). The seawater green and freshwater algae strains produced very low or negligible EPA, DHA and AA content.

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NEUROTRANSMITTER RECEPTORS: BINDING KINETICS, THERMODYNAMICS AND IONOPHORE FUNCTION

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GABAA, glycine, NMDA and 5-HT_3 type serotonin receptorionophore complexes have been compared. Pharmacological modulation of ionophore function has been correlated with allosteric binding interactions. The on and off rates of binding for channel blocking radioligands such as [^{35}S]TBPS or [^{3}H]TBoB for GABAA and [^{3}H]TCP for NMDA receptor-ionophores have revealed versatile allosteric interactions and reflected distinct ionophore states. Nonequilibrium [^{35}S]TBPS binding as a function of the occupancy of the benzodiazepine binding sites differentiate the whole spectrum of efficacies from full agonists to inverse agonists. The effects of GABA and the GABAA antagonist bicuculline on the dissociation phases of [3H]TBoB have been attributed to interconvertible multi-affinity states of the convulsant sites and to distinct ionophore states. The accelerating effects on TBPS dissociation differentiate CNS depressants from convulsants such as the anticonvulsant R(-) and convulsant S(+) enantiomers of 1-methyl-5-phenyl-5-propylbarbiturate (MPPB). The temperature dependence of the rates of TBPS dissociation revealed that R(-)MPPB accelerated the dissociation via decreasing its activation energy. Patch clamp electrophysiology of whole cortical neurones showed a stereoselective direct opening of the chloride ionophores by R(-)MPPB. Distinct thermodynamic parameters of binding for GABAA agonists and antagonists were correlated with their efficacies. This correlation has been extended for glycine and 5-HT_3 receptor binding. The distinct signs of the thermodynamic parameters of agonist and antagonist binding can be attributed to their distinct types of binding interactions.

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References

G. Maksay, *Neurochem. Int.*, in press.

DRUG METABOLISM AND SIGNAL TRANSDUCTION; DETOXIFICATION OR SIGNAL CONVERSION

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Drug metabolizing enzymes require cofactors formed at the expense of intermediates of various pathways in intermediary metabolism (e.g. NADPH - gluconeogenesis in phase I, UDP-glucuronate - glycogenolysis in phase II of drug metabolism). Therefore, drug metabolism represents a metabolic burden on liver metabolism, which is under a strict control. cAMP- and calcium-mediated signal transduction routes play a dominant role in regulation of metabolism in hepatocytes. There are several observations that drug metabolizing enzymes are direct or indirect targets of cAMP or calcium-dependent regulations. It may result in an altered balance of the phase I and phase II reactions and can cause an increased oxygenation and decreased conjugation. The increased formation of an oxygenated intermediate in the drug metabolism can cause several toxic effects (e.g. chemical carcinogenesis, oxidative stress).

Recently, several processes leading to the synthesis or degradation of various intercellular messengers - signals - have been shown to be catalysed by drug metabolizing enzymes (e.g. steroids, thyroxin, epinephrine). Therefore, a change in their activity can induce an alteration in the signal metabolism, as well. As drug metabolizing enzymes are mainly controlled at the level of gene expression, the induction state of the drug metabolizing enzymes (exposed mainly by xenobiotics) also may control the metabolism of several signals. The "original, biological" function of these enzymes is the participation in the "signal" metabolism, thus drug metabolism is under the control of a series of signal transduction routes, but at same time metabolism of various signal molecules is also affected.

NEW MACROMOLECULE CONJUGATES CONTAINING GnRH PEPTIDE HORMONE ANALOGS WITH SELECTIVE ANTITUMOR ACTIVITY. 1. SYNTHESES OF NEW GnRH ANALOGS AND THEIR CONJUGATES

