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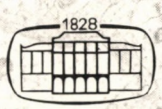

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AREAS OF DORMANT GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IN THE RAT BRAIN AS REVEALED BY AUTOMATED IMAGE ANALYSIS OF SERIAL CORONAL SECTIONS

Hajós, F. and Zilles, K.

Department of Anatomy and Histology, University of Veterinary Science, Budapest, Hungary,
C. u. O. Vogt Institut für Hirnforschung, Heinrich-Heine-Universität, Düsseldorf, Germany

Summary: Coronal vibratome sections of the rat brain were immunostained for glial fibrillary acidic protein (GFAP) a major cytoskeletal protein typical for astrocytes. Using computer-assisted image analysis of whole sections, a substantial heterogeneity in regional staining intensities was pointed out.
The middle layers of the neocortex, the thalamus and caudate-putamen and the tectal and tegmental part of the mesencephalon were found to show no detectable staining, while peak staining intensities were measured for the pallidum, septal triangular nucleus, hippocampus, medial geniculate nucleus and interpeduncular nucleus. In some of the negative areas neural lesioning induced the appearance of GFAP immunoreaction parallel to an up-regulation of GFAP synthesis. On this basis it was assumed that GFAP immunoreactivity is dormant in these astrocytes and plastic changes in the neuropil trigger its expression.

Key words: Brain - GFAP - distribution - image analysis - rat

Correspondence should be addressed to: Dr. Ferenc Hajós
Department of Anatomy and Histology, University of Veterinary Science; H-1400 Budapest, P.O.Box 2; Hungary

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INTRODUCTION

Studies on the immunohistochemical demonstration of glial fibrillary acidic protein (GFAP; Eng et al., 1971; Eng, 1985) in the mammalian central nervous system have revealed a substantial heterogeneity for this major cytoskeletal protein among GFAP-immonstained astrocytes concerning their regional distribution (Bignami et al., 1972; Hajós and Kálmán, 1989; Kálmán and Hajós, 1989; Kamo et al.,1984, Tugo et al.,1982). Although in some cases variations could be ascribed to factors extrinsic to the brain, such as different hormonal states (Salm et al., 1983; Tranque et al., 1987, García-Segura et al., 1994), a remarkable consistency appeared to exist in regional GFAP-patterns. Accordingly, certain areas contained densely packed immunostained astrocytes, while in others astrocytes were scarcely distributed or apparently lacking. These unstained territories, however, were shown with routine histology to contain astrocytes with similar structure, shape and distribution than in many other heavily stained regions (Wree et al.,1980) implying the assumptions that (i) they may - at least immunochemically - differ from the rest of the population, and (ii) that GFAP may not be as a reliable astrocyte marker as generally claimed.

To investigate this problem, comparative GFAP maps of coronal serial sections of the brain would be helpful, but conventional drawing techniques are, for this purpose, of limited value since they do not reflect differences in regional staining intensities. Attempts to produce maps expressing in addition to simple distribution-plots also a comparison of regional staining intensities, brought about inconsistent results. Under low magnification showing large areas, GFAP immunostaining can neither be recognised nor photographed, so one has to rely on the visual impression of staining intensity gained in higher-power microscopic fields. Extrapolated summarising drawings hence mirror the subjective assessment of the investigator.

Recent development in computer-assisted image analysis of histological preparations offers a method to map in objective terms the distribution of immunohistochemical reactions together with their comparative regional intensities recorded as grey-level values. An automated scanning of the preparation under medium power yields series of digitised images corresponding to packing densities of GFAP immunoreactive elements per measuring field (areal fraction). From the resulting data matrix, the preparation can be reconstructed in any size required for illustration, while numerical values expressing areal fractions can be converted into various graphic code systems. Moreover, this method is independent from section thickness, background staining and variations of staining intensity between series of reactions.

In the present study we utilised the potentials of computer-assisted image analysis to produce objective GFAP distribution-maps of whole coronal sections of the rat brain, which express also the differences in regional immunostaining intensities. Our aim was to delineate the regions with different GFAP reactions, and to investigate the reasons and possible significance of this heterogeneity.

MATERIALS AND METHODS

Adult male albino rats of 200g body weight were perfused under Avertin anaesthesia with a mixture of paraformaldehyde and picric acid (4% each) in phosphate buffer (PB;) 0.1M, pH 7,4) through the aorta. Brains were excised from the skull and kept in the above solution for overnight, then 40 μm thick serial sections were cut in the coronal plane on a vibratome and processed for GFAP immunohistochemistry.

Immunostaining for GFAP was carried out on freely floating vibratome sections. To exhaust endogenous peroxidase activity, sections were treated for 5 min with 3% H_2O_2. After a short rinse in three changes of PB, non-specific immunoreactivity was suppressed by 20% normal goat serum in which sections were kept for 2 h at room temperature. Incubation with the primary antiserum lasted for 36 to 48 h at 4°C , under vigorous shaking. A monoclonal antiserum to GFAP (Boehringer) was used in a dilution of 1: 200. As second antibody, biotinylated rabbit IgG (1 : 80) and peroxidase labelled streptavidin (Amersham; 1: 40) were used. The immune-complex molecule was visualized by the 3'3'-diaminobenzidine-tetrahydrochloride (DAB)-reaction. Sections were mounted on slide glasses in a rostro-caudal sequence and coverslipped under DPX. In some cases, a fluorescent conjugate-labelled second antibody was used for fluorescence microscopy.

For immunohistochemical control, sections were incubated with preabsorbed antiserum or by omitting the primary antiserum. The specificity of the antiserum was proven by the consistently negative reaction under these conditions.

Image analysis was performed after studying the preparations in a light and microscope. Slides were transferred to an IBAS image analyser (Kontron). Full coronal sections were scanned with a computer-controlled scanning procedure (Schleicher et al., 1986). The microscopic images (12,5-fold objective lens) were digitised into grey level images (8 bit grey level resolution) using a TV camera. In these images, GFAP-positive structures were segmented by adaptive thresholding (Rosenfeld and Kak, 1976) and the areal fraction was measured in square measuring fields 40 x 40μm in size. According to the x,y count of the measuring field, data were stored in a data matrix which represented the spatial distribution of the GFAP-positive structures in the specimen. The data matrix was plotted by subdividing the range of the areal fraction values (0% to 100%) into 11 subranges, each of which could be plotted in a distinct density pattern or in pseudocolours indicating increasing areal fraction values along a blue-green-yellow-red colour-gradient.

RESULTS

The overall appearance of the GFAP immunostaining was studied in fluorescence preparations (Fig. 1). Most astrocytes were seen as stellate cells with numerous processes. Occasionally long processes could be observed running to blood vessels which were outlined by their surrounding perivascular glia. Deviations from this general form were encountered in some specific regions, corroborating our earlier findings (Hajós and Kálmán, 1989; Kálmán an Hajós, 1989). Accordingly, regions were defined where astrocytes possessed instead of the usually long, slender processes, short and thick processes.

Computer plots of whole vibratome sections immunostained with GFAP demonstrated marked variations in the regional intensity of the immunoreaction. Typical differences will be described in coronal sections cut at the following levels: (i) anterior commissural, (ii) mid-hypothalamic, (iii) splenial and (iv) mid-mesencephalic.

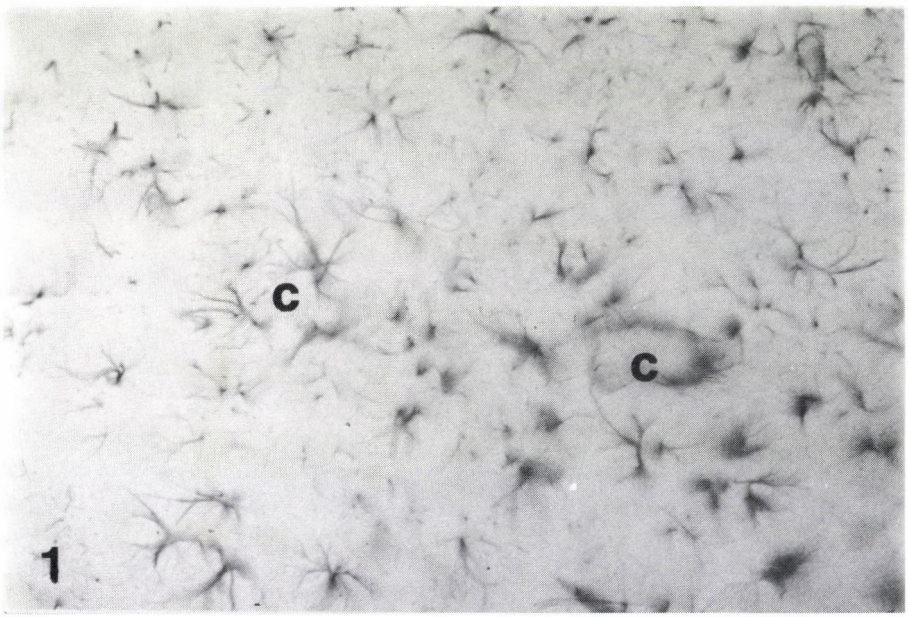

Fig.1: GFAP-immunoreaction of astrocytes in the piriform cortex as revealed by immunofluorescence. Note the stellate cells with delicate processes. Some long processes approach capillaries (C). x320

(i) At the level of the anterior commissure (Fig.2) the strongest GFAP staining was observed in the core of the globus pallidus and in the septal triangular nucleus. Periventricular and surface areas and the white matter of the cingulum were also intensely stained. The cortex showed a marked difference between its parts dorsal and ventral to the rhinal sulcus. Dorsally to the rhinal sulcus, in the neocortex, practically no staining occurred in layers II-V, while ventral to the rhinal sulcus, in the piriform cortex, an evenly distributed, medium-grade immunoreaction was detected. Further conspicuously unstained areas were observed in the medial septum, anterior hypothalamus, preoptic area and caudate-putamen. Particularly the latter was in sharp contrast to the high intensity of staining in the neighbouring pallidum.

(ii) At the mid-hypothalamic level (Fig.3) the distribution of staining between neocortex and piriform cortex was found as in the previous section. Here the layers of the dorsal hippocampus appeared as intensely stained elements with a prominent pyramidal layer. As in the previous section, periventricular and surface areas were intensely immunoreactive. The thalamus was almost entirely negative, while in the ventral hypothalamus staining was most intense.

(iii) In a next section through the splenial region of the corpus callosum (Fig.4) the above described difference between the staining of neocortex and piriform cortex was unaltered. The entire hippocampus fell in the plane of sectioning and its dorsal part showed a stronger reaction than the ventral. An interesting layering of GFAP was seen in the corpus callosum. In this coronal plane the caudal part of the diencephalon, and ventrally the rostral structures of the mesencephalon were noticed. The diencephalon was almost completely unstained, mesencephalic structures, particularly the interpeduncular nucleus displayed an intense staining.

(iv) In the section through the mid-portion of the mesencephalon (Fig.5) again a partially unstained occipital neocortex and a medium-stained piriform cortex occurred. At this level, the hippocampus was found intensely labelled. In the mesencephalic cross section, in sharp contrast to the negative tectal and tegmental areas, the interpeduncular nucleus, the substantia nigra and the medial geniculate nucleus were conspicuously stained. The interpeduncular nucleus proved to be one of the largest areas with a peak immunoreaction. It is also worth of notion that in the medial geniculate nucleus staining outlined layers with a latero-medially decreasing staining intensity.