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The use of agonists and antagonists of gonadotropin releasing hormone (GnRH) is one of the recent approaches in cancer research (1). We have synthesized some GnRH antagonist analogs, one of them, Ac-D-Trp$^{1-3}$,D-Cpa^{2},D-Lys^{6},D-Ala^{10}-GnRH (MI-1544) exhibits excellent endocrine (2) and significant antitumor effect (3). Ac-D-Trp$^{1-3}$,D-Cpa^{2},Lys^{5}(ßAsp(a-DEA))^{6},Gln^{8},D-Ala^{10}GnRH (MI-1892) has no endocrine (4), but specific antitumor effect (3). Lamprey GnRH-III, Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2 (5) isolated recently does not exhibit endocrine effect in mammals, but has strong, specific antitumor activity on human tumor cell lines (6). We have found that the in vitro and in vivo antitumor activity of GnRH analogs was enhanced by their conjugation to a macromolecular, poly-anionic carrier (7). These analogs - having free e-amino group in the Lys moiety of the molecules - were coupled to N-vinyl-pyrrolidone-co-maleic acid polimer (NVP-MA) through a peptide spacer. NVP-MA-GFLGONp (Mw: appr. 10000 D) was used for coupling. We suppose that these compounds may become new therapeutic compounds for the treatment of prostate, endometrium and breast cancer.

References

1. Miller, W.R., Steroid Biochem. Molec. Biol. 1990, 37, 467-480.
2. Kovács, M., et al., Peptides 1989, 10, 925-931.
3. Pályi, I., et al., Cancer Detection and Prevention, 1996, 20, 146-152.
4. Mező, I., et al., Biomedical Peptides, Proteins & Nucleic Acids (in press)
5. Sower, S.A., et al., Endocrinology 1993, 132, 1125-1131.
6. Mező, I., et al., Peptides (Proc. 14th Ann. Peptide Symposium) (in press)
7. Mező, I., et al., Peptides 1994, Escom, Leiden 1995 pp. 763-764.

BIOCHEMISTRY OF A NEW ANTI-MITOTIC DRUG

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The numerous anti-mitotic drugs already developed and therapeutically used in cancer chemotherapy belong to different classes based on their chemical structure and other distinctive features. Bis-indol alkaloids (e.g., vinblastine, vincristine, navelbine) are important group of anti-mitotic agents. Toxic side effects present a major problem in the administration of these drugs. Several hypotheses have been put forward to account for these phenomena, however, no molecular mechanism has been identified so far. The methodologies and techniques developed by us and applied successfully in several previous drug studies [1-6] were synthesized into complex screen systems for analysis of anti-microtubular and anti-calmodulin activities of drugs at molecular level. The combination of these cellular and biochemical methodologies rendered it possible to identify a new mechanism of action of a new drug that gives innovative hints to suggest chemotherapeutic strategies for circumvention of toxic side effects. KAR-2 (3"-(ß-chloroethyl)-3-spiro-2",4"-dioxo-5-oxazolidino-4'-deacetoxy-vinblastine) a new semisynthetic bis-indol derivative (Hung. Patent Desc. 978/95) was synthesized the characteristics of that are summarized as follows: i) it interacts or overlaps with the vinca site domain of tubulin; ii) it is active in anti-microtubular test and exhibits higher affinity to tubulin than vinblastine; iii) its target in cells is the cytoskeleton; iv) its anti-microtubular activity is highly dependent on the organization state of microtubules; v) it binds to calmodulin as vinblastine, however, the interaction is virtually Ca^{2+}-independent; vi) in contrast to vinblastine, it does not exhibit any anti-calmodulin activity; vii) it suspends anti-calmodulin activity of drugs by acting as "calmodulin liberator"; viii) its cytostatic effect is proved by *in vivo* experiments in mouse leukemia P388 test; ix) it is less toxic than vinblastine. Therefore, the new compound, KAR-2 is unique among the bis-indols with its significant antimicrotubular potency and lack of anti-calmodulin activity and toxicity.

References

1. Liliom, K., A. Lehotzky, A. Molnar and J. Ovádi. (1995). *Anal. Biochem.* 228, 18-26.
2. Liliom, K., F. Orosz, L. Horváth and J. Ovádi. (1991). *J. Immunol. Methods.* 143, 119-125.
3. Molnár, A., K. Liliom, F. Orosz, B. G. Vértessy and J. Ovádi. (1995). *Eur. J. Pharmacol.* 291, 73-82
4. Orosz, F., T. Y. Christova and J. Ovádi. (1988). *Mol. Pharmacol.* 33, 678-682.
5. Orosz, F., K. Liliom, N. Barkhudaryan, L. Horváth and J. Ovádi. (1992). *Biochemical J.* 284, 803-808.
6. Orosz, F., M. Telegdi, K. Liliom, M. Solti, D. Korbonits and J. Ovádi. (1990). *Molec. Pharmacol.* 38, 910-916.

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NEW MACROMOLECULE CONJUGATES CONTAINING GnRH PEPTIDE HORMONE ANALOGS WITH SELECTIVE ANTITUMOR ACTIVITY. 2. EFFECT OF NEW GNRH ANALOGS AND THEIR CONJUGATES ON HUMAN BREAST, PROSTATE AND ENDOMETRIUM CELL LINES IN VITRO

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Cell lines *in vitro* are generally used for prescreening of new potential anticancer compounds. However, the extrapolation of *in vitro* effect to the *in vivo* conditions is questionable, due to the high false positive and false negative ratios. In our present studies, we tested new GnRH analog agonists, antagonists and their conjugates on human breast (MCF-7, MDA-MB-231), prostate (PC3, LNCaP) and endometrium (Ishikawa) cancer cell lines, which had been shown to have GnRH receptor binding sites. This fact ensured us that our positive results might be extrapolated to positive *in vivo* results. The GnRH hormone peptide analogs may exert their antitumor effect not only by indirect way as chemical castration but they have a direct and selective antitumor effect. This can be detected by using *in vitro* tests. We tested the effect of MI-1544 human, MI-1892 chicken GnRH antagonists, the sea lamprey GnRH-III containing only L-amino acids, and of their conjugates on our *in vitro* tests using cell proliferation (SRB) and clonogenic assay. The antagonists inhibited cell proliferation by 1238% and they inhibited colony formation by 10-45% at 50 mM doses, depending on the particular cell lines. Conjugation of these analogs with the copolymer through a tetrapeptide spacer dramatically enhanced the antitumor activity of all analogs. Their antiproliferation effect was 45-90% and the inhibition of colony formation reached 95100%. The conjugates inhibited the cdc25 phosphatase, which has a role in the G2/M transition of the cell division cycle (EORTC-NDDO study). These new compounds may serve for the treatment of GnRH receptor-containing tumors and they can help to delay the traditional therapy with cytostatics of severe side effects.

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ABSENCE OF MHC CLASS II MOLECULES IS DUE TO REPRESSION OF TRANSCRIPTION BY NEGATIVE REGULATORY ELEMENTS

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Expression of the major histocompatibility complex (MHC) class II antigens is tightly regulated in a diverse number of antigen-presenting cells and are critical for initiating immune responses by CD+ T lymphocytes. Aberrant MHC-II expression have been detected in different pathological events including diseases of central nervous system and fetal abortion. We have investigated the mechanism of suppression of MHC-II gene expression in cells where normally these molecules are not expressed, including rat microglia and trophoblast cells. MHC-II IAa promoter fragments were subcloned into chloramphenicol acetyl transferase (CAT) reporter plasmids and transiently transfected into IAoc negative cells. Using deletional analysis, we have identified at least two negative response elements between 1200bp and -400bp, which regulates class II expression in these cells. Gel-shift assays demonstrate the presence of nuclear factors which bind the DNA in the region suspected of having a negative regulatory effect on the expression of class II genes.

CARNITINE BIOSYNTHESIS: THE EFFECT OF VALPROATE AND INTERMEDIATES OF PHENYLALANINE CATABOLISM

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It was postulated that anticonvulsive drug, Valproic acid (VPA) inhibits the biosynthesis of carnitine, contributing in this way to carnitine deficiency associated with VPA therapy. The conversion of [^{3}H]butyrobetaine into [^{3}H]carnitine was determined 60 min following a single intraperitoneal (i.p.) dose of 1.2 mmol per kg VPA in rats. The fraction of radioactivity found in [^{3}H]carnitine in the liver decreased from 63.2±1.50% to 39.2±1.11% (mean±SEM). Total carnitine in the liver also decreased from 181±11.8 nmol/g to 152±8.11 nmol/g, while the butyrobetaine level increased from 5.01±0.71 nmol/g to 8.22±0.82 nmol/g (mean±SEM). VPA exhibited a dramatic effect on the conversion of unlabelled loading amount of butyrobetaine, as well. These data together prove that VPA reduces the flux through the butyrobetaine hydroxylase (EC 1.14.11.1.). The drug, however, *in vitro,* directly did not inhibit the enzyme.