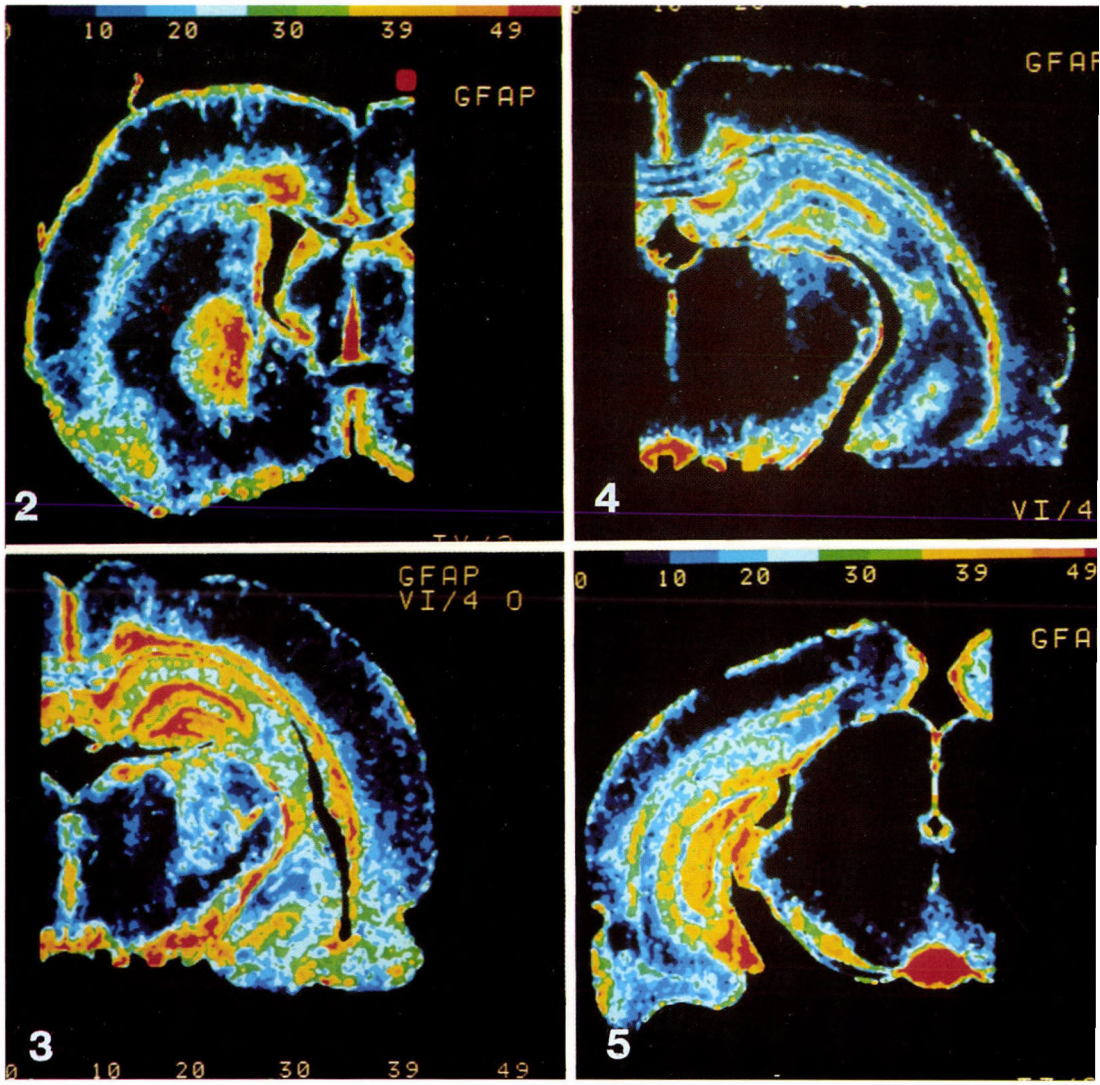

Fig. 2: Distribution of GFAP areal fractions in a coronal section at the level of the anterior commissure. Regions of highest areal fraction (red) are the septal triangular nucleus, the core of pallidum and the cingulate white matter.

Fig. 3: Distribution of GFAP areal fractions in a coronal section at the mid-hypothalamic level. Structures in the hippocampus, cingulate white matter, and at the basal hypothalamus and mesencephalon have the highest areal fractions indicated by red.

Fig. 4: Distribution of GFAP areal fractions in a coronal section at the splenial level. In the corpus callosum GFAP immunoreactivity is arranged in regular layers. The cingulum and the basal mesencephalon contain the highest areal fraction values (red).

Fig. 5: Distribution of GFAP areal fractions in a coronal section at the mid-mesencephalic level. The ventral hippocampus, and in particular the interpeduncular nucleus are conspicuous by their high GFAP areal fractions (red).

DISCUSSION

Although the uneven distribution GFAP immunostained elements throughout the rat brain is evident from recent (Bignami et al., 1972; Kamo et al., 1984; Tugo et al., 1982) as well as from our present findings, still the wide range of variations revealed by computer-assisted image analysis is surprising. According to routine histological studies, astroglia are present in all central nervous system regions (Wree et al, 1980), including the subtypes such as protoplasmic and fibrous astrocytes distinguished by classical studies. Initially, GFAP immunoreactivity was ascribed to glial intermediate filaments a predominant cytoplasmic feature of fibrous astrocytes (see for references Privat and Rataboul, 1986), but recent immunohistochemical studies have revealed this protein in a number of typical protoplasmic astrocytes (Connor and Berkowitz, 1985) , while biochemically GFAP was shown also in the filament-independent, soluble cytoplasmic compartment (Patel et al., 1985). Hence there is no histological reason which may explain the lack of immunoreactivity in some astrocytes.

The present observation of whole areas without detectable GFAP immunostaining calls for interpretation. Similar areas have recently been described (Tugo et al., 1982; Kamo et al., 1984) but not in the context of an objectively mapped distribution and were not commented upon. In a previous work (Zilles et al., 1991) we made an attempt to interpret regions of high immunoreactivity as surface-derived structures. As to our present findings, we assume that GFAP-negative territories contain astrocytes with dormant immunoreactivity. This assumption is supported by our recent work on the lesioning of the geniculo-cortical pathway by which we were able to elicit a marked GFAP reaction in the visual cortical layers which were negative in the control (Hajós et al., 1990a). A similar increase in GFAP immunoreactivity could be evoked in the spinal cord by dorsal root transection (Hajós et al., 1990b). Biochemical studies in progress in our laboratory argue for a net increase in the GFAP protein in these territories. It seems therefore, that GFAP synthesis can be triggered in astrocytes by influencing their neuronal environment. Indeed, by using in situ hybridization Lewis and Cowan (1985) observed a high concentration of GFAP mRNA in superficial layers of the cerebrum, in contrast to other astrocytes of the grey matter. This result suggests that even under normal conditions a differential rate of GFAP turnover or translational control may exist between brain regions.

The regional distribution of astrocytes with this dormant capacity of GFAP expression is difficult to explain because regions of most diverse nature share intense or lacking GFAP staining. While the lack of GFAP staining of the middle layers of the neocortex, thalamus

and caudate-putamen would argue for an involvement of areas receiving massive afferent input, the intense reaction of the hippocampus does not fit into this hypothesis. Nevertheless, the fact that GFAP immunoreaction triggered by neuronal injury proved to be transient (Kálmán et al., 1993), moreover that it appeared to indicate a hypertrophy rather than proliferation of the astroglial population (Hajós et al., 1993), suggests that the phenomenon is indicative of a high potential for structural plasticity within the region. In this context, the temporary up-regulation of GFAP synthesis coupled to the loss of afferent input in a particular region, can be regarded as part of a space-sustaining glial hypertrophy necessary in the traumatised neuropil until it settles in a rearranged form.

ACKNOWLEDGEMENTS: This work was supported by grants OTKA 1245 of the Hungarian Scientific Research Fund and by Zi 192/5-3 of the Deutsche Forschungsgemeinschaft.

REFERENCES

Bignami, A, Eng, L.F., Dahl, D and Uyeda, C.T. (1972) Localization of glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res. 43, 429-435.

Connor, J.R. and Berkowitz, E.M. (1985) A demonstration of glial filament distribution in astrocytes isolated from rat cerebral cortex. Neuroscience 16, 33-44.

Eng, L.F. (1985) Glial fibrillary acidic protein (GFAP): The major protein of glial intermediate filaments in differentiated astrocytes. J. Neuroimmunol. 8, 203-214.

Eng, L.F., Vanderhaegen, J.J., Bignami, A and Gerstl, B (1971) An acidic protein isolated from astrocytes. Brain Res. 28, 351-354.

Garcia-Segura, L.M., Chowen, J.A., Párducz, Á. and Naftolin, F. (1994) Gonadal hormones as promoters of structural synaptic plasticity: cellular mechanisms. Progr. Neurobiol. 44, 279-307.

Hajós, F and Kálmán, M (1989) Distribution of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the rat brain. II. Mesencephalon, rhombencephalon and spinal cord. Exp. Brain Res. 78, 164-173.

Hajós, F., Kálmán, M., Zilles, K., Schleicher, A. and Sótonyi, P (1990a) Remote astrocytic response as demonstrated by glial fibrillary acidic protein immunohistochemistry in the visual cortex of dorsal lateral geniculate nucleus lesioned rats. Glia 3, 301-310.

Hajós, F., Csillik, B. and Knyihár-Csillik, E. (1990b) Alterations in glial fibrillary acidic protein immunoreactivity in the upper dorsal horn of the rat spinal cord in the course of transganglionic degenerative atrophy and regenerative proliferation. Neurosci. Lett. 117, 8-13.

Hajós, F., Gerics, B., and Turai, É. (1993) Astroglial reaction following Wallerian degeneration in the rat visual cortex: proliferation or hypertrophy? Neurobiology 1, 123-131.

Kálmán, M and Hajós, F (1989) Distribution of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the rat brain. I. Forebrain. Exp. Brain Res. 78, 147-163.

Kálmán, M., Csillag, A., Schleicher, A., Rind, C., Hajós, F. and Zilles, K. (1993) Long-term effects of anterograde degeneration on astroglial reaction in the rat geniculo-cortical system as revealed by computerized image analysis. Anat. Embryol. 187, 1-7.

Kamo, H., Haebara, H., Akiguchi, J., Kameyama, M., Kimura, H. and McGeer, P.L. (1984) A distinctive distribution of reactive astroglia in the precentral cortex in amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) 74, 33-38.

Lewis, S.A. and Cowan, N.J. (1985) Temporal expression of mouse glial fibrillary acidic protein mRNA studied by a rapid in situ hybridization procedure. J. Neurochem. 45, 913-919.

Patel, A.J., Weir, M.D., Hunt, T., Tabourdin, C.S.M. and Thomas, D.G.T. (1985) Distribution of glutamine synthetase and glial fibrillary acidic protein and correlations with glutamate decarboxylase in different regions of the rat central nervous system. Brain Res. 331, 1-10.

Privat, A., Rataboul, P. (1986) Fibrous and protoplasmic astrocytes. In: Fedoroff, S., Vernadakis, A., Astrocytes. Vol.1.pp 105-130, Academic Press, New York.

Rosenfeld, A. and Kak, A.C. (1976) Digital Picture Processing. Academic Press, New York

Salm, A., Smithson, K.G. and Hatton, G.I. (1983) Lactation associated redistribution of the glial fibrillary acidic protein within the supraoptic nucleus: an immunocytochemical study. Cell Tiss. Res. 242, 9-15.

Schleicher, A., Zilles, K. and Wree, A. (1986) A quantitative approach to cytoarchitectonics: software and hardware aspects of a system for the evaluation and analysis of structural inhomogeneities in nervous tissue. J. Neurosci. Meth. 18, 221-235.

Tranque, P.A., Suarez, I., Olmos, G., Fernandez, B. and Garcia-Segura, L.M. (1987) Estradiol-induced redistribution of glial fibrillary acidic protein immunoreactivity in the rat brain. Brain Res. 406, 348-351.

Tugo, M., Kumashiro, M., Kimura, M. and Maeda, T. (1982) Distribution of glial fibrillary acidic protein in the rat brain studied by immunohistochemistry. J. Histochem. Cytochem. 30, p561.

Wree, A., Zilles, K. and Schleicher, A. (1980) Analyse der laminären Strukturen der Area striata mit verschiedenen stereologischen Messmethoden. Verh. Anat. Ges. 74, 727-728.

Zilles, K., Hajós, F., Kálmán, M. and Scleicher, A. (1991) Mapping of glial fibrillary acidic protein-immunoreactivity in the rat forebrain and mesencephalon by computerized image analysis. J. Comp. Neurol. 308, 340-355.

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ABSTRACTS

from the

Second Congress of the Hungarian Neuroscience Society

Szeged, 26-28 January 1995

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INTRODUCTION

The abstracts in this issue summarise the 2nd Annual Conference of the Hungarian Neuroscience Society (HNS), held in Szeged at the Biological Centre of the Hungarian Academy of Sciences. The host institution (honoured by the Assembly of Members during the 1st conference in the last year earlier in Pécs) was the Department of Comparative Physiology at the Attila József University of Sciences, Szeged.

The meeting was co-organized by the Biological Research Centre, and the Albert Szent-Györgyi Medical University of Szeged, and sponsored by grants from the Hungarian Committee of the Decade of the Brain Foundation, the Scientific and Cultural Council of the City of Szeged, the Foundation for Szeged, the Pick Company, and the Zenon Biotechnological Ltd.

The conference subject was in part selected by the Chairman of the Conference through the way of 16 invited lectures, and based on the shared scientific interest of the community explored before the meeting. The number of registered participants (over 220) reflected the constantly increasing interest of the Hungarian neuroscience community. Although our society was founded in this form two years ago, its predecessor have organized similar meetings in each year under the auspices of the Biological Section of Hungarian Academy of Sciences, and the Hungarian Physiological Society since 1973. For many years the number of participants has been increased continuously and the vivid, friendly atmosphere of these meetings was deepened. In line with these traditions the

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number of registered participants, and scientific presentations at the Szeged meeting surpassed the figures of the last meeting by about 20%. Through the two and half day altogether 62 oral presentations and 77 posters contributed to the program. In addition we held a memorial symposium which honoured János Szentágothai, the great neuroanatomist by six lectures of his pupils.