Searching for the mechanism of action we found that VPA decreased the level of a-ketoglutarate (a-KG, a co-factor of butyrobetaine hydroxylase) from 73.5±2.90 nmol/g to 52.9±2.2 nmol/g (mean±SEM) in the liver. The level of l-glutamate showed a rather dramatic decrease in the liver.

We tested three intermediates of phenylalanine catabolism. The phenylacetic acid was the most effective which in a single i.p. dose of 1.2 mmol/kg decreased the butyrobetaine-to-carnitine conversion to 33.7%. vs. the 53.9% control value. The two others (phenylpyruvic acid, 50.5% vs. 57.5% control; homogentisic acid, 51.6% vs. 62.9%) were also effective, but to a lesser degree.

THE ROLE OF THE MULTIDRUG RESISTANCE PROTEIN IN THE BLOOD BRAIN BARRIER

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The blood-brain barrier (BBB) renders the brain tissue inaccessible to many toxic compounds, but also to potentially useful drugs. Most hydrophobic compounds can readily pass the BBB, but several such pharmacologically active molecules are practically unable to cross this barrier. Compounds included in this group, e.g. antitumor drugs, cyclosporins, steroid hormones, some dopamine antagonists, ß1 receptor antagonists like salmeterol and atenolol, opiate receptor agonists, H1 receptor antagonists like astemizole or terfenadine, cephalosporin antibiotics, antifungal agents like itroconasole, or antihelmintic agents like ivermectin, have been shown to interact with the multidrug resistance protein (MDR1 or P-glycoprotein), which causes resistance in cancer against chemotherapy. Recent data indicate that the apparent non-penetrability of the above agents through the BBB is due to their ATP-dependent extrusion by MDRl, present at the apical membrane of the brain capillary endothelial cells.

Based on these findings, the selective inhibition of the MDRl transporter may render the human BBB permeable to selected compounds, thus may help the development of the therapeutic effect of such compounds in the brain. Candidate pharmacological agents in this respect are cytotoxic compounds for treating brain tumors, antibiotics and antifungal agents against brain infections, as well as antipsychotic, antiparkinsonism, and antidepressant compounds. In several cases the protection of the brain against the entry of a pharmacologically active agent is an advantage (this is the case e.g. with H1 receptor antagonists for treating asthma, or with antihelmintic agents). Thus the rapid and simple screening of the interaction of newly developed hydrophobic drugs with the human MDRl protein may help the estimation of their entry through the BBB and, consequently, their potential therapeutic usefulness. Recently we have developed two assay systems applicable for the rapid in vitro screening of drug interactions with the human MDRl protein, which could be applied for this purpose. We have also developed a group of molecules with original structures (REVERSINs) which are proposed to selectively prevent MDRl-dependent multidrug resistance. The REVERSINs are non-toxic, hydrophobic small peptide derivatives, which strongly compete with the transported drugs on the multidrug transporter, thus may help their permeation through the BBB.

LIPOIC ACID DERIVATES PROTECT HEARTS FROM POSTISCHEMIC INJURIES

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Lipoic acid is a constituent of the α-ketoacid dehydrogenase complexes, and also occurs as free acid in mammals at very low concentration. Lipoic acid and its derivates can be reduced *in vivo* by lipoamide dehydrogenase forming dihydrolipoic acid derivates which can interfere in mitochondrial metabolism. In addition, dihydrolipoic acid derivates can serve as antioxidants protecting the heart from reactive oxygen related free radicals.