Neuroscience research continues to grow in Hungary and, in spite of the well known financial difficulties of the country, push the boundaries of our knowledge outward to new frontiers. It is the commitment of HNS to contribute for and help to integrate these efforts by the system of annual conferences. The next will be held in Tihany, in 1996.

Prof. Dr. Attila Baranyi
Chairman of the 2nd Annual
Conference

Neurobiology 3 (1), p. 17, 1995

EFFECTS OF TUMOR NECROSIS FACTOR α ON THE BLOOD-BRAIN BARRIER IN VIVO

Ábrahám, C.S.[1], Deli, M.A.[2], Joó, F.[2], Megyeri, P.[1] and Torpier, G.[3]

[1]Department of Pediatrics, Albert Szent-Györgyi Medical University;
[2]Laboratory of Molecular Neurobiology, Biological Research Center, Szeged, Hungary;
and [3]INSERM U 325 & SERLIA, Institute Pasteur, Lille, France

Tumor necrosis factor α (TNFα) plays a crucial role in the pathogenesis of the central nervous system infections. TNFα can pass the blood-brain barrier (BBB) by a bidirectional transport mechanism [1]. There is a controversy, however, about the effects of TNFα on the vasogenic brain edema formation. Intracisternal TNFα injection resulted in a dose-dependent opening of the BBB in newborn pigs [2], but the permeability of BBB for albumin did not change after intravascular administration in the mouse [1]. The aim of the study was to compare the changes in the blood-brain barrier (BBB) permeability after intracarotid and intracisternal injection of TNFα in newborn pigs. 48 piglets (4-8 h; 1,320-1,510 g; pentobarbitone anesthesia: 30 mg x kg^{-1}) of either sex were included into the study. The internal carotid artery was catheterized through the external branch, recombinant human TNFα was injected into the vessel in the following doses: 0; 10,000; and 100,000 IU, respectively (in 0.5 ml isotonic saline, 1 min), then the external carotid artery was ligated. In the other group, the animals were given intracisternal injection of TNFα (the same doses in 0.5 ml age- and species-specific cerebrospinal fluid). Before, as well as 4, 8, and 16 h after the challenge, the BBB permeability was determined concomitantly for a small (sodium fluorescein, SF, mw: 376 Da) and a large (Evans blue/albumin, EBA, mw: 67 kDa) tracer (2%, 5 $mlxkg^{-1}$, 30 min circulation time for both dyes) in parietal cortex, hippocampus, striatum, internal capsule and cerebellum of piglets. After the removal of the intravascular dyes by perfusion, cryostate sections from different brain regions were viewed for optical sectioning with a Leica confocal laser scanning microscope equipped with an argon/krypton ion laser (SF: green, EBA: red fluorescence). In the morphological study, a diffuse BBB opening for SF and a moderate perivascular extravasation for EBA were seen in TNFα-treated piglets after both challenges. A dose-dependent increase in the BBB permeability was also found by spectrophotometry both for the SF (excitation: 440 nm, emission: 525 nm) and for the EBA (absorbance at 620 nm) in the brain regions examined. Our data obtained on an *in vitro* reconstituted model of the BBB [3] suggested that a rearrangement of endothelial actin filaments may take a part in the increased BBB permeability. We conclude that significant increases in the concentration of TNFα in either side of the BBB may result in brain edema formation during neonatal sepsis and bacterial meningitis.

References: 1. Gutierrez, E.G. *et al.* (1993) *J. Neuroimmunol.* **47**:169-176.
2. Megyeri, P. *et al.* (1992) *Neurosci. Lett.* **148:** 137-140.
3. Deli, M.A. *et al.* (1995) *J. Neurosci. Res.* in press.

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INTERNEURONS SPECIALIZED TO CONTROL OTHER INTERNEURONS IN THE HIPPOCAMPUS

Acsády, L., Gulyás, A.I. and Freund, T.F.

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest
PO Box 67. H-1450

Cortical networks have long been thought to consist of two basic components: excitatory and inhibitory neurons. Activity of the profusely interconnected ensembles of excitatory cells is governed by inhibitory interneurons, which, by innervating different parts of the excitatory cells, can exert various inhibitory functions. Here we demonstrate that, at least in the archicortex, a third component has to be added to any wiring diagrams: three interneuron populations specialized to control GABAergic neurons responsible for dendritic inhibition of pyramidal cells, have been identified.

Type I interneurons contain the calcium binding protein calretinin and innervate calbindin-containing interneurons that exert inhibition in the Schaffer-collateral termination zone. Type I interneurons are also linked to each other via multiple axodendritic and dendrodendritic contacts. The VIP-immunoreactive Type II interneurons have a dendritic tree restricted to str. lacunosum-moleculare of CA1, thus allowing a selective activation by the entorhinal cortex. Their postsynaptic targets are similar to those innervated by Type I interneurons. In Type III interneurons VIP and calretinin are colocalized. These cells project to the border of str. oriens and alveus, and innervate horizontal interneurons of this sublamina. These horizontal interneurons were shown to send their axons to str. lacunosum-moleculare, and control the efficacy of the entorhinal afferents.

These results demonstrate that in the hippocampus different interneuron types evolved to selectively control specific subsets of GABAergic neurons. These interneuronal networks may participate: 1) in the synchronization of dendritic inhibition; 2) in the input-selective disinhibition of principal cells; and 3) in the synchronization of GABA-A receptor-mediated oscillations.

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Neurobiology 3 (1), p. 19, 1995

MODELING MITRAL AND GRANULE CELLS OF THE OLFACTORY BULB WITH THE 'NEURON' SOFTWARE TOOL

Adorján, P.[1,2], Aradi, I.[1,3], Barna, Gy.[1], Érdi, P.[1] and Grőbler, T.[1]

[1]Dept. Biophysics, KFKI Res. Inst. for Particle and Nuclear Physics of the Hungarian Academy of Sciences, H-1525 Budapest, P.O.B. 49, Hungary;
[2]L. Eötvös Univ., Budapest; [3]Dept. of Comparative Physiology, L. Eötvös Univ., Budapest

Stimulations of detailed models of single mitral and granule cells of the vertebrate olfactory bulb have been done using morphological and physiological data. Morphological data used to describe the basic structure of each cell types are: some size, length of interbranch segments, diameter of branches, branching probabilites, and density of and size of any spines. Passive and active membrane properties were either the same as used by Bhalla and Bower (1993) or were calculated by parameter estimation techniques.

In accordance with the assumptions of Bhalla and Bower (1993) the mitral and granule cells contain six and four channel types, respectively, and the Hodgkin-Huxley formalism has been used for the kinetics of all channels.

Preliminary stimulation experiments have been done to describe the dynamics of the system of a pair of mitral and granule cells coupled by dendro-dendritic synapses. The stimulation of larger network may lead to the substantial extension of the results of our earlier (Érdi et al. 1993) "whole bulb" model built from more simple "fire-and integrate type" neurons.

Computer stimulations have been done by the NEURON stimulation program (Hines 1993).

References:

Bhalla, U.S. and Bower, J. M.: Exploring Parameter Space in Detailed Single Neuron Models: Stimulations of the Mitral and Granule Cells of the Olfactory Bulb. J. Neurophysiol. 69 (1993) 1948-1965

Érdi, P., Grőbler, T., Barna, G. and Kaski, K.: Dynamics of the Olfactory Bulb: Bifurcations, Learning and Memory. Biological Cybernetics 63 (1993) 57-66

Hines, M.: The NEURON Stimulation Program. In: Neural Network Stimulation Environments. J. Skrzypek (ed.), Kluwer Acad. Publ. Norwel, MA, 1993

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A CLINICALLY APPLICABLE METHOD TO EVALUATE VISUO-COGNITIVE PROCESSING

Antal, A.[1,2], Bodis-Wollner, I.[2], Pfeiffer, R.[3]

[1]Department of Physiology, Albert Szent-Gyorgyi Medical University, H-6720, Szeged, Hungary

[2]Department of Neurology, SUNY, Health Science Center at Brooklyn, New York, U.S.A.

[3]Department of Neurology, University of Tennessee, Memphis, U.S.A.

In the last two decades the late components of the event-related brain potentials (ERPs) are being applied as a measurement tool for the evaluation of cognitive capability in normal and clinical populations. The most extensively studied P300 is one of the late components of ERPs which relates to the cognitive aspects of discriminating target from non-target stimuli, indexes categorization processes and the context updating of memory. Previous studies which investigated P300 using visual stimuli in oddball paradigm mainly utilized letters, numbers or geometric shapes. Using these complex tasks the variability of normal data is high. In our study we used simple sinusoidal grating patterns differing only in spatial frequency (0.5 and 1 cycles/degree) with known optical and physiological properties and established normative parameters for the major deflections of visual ERP in 41 healthy subjects (age range: 22-77 years). Using this paradigm we confirmed a positive correlation between age and visual P300 latency and reported the existence of a significant negative deflection (N200) preceding the P300 component of which permanence was controversial in the literature. We described the relationship of primary and cognitive ERPs by calculating the latency difference and the amplitude ratio between primary visual (P100) and late cognitive (P300) components. Our findings recommend the simple sinusoidal grating paradigm for dissecting primary changes from visuo-cognitive ones in normal aging. From the point of view of task difficulty the spatial frequency discrimination presents an easy task and it can be useful for patients with presumed cognitive difficulties.

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THE INNERVATION OF THE RAT SPINAL CORD BY AXONS DESCENDING FROM THE LOCUS COERULEUS-SUBCOERULEUS COMPLEX

Antal, M. and Polgár, E.

Department of Anatomy, University Medical School of Debrecen, H-4012 Debrecen, Hungary

The spinal projections of noradrenergic brainstem nuclei, locus coeruleus and locus subcoeruleus, that influence complex sensory and motor activities in the spinal cord were studied in the rat by using the anterograde neural tracer Phaseolus vulgaris leucoagglutinin (PHA-L). After injecting PHA-L unilaterally into the nucleus coeruleus and nucleus subcoeruleus, labelled fibers and terminals were detected at cervical, thoracic as well as lumbar segments of the spinal cord. Most of the fibers from both nuclei descended in the ipsilateral ventral and contralateral dorso-lateral funiculus, and terminated in the ventral gray matter (laminae VII, VIII, X). Substantial projection to the superficial dorsal horn was revealed only from the subcoeruleus complex.

To study the postsynaptic targets of these descending fibers, sections were stained for both PHA-L and calbindin-D28k (CaB), a calcium-binding protein that have been reported to be a marker of certain subsets of spinal neurons including stalked cells and supraspinally projecting neurons in the dorsal horn and Renshaw-cells in the ventral horn. All of the aforementioned types of CaB-immunoreactive neurons were found to receive contacts from fibers descending from the investigated noradrenergic brainstem nuclei.

Synaptic contacts of labelled terminals in laminae I-II and laminae VII-IX as well as GABA and glycine immunoreactivities of both the terminals and their postsynaptic targets were also investigated in a correlative electron microscopic study. Coerulo-spinals terminals were primarily engaged in synaptic contacts with dendritic shafts both in the dorsal and ventral horns, but they also established synapses with somata and occasionally with dendritic spines. The majority of coerulo-spinal terminals formed asymmetric synapses in the superficial dorsal horn, whereas they were engaged almost exclusively in symmetric synapses in the ventral gray matter. All of the labelled terminals as well as their postsynaptic targets were negative for GABA. Glycine-immunoreactivity, however, was detected in 12% of the coerulo-spinal terminals and 23% of their postsynaptic targets, indicating that glycinergic mechanisms may play some role in coerulo-spinal synaptic transmission.

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PARADOXICAL THERMOREGULATORY RESPONSES OF COLD-ADAPTED RATS TO ACUTE COLD EXPOSURE

Balaskó, M., Székely, M., Szelényi, Z.

Department of Pathophysiology,
University Medical School of Pécs, H-7643 Pécs, Hungary

It has been observed that cold-adapted (CA) rats in response to acute cold (5C°) exposure (CE) increased their metabolic rate to such an extent that their core (colonic/aortic) temperature (T_c) became significantly elevated. T_c remained high throughout the 60 min of CE. A reverse paradoxical response was observed when returning to thermoneutrality (TN:25 C°). With repeated exposures the phenomenon was even more pronounced. In contrast, non-adapted (NA) rats when confronted by cold-stress of similar severity (15C°) let their body temperature fall which was normalised slowly and gradually upon returning to TN (30 C°) environment.