Effect of lipoic acid derivates on the carbohydrate and fatty acid oxidation were studied *in situ* perfused rat heart. It was observed that lipoarnide (0.25 mM) increased the carbohydrate oxidation with 103% in postischemic myocardium, and parallel promoted the dephosphorylation and 47% activation of pyruvate dehydrogenase complex. Furthermore, lipoalnide caused a 64% activation of the citric acid cycle in postischemic rat heart improving the mitochondrial energy production of postischemic myocardium.

Ischemia followed by reoxigenation increases the oxygen related free radical formation in heart, and the free radicals cause damages in both lipid and aqueous phases. Ubiquinone is a constituent of the respiratory chain in lipid membranes and can form a semiquinone free radical which can be detected in deep-freeze hearts by Electron Paramagnetic Resonance (EPR) spectroscopy. Because the semiquinone free radicals are relatively stable they can be used to monitor the free radical formation in lipid phase. EPR data showed that the ischemia reoxigenation caused 328% increase in the semiquinone concentration, and lipoamide (0.2 mM) decreased the semiquinone concentration with 93% in postischemic rat heart. These data show that lipoic acid derivates protect the heart from postischemic oxidative injuries, which explain why these compounds improve the recovery of postischemic myocardium.

TRANSGENIC APPROACHES TO GENERATE ANIMAL MODELS FOR NEUROLOGICAL DISEASES

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Animal models are an essential means by which the pathogenesis of different CNS disorders can be studied. They are also important for developing and testing potential new therapeutic strategies to prevent or counteract pathological consequences of neurological diseases.

Recently introduced techniques based on reverse genetic approaches such as transgenesis and gene knockout are proven to be especially useful in generating genetically modified mouse models that recapitulate pathologic and/or phenotypic characteristics of CNS disorders.

When the molecular pathology is based on altered level of gene expression, transgenesis is utilized to generate mouse lines that show overexpression or reduced expression of the affected gene products. Transgenic mice that overexpress a mutant form of a gene can also be generated if a neurological disease is based on toxic gain of function.

In the case of human CNS disorders based on loss of function, mutations, the most appropriate models are produced by in vivo targeted inactivation of genes through homologous recombination. This gene knockout strategy can also be utilized to replace the normal alleles with a mutated gene.

Several examples of transgenic models will be reviewed that help to clarify the underlying mechanisms of Alzheimer's disease.

Transgenic mice with reduced glucocorticoid-receptor levels will also be discussed as potential models for human depression based on the increased activity of the hypothalamic-piturity-adrenocortical system.

Finally, targeted disruption of the genes for two serotonin receptors will be reviewed that have confirmed pharmacological experiments and revealed unexpected behavioral roles of serotonin.

IN VITRO METABOLISM STUDIES

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Xenobiotic metabolising enzymes, which have classically differentiated from the enzymes of endobiotic metabolism, can be found in most of the tissues, but mainly in the organs which are the first or second protective lines in the body. The most important metabolising organ is the liver, but the gastrointestinal tract, olfactory epithelium, lungs, skin, placenta, kidneys, brain and urinary bladder also have a considerable metabolic activity.
Generally, the aim of metabolism studies is to help the selection of toxicological species, and to find correlation between the presence of the parent compound or its metabolite(s) and the pharmacological or toxicological activities. For studying metabolism many techniques can be used, including the in vitro methods such as organ perfusion, tissueslices, isolated cells, tissue homogenates, cellular subfractions (S9), microsomal preparation, isolated-purified iso-enzymes or any of the aboves combined with gene technology.
CHINOIN makes efforts to develop new, new type, effective and selective drugs with known mechanism of action. The first screen of the candidates is done in vitro on a selected molecular target (enzyme, receptor, ion-channel) which is followed by further in vitro, ex vivo and in vivo tests. A potential drug having in vitro activity on the selected molecular target can be active in vivo if it can be absorbed, penetrates through the membranes to reach the target, and is stable enough to act on the target. A potential drug being active in animal experiments can be active in the man if there is no significant difference in the qualitative and quantitative metabolism between the species. For these reasons, in CHINOIN, in vitro metabolism studies using microsomal preparations obtained from the pharmacological species and men has been involved in the drug discovery processes. The first results are promising, however including other in vitro techniques such as the absorption model using Ca-Co-2 cells seems to be important, too.

PEPTIDES AND EXCITOTOXINS IN THE PATHOGENESIS OF NEUROLOGICAL DISORDERS

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Neurodegenerative disorders are characterized by delayed onset of neuronal cell death in circumscribed group of neurons (1,2,3,4). The possibility that a defect in energy metabolism could result in excitotoxicity was originally demonstrated by the work of Novelli et al. (5). The mechanism for this effect was hypothesized to be a consequence of a reduction in ATP, which is crucial for maintaining the normal resting potential of the cell membrane. Amimooxyacetic acid (AOAA) (an inhibitor of several pyridoxal phosphat-dependent enzymes in the brain) could produce NMDA receptor-mediated excitotoxin lesions. In our experiments intrastriatal injection of AOAA result in both acute and chronic behavioral changes which are attenuated by pentobarbital anesthesia. Freely moving animals microinjected with AOAA had impaired leaming activity in an active avoidance conditioning test, and showed reduced striatal concentrations of substance P and GABA. Furthermore, excitotoxicity plays a role in the pathogenesis of neurodegenerative disorders. Concerning Huntington's disease (HD). Initial studies showed that kainic acid lesions could mimic many of the characteristic histologic and neurochemical features of HD (1,2). These lesions, however were an imperfect model because did not spare NADPH-diaphorase (somatostatin containing) neurons, which are a class of aspiny striatal interneurons that are strikingly spared in HD. Later studies suggested that quinolinic acid as well as other NMDA-receptor agonists resulted in an improved model of HD, in which there was relative sparing of the NADPH-diaphorase neurons (1). Pharmacological manipulation can markedly increase brain tissue or cerebrospinal fluid concentrations of kynurenic acid (endogenous excitatory amino acid antagonist in the central nervous system). Strategies to inhibit the effects of excitotoxins and improve energy metabolism could prove efficacious in attempting to slow or halt the neurodegenerative processes (1,2,4).

References

(1) Beal, M.F.: Neurochemistry and toxin models in Huntington's disease. Curr. Opinion Neurol. 7:542-547, 1994.

(2) Vécsei, L., Freese, A., Swartz, K.J., Beal, M.F.: Neurological Disorders: Novel Experimental and Therapeutic Strategies. Horwood, Chichester, pp. 1-273, 1992.

(3) Vécsei, L., Klivenyi, P.: Somatostatin and Alzheimer's disease. Arch. Geront. Geriatr. 21:35-41, 1995.

(4) Vécsei, L., Beal, M.F.: Huntington's disease, behavioral disturbances and kynurenines: preclinical findings and therapeutic perspectives. Biol. Psych. in press 1996.

(5). Novelli, A., Reilly, J.A., Lysko, P.G., Henneberry, R.C.: Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 451:205-212, 1988.

THE IMPORTANCE OF THE INVESTIGATION OF CYTOCHROME P-450 AND CONJUGATING ENZYMES IN DRUG DEVELOPMENT

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In order to evaluate the results of toxicity testing and to establish their relevance to humans the metabolic investigations of the drugs is required. In vivo studies are rather time consuming and in humans they can only be made after the first human investigations. In vitro metabolic studies, however, can be carried out rapidly at the early phase of drug development and even human tissues can be used. Cytochrome P-450 and the conjugating enzymes of the liver are primarily responsible for the metabolism and for the elimination of drugs. However these enzymes are heterogeneous, their activities may differ from species to species and they sometimes display different phenotypes in the human population. Moreover the result of these enzyme activities are not always detoxication, there are growing evidence that these enzymes may also be responsible for the production of toxic compounds, and thus responsible for the toxicity of drugs.

The (in vitro) investigation of these enzymes allows:

- to determine the metabolic profile of a drug in different animal species (generally used in toxicology) and in humans
- to identify the structure of metabolites by spectroscopic methods
- to identify the enzymes responsible for the formation of metabolites
- to study drug interactions
- to study the induction of drug metabolizing enzymes
- to detect unstable metabolites.