In our present investigations the mechanism of CE-induced paradoxical response was further studied. In some experiments CA rats were exposed to cold for 180 min and we hoped to get an answer to the question whether it is the dynamics or the extent of the CE which is the more important factor in the development of paradoxical reaction. In other experiments pharmacological approach was used. An antipyretic dose (10mg/kg) of indomethacin, potent inhibiting doses (100 μg/kg) of peripheral or central cholecystokinin (CCK) receptor antagonists (L-364,718 or L-365,260, respectively) were given subcutaneously, or 10 mg/kg of α-adrenergic receptor antagonist phentolamin was injected into one of the lateral cerebral ventricles (ICV) half an hour prior to CE to decide the possible role of prostaglandins (PG), CCK and central adrenergic activity in the development of CE-induced T_c elevation in CA rats.

In experiments with long-term CE the T_c remained elevated throughout 180 min of CE and at the end of the third hour the body temperature was still significantly higher than before CE or in control animals without CE.

Neither CCK type-A receptor antagonist, nor CCK type-B receptor antagonist could prevent the development of the paradoxical phenomenon. Indomethacin could not alter the CE-induced paradoxical response either. Phentolamin did inhibit the rise of T_c, but it also suppressed the PG-induced fever-like rise of body temperature, so it may not be a specific effect.

The CE-induced rise in T_c suggests that the peripheral thermoreception in CA rats has a great role, exceeding the role of central thermosensors. Our data suggest that neither PGs nor CCK has important role in the development of this phenomenon. Preliminary data show that CE-induced T_c rise can be observed even in starved CA rats, though their initial body temperature is lower than that of control animals.

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ACUTE EFFECTS OF β-AMYLOID PEPTIDES ON THE ELECTROPHYSIOLOGICAL ACTIVITY AND SYNAPTIC RESPONSES OF CAT NEOCORTICAL NEURONS *IN VIVO*

Baranyi,A., Mari,Z., Szabó,E., Márki-Zay,J., +Soós,K., +Varga,J. and +Penke,B.

Department of Comparative Physiology, Attila Jozsef University of Szeged, H-6726 and +Department of Medical Chemistry,Albert Szentgyörgyi Medical University of Szeged, Hungary

β-amyloid peptides (BAP) may have an important pathogenic role in the Alzheimer disease, when its normally low extracellular concentration increases with orders of magnitude in different parts of the central nervous system. Earlier in vitro studies described neurotoxic effects of the peptide and its fragments. The mechanisms of BAP neurotoxicity, however, and especially actions of elevated BAP levels on electrophysiology of *in vivo* cortical neurons at cellular and synaptic levels have remained unknown. Effects of BAP 1-42/1-40, 25-35, 31-35 and 32-35 were studied in intracellular microelectrode recordings from the motor cortex of anesthetized cats. Peptides were pressure injected (50-150 femtoliters of 100 uM peptide solution) juxtasomatically via an extracellular pipette of the combined microelectrode to the intracellularly recorded neuron (1). All peptides injected extracellularly induced, in minutes, transient depression of spontaneous and ic.current-induced firing activity, and hyperpolarization of membrane potential. Thereafter all components of excitatory postsynaptic potentials (EPSPs to thalamic VL and pyramidal tract, PT stimulation) increased very prominently and remained potentiated until the end of recordings (up to 3 hrs). The slow afterhyperpolarization component of action potentials, the slow components of IPSPs, and the depolarizing inward rectifications to ic.current injections were also augmented, Although these latter parameters approached preinjection values 20-30 minutes after BAP injection, the potentiation of EPSPs unchanged. The actions of BAPs were observed in all cell types and synaptic pathways tested.Similar effects were not induced in case of control injections with the vehicle solution, with intracellular injections of the peptides, and they were absent following ic. injections of 0.25M EGTA and BAPTA into the recorded neurons.

The results indicate that sudden elevation in extracellular BAP levels induce prominent, fast changes in the intracellularly recorded elctrophysiological parameters of neocortical neurons.

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Ref.1. Baranyi,A. and Chase,M. Brain Research, 307:233-245, 1984.

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CHANGE OF AMINO ACID CONCENTRATIONS IN CISTERNAL CSF AND SERUM OF PATIENTS WITH ESSENTIAL TREMOR

Baranyi, M.[1], Mály, J.[2] and Vizi, E. S.[1]

[1]Institue of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest,PO.Box 67, Hungary and[2] Department of Neurology, County Fehér, Székesfehérvár, Hungary

A newly developed high performance liquid chromatographic method was used for determination of abnormalities in free amino acids in cisternal CSF and serum from patients with essential tremor.

The trend of the changes of amino acids concentrations in both biological fluid were similar, with exception of aspartate and tyrosine. This oposition may be indicate that the decreasing in CSF is indipendence from the serum. At slight inhibitory amino acids, as glycine and serine reduced values were observed. Significant decrease was detected in the concentration of glutamine ($p < 0.001$) in the serum, while the decrease was a slight in CSF.

The increase in the concentration of glutamate together with the reduced levels of GABA, glycine and serine in CSF may be the neurochemical basis of the central oscillation in essential tremor. Synthesis of glutamate aspartate and from the glutamate glycine, serine GABA derived from glutamine.

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ANALYSIS OF FAST AND SLOW MYOGRAPHIC ACTIVITY OF THE SMALL INTESTINE BY A NEW EXTRACELLULAR GI AMPLIFIER

Bárdos, Gy.

Psychophysiology Research Group, Hungarian Academy of Sciences, Múzeum krt. 4/A, H-1088, Budapest, Hungary

Recording intestinal motility in the freely moving animals has always been a problem. Implanted mechanical devices usually occlude normal passage, while transducers inserted temporarily required significant restriction of the animal. At the same time, changes of the electric potentials accompanying motor events in the gastrointestinal system were also in the focus of some interest; however, correlation between slow and fast potential changes and the respective movements has not yet been clarified. The method reported here intends to solve both problems.

Silver/silverchloride electrodes are implanted into the superficial outer layers of the intestine; leads are attached to a plug cemented onto the skull. In the pilot experiment reported here two pairs of electrodes were sutured into an isolated small intestinal loop (Thiry-Vella fistula); in some experiments a balloon filled with water was also inserted into the loop.

Potential changes are fed into an EXP-02G type two-way extracellular amplifier (EXPERIMETRIA, Budapest). The amplifier conditions the signals in a very special way: on one channel the slow potentials, on the other channel the fast signals are filtered out; thus both types are derived from one and the same pair of electrodes. By attaching the inserted balloon to a pressure transducer movements and electric changes could be correlated. The balloon is also used to mechanically distend the gut.

Preliminary results show that this device can record both types of electric signals simultaneously; the pattern changes if the loop is distended. However, a special care has to be paid to the artefacts brought about by the gross movements of the animals, especially to head-shakes, rearing and grooming; special wiring is required to ensure longevity of the implants.

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FAILURE OF N^{ω}-NITRO-L-ARGININE, A NITRIC OXIDE SYNTHASE INHIBITOR TO AFFECT CAPSAICIN-SENSITIVE NASAL VASCULAR AND SECRETORY RESPONSES

Bari, F., Domoki, F., Boros, K. and Jancsó, G.

Department of Physiology, Albert Szent-Györgyi Medical University,
H-6720 Szeged, Dóm tér 10, Hungary

Local application of capsaicin and related chemical irritants onto the nasal mucosa produces marked vascular responses, including vasodilatation and plasma extravasation and enhanced secretory activity. Peptide-containing sensory nerves play a crucial role in these reactions. Nitric oxide (NO) is generated by several different cell types, including neuronal, endothelial and mast cells in the nasal mucosa, and may play a role in the functions of the upper airways. The aim of the present experiments was to examine the possible mechanisms of nasal vascular and secretory responses mediated by capsaicin-sensitive sensory nerves. Experiments were carried out on anaesthetized rats (Nembutal, 45 mg/kg). Nasal secretory activity was measured by gravimetry. Preweighed strips of filter paper were inserted into the nasal cavity and changed at 3-min intervals. Nasal plasma extravasation was induced by instillation of capsaicin into the nose (25.6 nmol in 40 μl solution/nostril) after a previous i.v. injection of Evans blue or colloidal silver solution. The amount of extravasated Evans blue bound to serum albumin was measured by spectrophotometry. Localization of the leaky blood vessels after capsaicin treatment was revealed by analysis of transparent whole-mount preparations of nasal mucosa. Local application of capsaicin (200 pmol) resulted in a marked increase in nasal secretion (306 $\pm$ 23% of the basal level). Acute or chronic inhibition of nitric oxide synthase by N^{ω}-nitro-L-arginine (NNLA) did not affect the secretory response to capsaicin, whereas it was significantly attenuated by pretreatment with ruthenium red (2.2 mg/kg, s.c.). Capsaicin induced a marked increase in the dye content of the nasal tissues, which was not affected by pretreatment with NNLA (20 mg/kg i.v.). Light microscopy revealed that intranasal capsaicin treatment resulted in intense labelling with colloidal silver of numerous small blood vessels in the anterior part of the septal mucosa. Pretreatment with NNLA (20 mg/kg) failed to change this reaction. Although NO has been implicated in some of the (vascular) effects of capsaicin, the present findings indicate that NO may not be significantly involved in the mediation of the secretory and vascular effects of capsaicin.

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SODIUM-CHANNEL BLOCKADE AND ANTICONVULSANT ACTIVITY

Bence, J., Molnár, P. and Erdő, S.L.

CNS Pharmacology Laboratory, CHINOIN Co. Ltd., Tó u. 1-5, H-1045, Budapest

Clinically active antiepileptic drugs , phenytoin and carbamazepine are considered to act by the blockade of the voltage-dependent sodium channel in the CNS.

In the present study we compared the anticonvulsant activity and the sodium channel blocking effect of four selected compounds: phenytoin, carbamazepine, lidocaine and vinpocetine.

The effects of the drugs on **sodium currents** were studied by whole cell **patch clamp** technique in primary cultures of the rat cerebral cortex.

Phenytoin, lidocaine, carbamazepine and vinpocetine dose-dependently inhibited voltage-sensitive sodium-channel currents with IC50s of 50.5 , 98.7 , 212.6 , and 44.2 μM, respectively. The effects of all compounds were voltage-dependent, drugs shifted the steady-state inactivation to the hyperpolarizing direction.

The **anticonvulsant activity** of the four compounds was investigated using the maximal electroshock (**MES) test**-model in mice. This test was carried out under the following electroshock parameters: 60 mA, 60 Hz, 0.3 sec, corneal electrodes. Vinpocetine was solved in 20% ascorbic acid and further diluted with phys. saline, and administered i.p. 60 min before shock. Other drugs were suspended in phys. saline with CMC (0.5%), and injected 30 min before induction of convulsion.

All of the four compounds proved to be active in inhibiting the electric shock induced seizures with the following ED50 values: phenytoin: 2.5, lidocaine: 5.6, carbamazepine: 6.9, and vinpocetine: 41.7 mg/kg.

Conclusions: (1) The results of phenytoin, lidocain and carbamazepine on the MES-test and patch clamp studies corresponded closely, the order of potency of these three drugs in inhibiting shock-induced seizures and blocking the voltage-dependent sodium channels was the same.

(2) Vinpocetine proved to be a potent sodium-channel blocker, but its in vivo anticonvulsant potency was low. (This discrepancy could not be caused by poor in vivo absorption, because vinpocetine easily penetrates the brain)

(3) The difference between the in vivo anticonvulsant activity and sodium-channel blocking effect calls attention to other mechanisms that may play a role in the antiepileptic efficacy. Further studies are needed to clarify these processes.

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7-NITROINDAZOLE, AN INHIBITOR OF NITRIC OXIDE SYNTHASE, PREVENTS LIPOPOLYSACCHARIDE-INDUCED FEVER RESPONSE IN RABBITS

Bencsics, Á. and Vizi, E.S.

Institute of Experimental Medicine, Hungarian Academy of Sciences
1450 Budapest, P.O.Box 67, Hungary

Exogenous pyrogens, e.g., bacterial lipopolysaccharides (LPS), stimulate macrophages to release endogenous pyrogens, e.g., TNFα, IL-1ß, and IL-6, which act in the hypothalamus to induce fever. We studied the possible involvement of nitric oxide (NO), a free radical gas produced endogenously, in the febrile response to LPS.

Rabbits were injected intravenously by LPS (2 μg/kg). Colonic (T_C) and skin (T_S) temperature was recorded every 15 min. Thirty min prior to LPS treatment, animals were pretreated intraperitoneally by either 7-nitroindazole (7-NI) (Lancaster, England), a neuronal nitric oxide synthase inhibitor *[Moore et al, Br.J.Pharmacol. (1993), 110, 219-224]* (50 mg/kg) or - respectively - by saline.

Intravenous LPS caused biphasic fever with peaks of T_C at 90-105 min and at 210-225 min. During both the first and the second rising phase of T_C, falls in T_S were observed with minimum values at approximately 15-30 min prior to the peaks of T_C. These observations are similar to those made by others *[Morimoto et al, Am. J. Physiol. (1988), 254, R633-R640]*.

Administration of 7-NI completely inhibited the effect of LPS on both the first ($p<0.01$) and the second ($p<0.001$) peak of the biphasic fever (Student t test for AUC). In contrast, the fall of T_S accompanying the first and the second rise of T_C in control animals was not affected significantly by 7-NI. During the recovery period of the control group, T_S in the 7-NI treated group remained at low level throughout the observation period.

Present results indicate that NO is involved in LPS-induced fever response: an effect which can not be explained with its peripheral vasodilatator effect. The molecular mechanism, the mode of action of NO remains to be elucidated.

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MULTISENSORY INTERACTION PROPERTIES IN THE CORTEX ALONG THE ANTERIOR ECTOSYLVIAN SULCUS OF THE CAT

Benedek, Gy.[1], Kadunce, D.[2], Wallace, M.[2] and Stein, B. E.[2]

Department of Physiology, [1]Albert Szent-Györgyi Medical University, H-6720 Szeged, Hungary and Department of Neurobiology and Anatomy, [2]The Bowman Gray School of Medicine, Winston-Salem, NC, USA

Multisensory interaction is a powerful mechanism in sensory perception. The purpose of this study was to study multisensory multisensory integration in the cortex along the anterior ectosylvian sulcus (AES) of the feline brain. The interaction between acoustic and visual stimuli was investigated in AES cortical cells sensitive to both modalities. Extracellular microelectrode recording was performed in halothane-anaesthetised, immobilized, artificially respirated cats. Altogether 29 auditory-visual single unit and an additional unimodal 17 visual as well as 4 unimodal auditory neurons were investigated. Our observations indicate that simultaneously presented double stimulation elicits mostly a facilitation of the sensory response. Spatially separate, simultaneous stimulation results in an inhibitory interaction in some cases, but no statistical difference was found between the multisensory integration effect of overlapping and separate bimodal stimulation. Our data provide evidence for the notion that AES cortex plays a role in multisensory integration of incoming signals. The intensity of multisensory integration seems to be determined by several factors including the spatial ones.

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CHARACTERIZATION OF [^{3}H]MET^5-ENKEPHALIN-ARG^6-PHE^7 BINDING TO OPIOID RECEPTORS IN RAT BRAIN MEMBRANE FRACTIONS

Benyhe, S.[1], Farkas, J.[2], Tóth, G.[2] and Wollemann, M.[1]

[1]Institute of Biochemistry and [2]Isotope Laboratory, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.Box 521, Hungary

A tritiated opioid radioligand, based on the amino acid sequence of the naturally occurring heptapeptide Tyr-Gly-Gly-Phe-Met-Arg-Phe, (Met^5-enkephalin-Arg^6-Phe^7, MERF), has been synthetized in order to investigate its binding characteristics to rat brain membranes. The radiolabelled compound, [^{3}H]Tyr-Gly-Gly-Phe-Met-Arg-Phe, has been prepared by catalytic dehalogenation in the presence of tritium gas and resulted in a product with 40 Ci/mmol specific radioactivity.

Receptor binding assays were performed in particulate membrane fractions from rat brain. Incubations were carried out in the presence of broad-spectrum peptidase inhibitors at 4 °C. Under these conditions the equilibrium binding was achieved in 30-40 mins, and approximately 90 % of the applied radioligand remained unchanged as determined by HPLC analysis. The apparent affinity (K_d value) of the compound calculated by Scatchard transformation of saturation binding data was 4.7 nM, and the maximal number of specific heptapeptide binding sites (B_{max}) was 157 fmol/mg protein. Similar results were observed in homologous competition binding studies. A number of receptor type-selective opioid ligands were tested by displacement experiments. The rank order potencies of the examined compounds revealed that [^{3}H]MERF recognition sites are certainly not μ and κ_1 sites, whereas these sites are characterized by a κ_2 and/or δ binding profile. Considering the discrepancies found in the affinity of some compounds, including naltrindol and norbinaltorphimine, the presence of a novel, MERF-selective "heptapeptide" binding site in rat brain membranes is also suggested. A majority of the competition curves could be resolved as two components: a high affinity, stereospecific opioid binding site, and a substantially lower affinity binding component, which could only be displaced with Met^5-enkephalin, dynorphin$_{(1-13)}$, and unlabelled MERF, but not by other opioid ligands, e.g. [D-Ala^2-(Me)Phe^4-Gly^5-ol]enkephalin, morphine, naloxone etc. The nature of the latter, possibly nonopioid component of [^{3}H]MERF binding will be studied by Phe-Met-Arg-Phe-NH_2 (FMRFamide) analogues because of their striking sequence homology with the carboxy terminal of the heptapeptide.

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MULTIPLE RECIPROCAL INTERACTIONS BETWEEN THE MEDIAL SEPTUM AND THE SUPRAMAMMILLARY NUCLEUS

Borhegyi, Zs., Acsády, L., Maglóczky, Zs. and Freund, T.F.

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest
PO Box 67. H-1450

The supramammillary nucleus (SUM) is known to be involved in the modulation of hippocampal theta activity. The SUM sends fibres directly to the hippocampus, and, among others, to the medial septum-diagonal band of Broca (ms-dbB) complex, where pacemaker neurons for theta generation are thought to be located. We examined using double retrograde tracing whether the same neurons project to these two areas. We found that at least 20% of the SUM-hippocampal projection neurons innervate the ms-dbB as well.

Stimulation of the hypothalamo-septal pathway appears to facilitate theta-on cells and inhibit theta-off cells in the ms-dbB, suggesting that the projection is highly target selective. We studied this question in another set of experiments by combining anterograde transport of Phaseolus vulgaris leucoagglutinin (PHAL) with immunostaining for the putative postsynaptic neurons, i.e. for parvalbumin (PV) and choline acetyltransferase (CHAT) in the ms-dbB. The SUM-septal pathway showed no target selectivity. Single contacts, confirmed by electron microscopy, frequently occurred on distal dendrites of both cell types. Multiple contacts were occasionally found on PV-positive cell bodies.

To further elucidate the connections between the SUM and the ms-dbB a retrograde tracer was injected into the SUM. We found that septal PV-immunoreactive neurones send their axons into the SUM. These GABAergic cells are known to have numerous local axon collaterals, which are likely to be antidromically activated by SUM stimulation. Thus, inhibition activated antidromically, and excitation via orthodromic stimulation of SUM-septal fibres may explain the dual effect of SUM stimulation.

Akadémiai Kiadó, Budapest

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APPEARANCE AND SOME NEUROCHEMICAL FEATURES OF NITRERGIC NEURONS IN THE DEVELOPING ENTERIC NERVOUS SYSTEM OF THE QUAIL

[1]Boros, A., [1]Fekete, É., [2]Timmermans, J-P., [2]Adriaensen, D. and [2]Scheuermann, D. W.

Department of Zoology and Cell Biology, [1]József Attila University, Szeged, Hungary
Department of Morphology, Laboratory of Cell Biology and Histology, [2]University of Antwerp, Antwerp, Belgium

Using immunocytochemistry, NADPH-diaphorase (NADPHd) histochemistry, the appearance of nitrergic enteric neurons in different digestive tract regions of the embryonic, neonatal and adult quail (*Coturnix coturnix japonica*) was studied in whole mounts and sections. NADPHd was first detectable by embryonic day 4 in two distinct locations, namely the mesenchyme of the primordial gizzard and at the caeco-colonic junction. At embryonic day 6, nitrergic neurons had alrady begun to form a myenteric nerve network in the wall of the proventriculus, gizzard and proximal large intestine. By embryonic day 9, a myenteric network was visualized along the entire digestive tract of the quail. At the level of the stomach , this network was confined to the area covered by the intermediate muscles. By embryonic day 12-13, the NADPHd-positive myenteric neurons in the wall of the distal caeca also became organized into ganglia. From this developmental stage on, a submucous nitrergic nerve network, sandwiches between the *lamina muscularis mucosae* and the luminal side of the outer muscle layer, become prominent in the proventriculus and intestinal walls. In the adult quail, only a minority of the NADPHd-positive neurons stained for vasoactive intestinal polypeptide (VIP) along the intestine. VIP-immunoreactive (IR) cell bodies were frequent in the myenteric plexus but not in the submucous plexus, whereas there were considerable numbers of NADPHd-positive neurons in both plexuses. Nitrergic fibers were also observed in the outer muscle layer, but were almost absent from the lamina muscularis mucosae and lamina propria, in contrast to the dense VIP-ergic innervation encircling the bases of the intestinal crypts.

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EFFECTS OF OMISSION IN VOWEL SEQUENCE ON MAGNETIC FIELD RESPONSES

Csépe, V.[1], Kuriki, S.[2], Hirata, Y.[3] and Norio, F.[4]

[1]Institute for Psychology of the Hungarian Academy of Sciences, Budapest, Hungary; [2]National Institute for Physiological Sciences, Okazaki, Japan; [3]Research Institute for Electronic Sciences of the Hokkaido University, Sapporo, Japan; [4]Fujitsu Laboratories, Atsugi, Japan

The use of recently developed large scale multi-sensor magnetometers enables us to obtain temporally coherent data of neuromagnetic fields across many locations over the scalp in a short recording time. In our recent study waveforms and spatial distributions of the magnetic responses elicited by triplets of vowels and by omission in the vowel sequence were investigated.

Four normal hearing subjects participated in this study. Magnetic data were recorded through two 37-channel biomagnetometers (BTi Twin System) set over the left and the right temporal surface. Three different types of stimuli were given binaurally to the subjects in an active oddball paradigm. The frequent stimulus (probability 80%) consisted of three vowels, [aoi], meaning "blue" in Japanese. The target stimulus was composed by changing vowel [o]to the plosive-consonant-vowel [ka] and thus the meaning was changed to "red". The subjects' task was to count on the target occurring with 5% probability. The rare nontarget, [a_i] (probability 15%), was composed by omitting the [o] from the triplet.

The obtained source locations at the N100m ("m" for magnetic) peak to the three vowels in [aoi] were very close to each other over hemispheres. The responses to the closing vowel, [i], were enhanced in the right hemisphere (significantly larger dipole moment), probably associated with the sequence termination. Moreover, in the [i] responses the left-right latency difference was smaller than in the [a] and [o] responses where the difference was significant. The N100m source of the [a] and [o] vowels of the omission stimuli became more separated over the left than over the right temporal surface, especially in the anterior-posterior direction. The omission-related magnetic field had two prominent deflections, corresponding in latency to the electric N2b-P3a wave-complex. The monopolar distribution of the first deflection questions its identity with the mismatch negativity (MMN).

Our results demonstrated differences between the two hemispheres in analytical and integrative processing of speech stimuli. It is also demonstrated that the terminal phoneme has a special importance when sequences supposed to form a perceptual entity. The fact that we failed to find a reliable MMNm to the vowel-omission suggests, that the processes behind might be different from those being active in automatic detection of omission. It corresponds to those few results which show that omission-MMN is recordable under limited conditions.

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EXPRESSION OF THE KAPPA-OPIOID RECEPTOR IN THE HUMAN BRAIN TISSUE STUDIED BY IMMUNOCYTOCHEMISTRY AND "IN SITU" HYBRIDIZATION

Cserpán, E., Tóth, P., *Wevers, A., #Schmidt, P., *Schröder, H. and Maderspach, K.

Inst. Biochemistry, Biological Res. Ctr., Hungarian Acad. Sci., H-6701 Szeged, Hungary; University of Cologne, *Dept. Anatomy and #Ins. of Forensic Medicine, Cologne, Germany

Opioid receptor provide primary interaction sites of the human brain with opiates. The expression of kappa opioid receptor (k-OR) and its mRNA was studied by immunohistochemistry (a) and by "in situ" hybridization (b).

(a) Immunohistochemical studies using the monclonal antibody (mAb) KA8 [1] had shown k-OR-like immunoreactivity mainly localised on pyramidal neurons of layer II-III and V of human prefrontal cortex [2].

(b) The k-OR encoding sequence, determined by Yasuda and coworkers [3] was used to construct oligonucleotid probes (sense and anti sense) 48 mer, 3' CCCCCTGAGGTACCACTCACGACGTCGACCTTTGGCTCCTCGCTACCG 5' and 3'-endlabeled with digoxigenin-UTP, which were used for "in situ" hybridization.

According to the results k-OR mRNA was expressed in the prefrontal cortex mainly in the pyramidal neurons of layer II-III, V and multiform neurons of the layer VI. No labeled astrocytes were seen. In the motocortex some neurons in deeper layer II and the Betz giant pyramidal neurons heavily labeled. In the primary visual cortex, again pyramidal-like neurons expressed k-OR mRNA mainly in the layer VI and numerous giant stellate cells. These findings are in agreement with the results of above immunohistochemical studies for the k-OR distribution in frontal cortex.

Our immunohistochemical and "in situ" hybridization studies have set the stage to proceed working on the comparison of receptor expression in the brain of opiate drug addicts as compared to present normal controls.