NEW MACROMOLECULE CONJUGATES CONTAINING GnRH PEPTIDE HORMONE ANALOGS WITH SELECTIVE ANTITUMOR ACTIVITY. 3. MECHANISM OF ACTION *IN VITRO* AND *IN VIVO* OF NEW GnRH ANALOGS AND THEIR CONJUGATES

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Radioreceptor assay with (^{3}H-Pro)^{9}-GnRH-III lamprey GnRH analog, iodine-labeled ^{125}I-Mr-1544 human GnRH antagonist and their conjugates (P-X-^{3}H-GnRH-III and P-X-^{125}I-MI-1544) on membrane suspension of breast (MDA-MB-231, MCF-7), prostate (PC3, LNCaP) and endometrium (Ishikawa) cancer cells showed presence of high (Kd=1-5 nM) and low (Kd=0.3-0.8 μM) affinity binding sites. Comparing the Kd values of high affinity binding sites for labeled compounds, the conjugates seem to have the highest affinity to the receptor. Both lamprey GnRH-III peptide hormone as well as GnRH analog-macromolecule conjugates are taken up by the cancer cells *via* a process of receptor-mediated internalization. This mechanism seems to be target cell-specific, since binding was displacable by unlabeled GnRH-III or GnRH analog-macromolecule conjugates. The labeled macromolecule (^{125}I-P) and macromolecule-spacer (^{125}I-P-X-OH) did neither bind to any of the five cancer cell surface, nor internalize in the cell by receptor - mediated process. Studying the organ distribution and blood clearence of the compounds given subcutaneously in MDAMB-231 human breast cancer xenograft-bearing mice, the conjugation of ^{125}I-M:I-1544 resulted in an alteration in the biodistribution of the free MI-1544 molecule. Again, biodistribution of ^{14}C-P-X copolymerspacer was altered when it was conjugated with MI-1544. In both cases, i.e. P-X-^{125}I-MI-1544 and ^{14}C-P-X-MI-1544 treatment, a longer blood survival, an enhanced whole body retention and a higher uptake by the tumor was observed. It is concluded that the active conjugate molecule acted supposedly not only as a prodrug. The elucidation of the mechanism might result in planning a new strategy of hormonal treatment for an improved clinical response.

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APPLICATION OF ANTISENSE OLIGODEOXYNUCLEOTIDES IN MOLECULAR PHARMACOLOGY, THERAPY AND RECEPTOR RESEARCH

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The use of oligonucleotides in therapy and research became an ever growing field the last decade. Antisense oligonucleotides inhibit the transcription of DNA or the translation of RNA to protein, forming complementary base pairs to nucleic acids. Triplex DNA may result of the blocking of the binding of different factors required for initiation of mRNA translation. Oligonucleotides activate also ribonuclease H which cleaves the mRNA. The optimal size of the oligonucleotides should be 15-20 oligomer. Increasing the length leads to reduction of uptake, decreasing it looses selectivity. Controls of mismatch bases should be included to test specificity. Affinity of oligonucleotides is very high comparing to conventional drugs acting on receptors. One base mismatch leads to 500-fold loss in affinity.

Among modified oligonucleotides phosphorothioates are the most stable. Therapeutical application of the antisense oligonucleotides is in the clinical trial stage. Most experiences are published with AIDS, leukemia, herpes simplex virus and cytomegalo virus infections and they are also used to block immune cell activity in transplants, autoimmune disease and rheumatoid arthritis, but continuous therapy is necessary.

Antisense oligodeoxynucleotides are modern tools in research of membrane integrated receptors, as the cloned sequences of the receptor proteins became known. 15-20-mer oligonucleotides from different regions of the cloned sequences of mu, delta and kappa-type opioid receptors caused partial blockade of agonist-provoked analgesia, radioligand binding, at the mRNA level. The blockade appeared gradually in 3-5 days, was of high pharmacological selectivity and reversible. The results strongly supported the functional heterogeneity of these receptors. The loss of function caused by the antisense blockade helps to explore the function of those receptors which have no selective ligands.

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