References:

[1] Maderspach, K. *J. Neurochem. 56 [1991] 1887-1904*
[2] Schmidt, P. *Brain Res. 654 [1994] 223-233*
[3] Yasuda, K. *PROC. Natl. Acad. Sci. U.S.A. 90. 6736-6740*

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EFFECT OF APOMORPHINE ON INNATE AND ACQUIRED COLOUR PREFERENCES OF QUAIL

Csillag, A.[1] and Kovach, J. K.[2]

[1]1st Department of Anatomy, Semmelweis University of Medicine, 1450 Budapest, Hungary and
[2]Menninger Clinic, Topeka, KS, 66601 U.S.A.

Artificially selected, newly hatched Japanese quail chicks exhibit divergent approach preferences for and imprintabilities to particular colours of otherwise identical visual stimuli. Medial diencephalic lesions, which impaired major tegmentostriatal (putative dopaminergic) pathways, have been found to attenuate the genetically selected preferences for stimulus colour. In the present study, we tested the short-term effects of the dopamine agonist apomorphine on the approach preference behaviour.

Day-old quail chicks genetically selected for red (RL) or blue (BL) preferences were injected intraperitoneally with apomorphine, and the birds' preference for their selected colours was assessed using a 7-choice mass screening apparatus, each of its 28 compartments offering a choice between the same pair of flickering colour stimuli, blue *vs* red, of equal size, luminance and intermittence. The birds' performance was tested before and 45 min, 2 h and 24 h after drug treatment, and evaluated by ANOVA.

In RL chicks, a transient, dose-dependent attenuation of the preference for red stimulus was observed after the administration of apomorphine. This effect was partially abolished when the apomorphine treatment had been preceded by administration of haloperidol, but not sulpiride. Apomorphine also attenuated the blue preference of BL birds to a lesser degree and this inhibition was also abolished by haloperidol. In quails selected for high imprintability (Hi), imprinting to red- or blue stimuli resulted in preferences that matched those of genetically selected RL or BL birds. Apomorphine strongly attenuated the acquired red preference of red-exposed Hi chicks and this effect was also blocked by haloperidol, but not sulpiride, treatment. Blue-exposed Hi chicks were less affected by apomorphine.

The results suggest a dopaminergic mechanism in the manifestation of visually guided approach preference, whether based upon genetic selection or environmental influence.

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TOPOGRAPHICAL CORRELATIONS BETWEEN CGRP AND THE NICOTINIC ACETYLCHOLINE RECEPTOR IN MEYNERT'S BASAL NUCLEUS AND IN THE PREFRONTAL CORTEX OF THE PRIMATE BRAIN

Csillik, B., Rakic, P., Goldman-Rakic, P. and Knyihar-Csillik, E.

Section of Neurobiology, Yale University Medical School, New Haven, CT, USA; Bay Zoltan Institute for Biotechnology, Szeged, Hungary (BC) and Department of Clinical Neurology, Albert Szent-Gyorgyi Medical University, Szeged, Hungary (EKC)

Micro-topographical correlations between calcitonin gene-related peptide (CGRP) and the nicotinic acetylcholine receptor (n-AChR) were studied in adult monkeys (Macaca fasciculata) in order to disclose the possibility of a functional linkage in the basal nucleus and in the cerebral cortex, contributing to the working memory. Investigations were performed with pre-embedding immunocytochemical techniques at light- and electron microscopic levels, using a polyclonal antiserum raised against CGRP in rabbit (Amersham) which recognizes both α- and β-CGRP but does not cross-react with calcitonin. For the immunohistochemical localization of n-AChR, two monoclonal antibodies, harvested from mouse (mAb-74.25) and rat (mAb-35) hybridoma cell cultures, and biotinylated α-bungarotoxin were used.

A close correlation was found between CGRP-immunoreactive varicous axons and n-AChR-spots on the surfaces of large cholinergic nerve cells in the basal nucleus. Distinct differences between the localization of α-bungarotoxin, mAb-74.25 and mAb-35 binding sites suggest pleiotropy or at least heterogeneity of n-AChR subunits. Since our earlier results proved convergence of CGRP-positive axons upon choline acetyltransferase (ChAT)-positive neuronal somata and dendrites, and the existence of cholinergic synapses on dendrites (auto- viz. homosynapses with autoreceptors) of cholinergic nerve cells, the extrapolation seems germane that, in manner similar or analogous to that prevailing in the neuromuscular junction, CGRP might play an important role in the regulation of expression and maintenance of the α-subunit of the n-AChR in the basal nucleus.

In the feline cerebral cortex, a widespread CGRP-immunopositive axonal system has been reported recently. In the primate prefrontal cortex, not only axons but

also numerous CGRP-positive nerve cells, mainly stellate and granular cells in lamina II and IV were observed which are engaged in multiple synaptic contacts with pyramidal cells. Though localization of n-AChR in the prefrontal cortex is similar to the axonal system exerting CGRP immunoreactivity, there are no n-AChR-immunoreactive perikarya present. On the other hand, mAb-74.25 immunopositive axonal systems are present not only in the prefrontal cortex, but also in the entire extent of the neocortex.

It is concluded that a functional linkage exists between CGRP and the n-AChR (possibly the α-subunit of the latter), both in the basal nucleus and in the prefrontal cortex. Accordingly, there is a good reason to assume that such functional linkage is essential for the normal function of the neuronal network subserving memory, and that disturbance of this functional linkage might be responsible for its impairment, characterizing e.g. Alzheimer's disease.

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OCTOPAMINERGIC NEURONS IN THE CENTRAL NERVOUS SYSTEM OF OLIGOCHAETA

Csoknya, M.[1], Lengvári, I.[2], Eckert, M.[3], Rapus, J.[3], Hámori, J.[1], Hiripi, L.[4], Elekes, K.[4]

[1]Department of Zoology, Janus Pannonius University, Pécs, [2]Department of Anatomy, University Medical School, Pécs, Hungary, [3]Institute of General Zoology and Animal Physiology, FSU, Jena, FRG, [4]Department of Experimental Zoology, Balaton Limnological Research Institute of Hungarian Academy of Sciences, Tihany, Hungary

The presence of octopamine in the nervous system of *Lumbricus terrestris* and *Eisenia foetida* was studied by immunocytochemistry utilizing paraffin-embedded serial sections and by high performance liquid chromatography (HPLC).

Octopamine immunoreactive perikarya were found in each part of the central nervous system of both species. In *Lumbricus* 96-102 labelled cells were found in the cerebral ganglion, 18 in the subesophageal ganglion and 14 in each of the ventral cord ganglia. The same figures of *Eisenia* were 88-98, 20-22 and 6, respectively. Sizes of octopaminergic cells varied according to their location, the largest cells being found deep in the cerebral ganglion and in the medial portion of the subesophageal as well as ventral cord ganglia. According to their morphological characteristics it is probable that octopaminergic cells are interneurons. On the basis of indirect evidence it is suggested that some octopaminergic neurons contain proctolin. Octopaminergic axons form an uneven network in the neuropil of all parts of the central nervous system.

High performance liquid chromatography assay revealed the presence of octopamine in each investigated part of the central nervous system, showing concentration values between 8.6 and 16.7 pmol/mg wet weight in both species. Concentrations of octopamine precursor tyramine are significantly lower in the central nervous system of *Eisenia* (< 0.5 pmol/mg wet weight), than in that of *Lumbricus* (0.67-2.0 pmol/mg wet weight). The metabolism of ^{3}H-tyrosine reveals that tyramine and octopamine are synthesized by tyrosine-decarboxylase and tyramine-β-hydroxylase enzymes, respectively. Based on these findings, the role of octopamine in central and peripheral regulatory processes of *Oligochaeta* is suggested.

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EFFECTS OF TUMOR NECROSIS FACTOR-α ON THE BLOOD-BRAIN BARRIER IN VITRO

Deli, M.A.[1], Dehouck, M.-P.[3], Descamps, L.[3], Cecchelli, R.[3], Ábrahám, C.S.[2], Joó, F.[1] and Torpier, G.[3]

[1]Laboratory of Molecular Neurobiology, Biological Research Centre, and
[2]Department of Pediatrics, Szent-Györgyi Medical University, Szeged, Hungary;
[3]INSERM U.325, Institut Pasteur, Lille, France

Tumor necrosis factor α (TNFα), a proinflammatory cytokine, is involved in the pathogenesis of CNS infections and several neurodegenerative diseases. The possible role of the blood-brain barrier (BBB), the active interface between the blood and brain, during these changes is controversial. It is disputed whether TNFα results in an opening of the BBB for different tracers or not in animal models. No data are available on the direct effect of TNFα on the permeability of cerebral endothelial cell monolayers cocultured with astrocytes, the *in vitro* reconstituted model of BBB. The aim of our present study was: (i) to elucidate if TNFα exposed to the luminal side of bovine brain capillary endothelial cells (BBCEC) could exert any influence on the penetration of small molecular weight tracers and (ii) to establish if possible changes in the permeability of cerebral endothelial cell monolayers were accompanied by a reorganization of certain cytoskeletal filaments being known to be related to the regulation of passage of substances through the BBB.

BBCEC at passages 4-6 were seeded on collagen coated cell culture inserts, which were placed into the astrocyte-containing multiwell dishes for a 2 week coculture period. In contrast to the finding on the endothelial cells of aortic arch origin, one-hour exposure of recombinant human TNFα (in concentrations of 50, 250, and 500 U/ml, respectively) to the luminal membrane of BBCEC did not change significantly the transendothelial flux of either ^{14}C-sucrose (m.w. 342 Da), or ^{3}H-inulin (m.w. 5 kDa) up to 4 h (*early phase*), except for a slight decrease ($p<0.05$ unpaired *t* -test) in sucrose permeation at 120 and 240 min with the highest dose of TNFα. On the other hand, 16 hours after the one-hour challenge with TNFα (*delayed phase*) at all three concentrations, significant ($p<0.001$ unpaired *t* -test) 2-fold increase was induced in the permeability of BBCEC monolayers for both markers. These changes of permeability were accompanied by a selective reorganization of F-actin filaments into stress fibers, while the intracellular distribution of vimentin remained similar to the control. In conclusion, the direct effect of TNFα on our *in vitro* BBB model was biphasic: it resulted in no change and/or decrease of permeability in the first 4 hours, and a significant increase of transendothelial flux as well as cytoskeletal changes 16 hours after the challenge.

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BASAL FOREBRAIN CORRELATES OF RHYTHMIC EEG ACTIVITY

Détári, L.[1], Rasmusson, D.D.[2] and Semba, K.[3]

[1]Department of Comparative Physiology, Eötvös Loránd University, Budapest, Hungary, Departments of [2]Physiology and [3]Anatomy & Neurobiology, Dalhousie University, Halifax, N.S., Canada

Several lines of evidence have shown that acetylcholine plays an important role in the regulation of cortical activity. Cortical acetylcholine release strongly increases during states characterized by EEG desynchronization and it exerts a powerful excitatory effect on pyramidal cells. The major source of this acetylcholine is the basal forebrain cholinergic system providing monosynaptic innervation for most of the forebrain.

In accordance with the release data, cortically projecting and other neurons (D-cells) were found to have selectively higher rate during cortical desynchronization compared to synchronization both in freely moving and anesthetized animals. In addition to these cells, a smaller number of neurons (S-cells) activated during synchronized states were consistently observed in these experiments.

Recent in vivo studies have shown that the basal forebrain cholinergic neurons are able to fire high frequency bursts triggered by a low threshold Ca^{2+} spike mechanism. In addition, the cells can generate a complex bursting pattern with 1-2 sec long activated periods recurring in every 5-8 sec.

The aim of the present experiments was to test the possibility that the fluctuating activity of the basal forebrain cholinergic neurons exerts a phasic effect on the cortical EEG, in addition to the generally accepted tonic influence.

In urethane anesthesia, 52 D-cells and 14 S-cells were recorded in the basal forebrain of 26 rats along with the cortical EEG. Two types of rhythmic activity were observed in the EEG: 1. at a deep level of anesthesia, few second long flat periods and large waves; 2. at a more superficial level, delta waves and short desynchronized periods alternated. Previous work has shown that the flat EEG periods in the first pattern correspond to a general inhibition in the cortex caused by potassium currents.The periodic blockade of these currents leads to the appearance of the large waves. Most of the D-cells were activated and most of the S-cells inactivated during the large waves in the first, and during the desynchronized periods in the second pattern. These observations suggest that basal forebrain cholinergic neurons indeed have an important phasic effect on EEG generation that might play a role in unanesthetized animals as well.

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THE NEUROMUSCULAR AND CIRCULATORY EFFECTS OF SZ-1677, A NOVEL STEROID TYPE NEUROMUSCULAR BLOCKING AGENT

Dóda, M.[1], Foldes, F. F.[2] and Vizi, E. S.[1]

[1]Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary and [2]Department of Anesthesiology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY 10467 USA

Searching for muscle relaxants with rapid onset and short duration of action, which are free of cardiovascular side effects, new steroid type muscle relaxants were synthesized and screened for neuromuscular and circulatory activity. Sz-1677, [1-3α-hydroxy-17ß (acetyloxy)-2ß -(1,4-dioxa-8-azaspiro [4,5] dec-8-yl)-5α -androstane-16ß -yl] -1-(2-propenyl)-pyrrolidinium Br was selected for further studies.

Cats of 2.5-3.5 kg body weight were anesthetized with i.p. chloralose and urethane, tracheotomized and ventilated with room air. The n. vagus was stimulated (20 Hz, 0.3 ms, 2 s, supramax V) and the blood pressure and heart rate were recorded. The sciatic nerve was stimulated (0.4 Hz, 0.2 ms, supramax. V) and contractions of the m. tibialis anterior were quantitated and recorded. The ED50 and ED90 values were calculated from the log dose-response regression lines. Drugs were administered intravenously.

SZ-1677 was about four times as potent as rocuronium. The time course of rocuronium was more rapid than that of SZ-1677, however, SZ-1677 even in 8xED90 dose does not inhibit the vagus induced depression of heart rate and blood presure.

8xED90 of SZ-1677 in contrast to rocuronium does not inhibit the vagus reflex therefore it is unlikely to cause elevation of heart rate or blood pressure in man.

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INHIBITION OF CUTANEOUS NEUROGENIC INFLAMMATION BY LIDOCAINE

Dux, M.[1], Jancsó,G.[1], Sann, H.[2]

[1]Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary, and [2]MPI f. physiologische u. klinische Forschung, W.G. Kerckhoff-Institut, Bad Nauheim, FRG

Axon reflex vasodilatation and neurogenic plasma extravasation are characteristic cutaneous vascular responses mediated by neuropeptides released from stimulated capsaicin-sensitive sensory nerve endings. Intracutaneous injection of local anaesthetics inhibits the axon reflex flare elicited by chemical irritants in the human skin. Early reports showed that local anaesthetics failed to affect the neurogenic inflammatory response evoked by chemical irritants in the rat skin. Accordingly, it has been suggested that the release of vasoactive peptides from sensory nerve endings is independent of an activation of Na^+ channels of afferent nerve terminals. Other studies, however, indicated that neurogenic plasma extravasation is inhibited by lidocaine in visceral organs. Therefore, the aim of the present study was to re-examine the effect of local anaesthetics on the neurogenic inflammatory response elicited by chemical irritants in the rat skin.

Experiments were performed on adult Wistar rats under chloral hydrate anaesthesia. Animals were given intracutaneous injections of lidocaine (0.1%, 0.25%, 0.5%, 0.75% or 1%) into separate regions of the shaved abdominal skin. Lidocaine was dissolved in Tyrode solution and injected in a volume of 0.1 ml. As a control, animals received an intracutaneous injection of 0.1 ml Tyrode solution. Cutaneous inflammatory reactions were evoked either by painting the shaved abdominal skin with mustard oil (5%), or by injecting histamine or the histamine liberator compound 48/80 intracutaneously into exactly the same areas of the skin where the lidocaine had been injected 15 min earlier. Evans blue dye (50 mg/kg) was injected intravenously 5 min prior to the application of an inflammatory stimulus. The rats were killed by bleeding. Skin samples of standardized size were excised at sites of injections, the extravasated dye was extracted and its amount was determined photometrically.

Painting the skin with mustard oil produced a deep blue colouration of the abdominal skin, indicative of an increased vascular permeability. Intracutaneous injection of lidocaine resulted in a dose-dependent inhibition of the inflammatory reactions elicited by mustard oil, compound 48/80 or histamine.

The present findings disclosed a dramatic reduction or even a complete abolition of the cutaneous neurogenic inflammatory response by lidocaine in the rat. The results suggest that the site of this inhibition is beyond the nerve terminal, presumably at the level of the endothelium of postcapillary venules.

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CHARACTERISTICS OF THE A-TYPE POTASSIUM CURRENTS UNDER VARIOUS ISOLATION CIRCUMSTANCES IN HELIX NEURONS

Erdélyi, L.

Attila József University, Department of Comparative Physiology, H-6701 Szeged, P.O.B. 533, Hungary

The A-type currents were investigated in identified Helix neurons under voltage-clamp circumstances in various isolation media. Among identified neurons the L and RPa2, 3; LPa4, the L and RPl1 or V1 neurons were examined. The isolation solution contained (mM): 80, NaCl; 4, KCl; 7 CaCl; 5 MgCl2; 5, Tris-HCl; pH=7.5 as normal solution; Tris-Cl substituted for NaCl in Na-free (Tris) and MgCl2 for CaCl2 in Na-, Ca-free (Tris) media. Furthermore, 160 mM mannitol and 80 mM LiCl substituted for NaCl in Na-free (Mann.) and Na-free (Li) solutions respectively. The identified neurons had resting membrane potentials of-50- - 55 mV and spike threshold potentials at about 30 mV more positive voltage. The cells were held at -50 mV holding potentials and the membrane currents were activated with depolarizing command pulses after conditioning hyperpolarization to -100 mV for 500 ms. The difference of currents activated with and without conditioning hyperpolarization was examined as isolated A-currents. A-current inactivation was studied at -30 mV command potential preceding with from -100 to -50 mV conditioning hyperpolarization. The time dependence of removal of inactivation (T1) and the time course of recovery from inactivation of A-currents (T2) were also estimated.

The peak amplitude of the A-currents decreased by 27.5 percent (mean at -30 mV, n=6) when the cell was soaked in Na-free (Tris) and increased by 27.8 percent (n=4) following 20 min exposure in Na-, Ca-free (Tris) media respectively. When Na-free (Mann.) solution was applied the peak amplitude increased by 13.7 percent (n=3). Na-free (Li) media decreased the peak amplitude by 32.5 percent (n=4) in the various identified neurons. Relative to the normal values, the time constant of decay (at -30 mV) decreased by 10 (n=5) and 41 percent (n=4) in Na-free (Tris) and Na-, Ca-free (Tris) solutions respectively. Na-free (Tris), Na-free (Mann.) decreased but Na-free (Li) increased the T1 and T2 values of the A-currents respectively. The voltage dependence of activation and inactivation of the A-currents was significantly modified in the Na-, Ca-free (Tris) solution.

It is concluded, that the A-current is down regulated by Na and Ca ions under physiological circumstances in the studied identified Helix neurons. Furethermore, Li ion looks like to be more effective than Na ion in this respect. In contrary to Aplysia, Helix pomatia L. neurons did not possess Ca-activated A-current (Junge, D.: Brain Res. 346, 294-300, 1985) or sodium activated potassium current components.

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RHYTHMOGENESIS, SYNCHRONIZATION AND CONTROL OF CHAOS: NEURAL MECHANISMS AND ALGORITHMS

Érdi, P.

Dept. Biophysics, KFKI Research Institute for Particle and Nuclear Physics of the Hungarian Academy of Sciences, H-1525 Budapest, P.O.B. 49, Hungary

Rhythmic activies in neural systems may occur both at single neuron level in consequence of the interaction of intrinsic currents or at network level due to some synchronization mechanisms (Gray 1994).

Since data of intracellular studies on the kinetics of voltage- and calcium-dependent ion channels start to be available for different cells, instead of simple single neuron models (as McCulloch-Pitts models, "integrate-and fire" models etc.) preferred earlier now detailed multi-compartmental models supplemented with kinetic equations for the recently discovered intrinsic membrane currents are the building blocks of (moderately large) network models. (see yet Adorján et al. this volume).

An analysis is presented to interpret the multiple rhythmicity of the cells of the thalamocortical circuit, and of the olfactory bulb from the point of view of dynamic system theory.Specifically, in the former case the hyperpolarization activated non-inactivating mixed sodium-potassium inward currents seems to be a bifurcation parameter driving the systems from the hyperpolarized resting state through delta and spindle frequences to the depolarized resting state.

Chaotic activities found in neural systems both at single-cell and population levels now are thouht to have both positive and negative functional role. 'Controlling chaos' is a concept initiated by the following question: how to stabilize unstable periodic orbits embedded within a strange attractor by making only small time-dependent perturbations in accessible system parameter? While control algorithms (e.g. proportional – feedback algorithm) were adopted for controlling systems "from outside", properly chosen learning rules can play the role of "internal" feed-forward control mechanisms.

Reference:

Gray, C. M.: Synchronous Oscillations in Neuronal Systems: Mechanisms and Functions. J. Computational Neurosci. 1 (1994) 11-38

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UP-REGULATION OF μ OPIOID RECEPTORS AND G PROTEINS IN THE BRAIN MICROSOMAL FRACTION OF MORPHINE TOLERANT RATS

Fábián, G., Bozó, B., Tombor, B., Szikszay, M.[1] and Szűcs, M.

Institute of Biochemistry, Biological Research Center Hungarian Academy of Sciences and [1]Department of Physiology, Szent-Györgyi A. Medical University, Szeged, Hungary

Prolonged exposure of cells to certain drugs induces adaptive changes resulting in tolerance or reduced responsiveness to this drug. The molecular mechanisms involved in the development of tolerance and dependence to the μ opioid receptor agonist morphine are largely unknown. Recently much attention has been directed toward postreceptor events. In the present work we aimed at studying alterations of G proteins as well as their coupling to opioid receptors after chronic morphine treatment in subcellular fractions of rat brain. We showed before that in rat brain μ opioid receptors are functionally coupled to pertussis toxin-sensitive G proteins (G_i/G_o) in synaptic plasmamembranes (SPM); and albeit present, uncoupled from G proteins in the microsomal fractions (MI). We hypothesized that the latters are candidates for desensitized, uncoupled opioid sites (1). Rats were rendered tolerant to morphine by s.c. injections of morphine twice daily for 5 days. GTP-γ-S binding, photoaffinity labelling, pertussis toxin-mediated ADP-ribosylation and Western-blotting experiments with specific antisera were used to investigate G-proteins. All these experiments supported the observation that chronic morphine treatment increased the level of α-subunits of G-proteins by 63% resp. 30% in SPM and MI, calculated from GTP-γ-S binding studies. The number of binding sites measured with the μ-opioid agonist peptide ligand, [^{3}H]DAMGO was elevated by about 50% in MI after morphine treatment. Moreover, these up-regulated sites displayed significant sensitivity to GTP, indicating that they are able to interact with G proteins, unlike the μ-sites detected in MI of untreated rats. Our results suggest that regulation of G-proteins (particularly G_i/G_o); moreover altered molecular interactions between opioid receptors and G proteins, especially of those localized intracellularly play a role in chronic morphine actions.

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(1) M. Szűcs and C.J. Coscia, J. Neurosci. Res. 31:565-572 (1992)

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NEUROCHEMICAL MODULATION OF CHEMOSENSITIVE NEURONS IN THE MONKEY PALLIDUM

Faludi, B.[1,2], Karádi, Z.[1], Hernádi, I.[2] and Lénárd, L.[1,2]

[1]Institute of Physiology, Pécs University, Medical School, H-7643 Pécs and
[2]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University, H-7643 Pécs, Hungary

The globus pallidus (GP), an extrapyramidal motor structure, plays important roles in the regulation of feeding (body weight and metabolic control, sensorimotor integration). Despite the large amount of relevant data, little is known about the feeding-associated neurochemical properties of pallidal neurons.

In these experiments, extracellular single neuron activity of pallidal cells was recorded by means of carbon fiber, multibarreled glass microelectrodes in alert rhesus monkeys during: 1) microelectrophoretic application of various neurochemicals (dopamine, noradrenaline, GABA, glucose, acetylcholine and NMDA), 2) taste- and 3) odor-stimulations.

Appx. 14% of GP cells were suppressed by microelectroosmotically applied glucose (these were assigned as GS units). About 17% of all pallidal cells tested responded to taste and odor stimuli. The taste- and odor-responsive neurons were mainly found among the GS cells. The GS and glucose-insensitive (GIS) cells and the taste-responsive and non-responsive GS neurons displayed differential sensitivities to catecholamines (CAs). NMDA caused the inhibition of two-thirds of the examined cells. In 1/4 of the tested neurons, there were biphasic activity changes after NMDA administration (initial facilitation followed by inhibition). The predicted excitatory effect of NMDA was found only in a minority (6%) of examined neurons.

Our findings demonstrated the existence of specific GS and taste- and odor-responsive neurons in the monkey GP. The CAergic responsiveness of the pallidal chemosensitive cells was similar to that of GS neurons of the lateral hypothalamic area and amygdala. The GS cells, through their differential neurochemical modulation, are postulated to be intimately involved in complex pallidal regulatory functions. Because of the exogen and endogen chemosensitive properties, the pallidal GS cells are thought to participate in feeding-related integrative mechanisms of the central glucose-monitoring network represented at different levels of the neuraxis.

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EFFECTS OF SYNTHETIC GALANINS ON ACETYLCHOLINE RELEASE FROM DIFFERENT AREAS OF RAT BRAIN

Farkas, Z., Kasa, P. and Balaspiri, L.*

Division of Alzheimer's Disease Research Laboratory, Department of Psychiatry and *Department of Medical Chemistry, Albert Szent-Györgyi Medical University, H-6720 Szeged, Hungary

Galanin (GAL) is a recently discovered neuropeptide that has been shown to be present in different areas and different neurons in the central nervous system (CNS). By immunocytochemical means, it has been revealed that the peptide is co-localized with acetylcholine (ACh) in many parts of the CNS. In vivo neuropharmacological experiments demonstrated the inhibitory effect of porcine GAL (pGAL1-29) on ACh release. Because of this inhibitory effect on ACh release, it is suggested that GAL antagonist may play a significant role in the treatment of Alzheimer's disease.

Our aim in these investigations was twofold: (1) to study the effects of various synthetic GALs and their fragments on the ACh release in different areas (dorsal hippocampus, ventral hippocampus, striatum and olfactory bulb) of the rat CNS, and (2) to compare the in vitro effects of GALs with the in vivo effects already described by several authors.

The effects various synthetic human GAL (hGAL) and (pGAL) sequences (hGAL1-30, hGAL1-19, hGAL1-15, hGAL17-30; pGAL1-29 and pGAL1-15) were studied on the ACh released from in vitro tissue sections from different areas of the rat brain. The effect of a GAL receptor antagonist, galantide (M15), was also investigated.

The results show that in in vitro experiments hGAL and pGAL and their fragments exert, different effects on the basal and the potassium-evoked ACh release from the rat striatum, dorsal and ventral hippocampus, and olfactory bulb. The GAL receptor antagonist M15 did not have any effect on the basal release in the striatum, hippocampus or olfactory bulb, but it inhibited the stimulatory effect of pGAL1-29 on the basal release of ACh. It is suggested that care must be taken in (1) extrapolating any data obtained in in vitro experiments to those obtained under in vivo circumstances, and (2) extrapolating any data obtained with pGAL and its fragments to results on hGAL and/or its fragments.

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A COMPUTER MODEL OF THE VISUAL CORTEX

Fehér, O. and Virág, T.

Department of Comparative Physiology, József Attila University,
H-6726 P.O.B. 533, Szeged, Hungary

In the computer program, written in Turbo-Pascal language the parameters of a neuron are stored in 4 memory adresses such as: state of excitation, threshold, number of discharged spike potentials and serial number of the neuron. The functioning of the neurons are laid down in macros, which are differentially built up according to the type of neuron. The input units are Golgi-interneurons, having circular visual field and localized histologically in sub-layer IV C. Different combinations of them give innervation for 6 types of orientation selective simple cells. They are identical with small pyramids and star pyramids of layer II and III. They give input for complex cells, playing role in feature extraction. All these neuronal processes are based on function of the retinal X-type ganglion cells. Retinal W-type ganglion cells feed small groups of afferent neurons which innervate the s.c. excentricity neurons, reflecting the dimensions of objects without resolving finer details. They get facilitating impulses from the simple cells. Orientation selectivity of simple cells is secured by function of inhibitory neurons fed by afferent cells in feed forward mode. The model is working with impulses coming from 252 retinal ganglion cells and includes cca 1000 neurons. In this form it is able to simulate orientation and direction selectivity, "on" and "off" type cells, recognizing simple figures in 2 dimension and some electrophysiological phenomena. It gives rational explanation for several sensory illusions,too.

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DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM IN THE HUMAN FETAL SMALL INTESTINE

Fekete, É.[1], Resch, B.[2], Timmermans, J-P.[3], Scheuermann, D.W.[3], Benedeczky, I.[1]

Department of Zoology and Cell Biology,[1] József Attila University, Szeged, Hungary
Department of Obstetrics and Gynecology,[2] Albert Szent-Györgyi Medical University, Szeged, Hungary and Laboratory of Cell Biology and Histology[3] University of Antwerpen, RUCA, Antwerpen, Belgium

The developing enteric nervous system of the human fetus has been examined by means of electron microscopy and neurone-specific enolase immunocytochemistry between 8 and 26 weeks of gestation, with special reference to the development of nerve-muscle contacts.

Prior to the 10th week of gestation, an unambigous morphological identification between nerve cells and surrounding mesenchyme cells could not be made, although some cells already expressed neurone-specific enolase immunoreactivity by the 8th week of gestation. Drastic changes in the morphplogical relation between the maturing myenteric plexus and the smooth muscle cells were noted within the fetal period examined. Between week 10 and 18 the elements of the myenteric plexus and the muscle cell processes from the circular muscle layer interdigitated. After week 18 the plexus became ensheated by supporting cells and connective tissue and interdigitation was no longer observed.

It is very probable that changing microenvironmental conditions cause this sequential pattern of nerve muscle contacts during prenatal development. Such changes may be essential for the morphogenesis or, more generally for the functional maturation of the external muscle coat and the interposed myenteric plexus.

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PROTEIN STRUCTURAL ASPECTS OF NEURONAL PLASTICITY

Friedrich, P.

Institute of Enzymology, BRC, Hung. Acad. Sci.,
Budapest, POB 7, H-1518

From the biochemical point of view, memory resides in altered protei (enzyme) structure in the appropriate synaptic location of neurons.

Short-term memory is generally thought to consist in the modification, maini by phosphorylation or proteolytic processing, of preexisting proteins (cf. Aplysi Hermissenda, Drosophila models). One of the main common pathways is th cAMP-cascade. In essence, the cAMP signal has to be prolonged, beyond the decay the messenger, probably by the concerted activation of adenylate cyclase throug receptor and Ca/CaM, and/or by the limited proteolysis of protein kinase A by the C activated neutral protease, μ-calpain in Drosophila. Short-term memory in Drosophi was also affected by the genetic elimination of a protein phosphatase type 1 catalyt subunit, in accord with the notion that learning/memory formation requires a delica balance of the opposing reactions of phosphorylation and dephosphorylation.

Long-term memory requires *de novo* protein synthesis (gene induction probably for the restructuring (enlargment) of synaptic contacts. In the transformatio also observed morphologically, the cytoskeleton is bound to be involved, in particul in the PSD, which is a network of cytoskeletal proteins harboring receptors and vario enzymes (protein kinases, phosphatases, etc.) According to current molecular models LTP, postsynaptic Ca induces the process, and maintenance may reside self-perpetuating autophosphorylation of Ca/CaM kinase II. This enzyme, along wi PKA, phosphorylates Glu (AMPA) receptor channels, which enhances conductivit thereby increasing synaptic strength. PKC seems also involved in the complex set reactions in LTP. Retrograde messengers, AA, NO, CO may account for the observe presynaptic component of LTP. An intriguing finding is that during LTP (in rat bra slices) the protein crosslinker enzyme, transglutaminase, becomes very active. If th turns out not to be an indicator of cell damage in the slice, it may contribute to t stabilization of synaptic protein assemblies by covalent side-chain crosslinking. Ge induction in long-term memory processes - from Aplysia through Drosophila mammals - seems to share the CREB system, indicating that cAMP generated at t periphery, or the catalytic subunit of PKA, does reach the nucleus.

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CONNECTIONS OF BLUE CONES IN THE *XENOPUS* RETINA: IMPLICATIONS FOR PROCESSING COLOR

Gábriel, R.[1] and Witkovsky, P.[2]

[1]Department of Zoology, Janus Pannonius University, H-7601 Pécs, Hungary and
[2]Departments of Physiology and Ophtalmology, New York University, New York, 10016, NY, USA

The so called chromaticity horizontal cells (C-HCs) of the *Xenopus* retina respond differentially to short and long wavelength light stimuli. They hyperpolarise by blue and green light flashes whilst give a similar amplitude depolarizing response to red light. Stell et al. (1) proposed that the depolarizing responses of C-HCs to red light depended on a feedback signal from luminosity horizontal cells (L-HCs) to short wavelength-sensitive cones in the retinas of lower vertebrates. We tested this hypothesis using electrophysiological methods combined with intracellular filling, electron microscopy and lectin cytochemistry. Since peanut agglutinin (PNA) labels red, but not blue-sensitive cones in the Xenopus retina (2) it became possible to trace the connections of PNA-labelled (=red-sensitive) and unlabelled (=blue-sensitive) cones to intracellularly filled C-HCs and L-HCs. We found that L-HCs received inputs at ribbon synapses from both spectral classes of cones whereas PNA-labelled (=red sensitive) cone bases were avoided by processes of intracellularly filled C-HCs. In turn, the blue-sensitive cones always contacted the intracellularly filled C-HC processes at ribbon synapses. Simultaneous recordings from C-HC/L-HC pairs established that when L-HCs were saturated by a steady bright red light, C-HCs responded alone to superimposed blue stimulus. Furthermore, C-HC responses to red flashes were delayed by about 30 msec with respect to that of L-HCs. On isolated patch-clamped L-HCs both gamma-aminobutyric acid and glycine opened cloride channels, whilst C-HCs were unresponsive for both amino acids. Since the C-HC does not respond to gamma-aminobutyric acid, the transmitter of L-HCs (3), the feedback synapse from L-HC to blue cones is the most plausible mechanism for creation of depolarizing responses in C-HCs.

1 Stell, W.K. Lightfoot, D.O., Wheeler, T.G. and Leeper, H.F. (1975) Science 190, 989-990
2 Zhang, J., Kleinschmiedt, J., Sun, P. and Witkovsky, P. (1994) Visual Neurosci. 11, 1185-1192
3 Cuningham, J., Neal, M.J., Stone, S. and Witkovsky, P. (1988) Neurosci. 24, 39-48

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THE EFFECT OF CORTICOSTERONE AND RU 28362 ON IMMORTALISED NEURONAL AND PRECURSOR CELL LINES

Gallyas, F.Jr.[1] and Tabira T.[2]

[1]Institute of Biochemistry. University Medical School of Pécs, H-7643 Pécs, Hungary and [2]Division of Demyelinating Diseases and Ageing, National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan

Glucocorticoids were reported to affect neurones of the central nervous system by a complex way. Adrenalectomy, i.e. deprivation of corticosterone (Cort), causes selective loss of granule cells of the dentate gyrus[1], while stress, i.e. elevation of circulating Cort, results in the death of pyramidal cells of the CA3 and CA4 region[2] in the hippocampus of rats.

Because of the too complex nature of the animal model and the heterogeneity of primary cultures, in an effort to evaluate the primary effect of Cort on neuronal cells, we studied the changes in survival and morphology on a panel of immortalised neuronal and precursor cell lines during Cort and RU 28362 (RU) treatment.

Our results showed that basically, Cort and RU did not differ in their action on any of the cell lines, raising the possibility that Cort exerted its effect via glucocorticoid II receptor-mediated mechanisms.

In case of the precursor cell lines, the treatment caused a 50-70% decrease in the cell number, almost regardless to the initial cell number and the presence or absence of serum. This decrease of viability was due to decrease of proliferation rate rather than to cell death, because there was no debris or dead cell at the end of the treatment, and the cell number increased during the treatment. Morphologically the cells became completely different from the control. The cell body was enlarged, flat, and was surrounded by a web-like "skirt" of multiple processes.

Cort and RU caused hardly any effect on either the viability or the morphology of the hybrid neuronal cell lines either in serum-containing or in serum-free medium. Further experiments are necessary to find the cause of this characteristic difference between precursor and hybrid neuronal cell lines.

References:
1/ McEwen et al. (1990) Biochemical Pharmacology 40 2393-2402
2/ Mizoguchi et al. (1992) Neuroscience Letters 138 157-160

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MORPHOLOGICAL CORRELATES OF METABOTROPIC GLUTAMATE RECEPTOR MEDIATED NEUROTRANSMISSION IN THE CENTRAL NERVOUS SYSTEM

Görcs, T. J.[1], Vidnyánszky, Z.[1], Négyessy, L.[1], Knöpfel, T.[2], Kuhn, R.[2] and Hámori, J.[1]

[1] Laboratory of Neurobiology, 1st Department of Anatomy, Semmelweis University Medical School, H-1094 Budapest, Hungary and

[2] Laboratory of Molecular and Cellular Biology, CNS Research, Ciba-Geigy, CH-4002 Basel, Switzerland

L-Glutamate is the major excitatory neurotransmitter in the central nervous system. Electrophysiological, pharmacological and molecular studies have demonstrated that glutamate acts on two major classes of receptors, ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs).

mGluRs couple to G-proteins and modulate the concentration of intracellular messengers. In recent years at least 7 members and several alternative spliced variants of the mGluR family have been cloned. Although no specific agonists or antagonists for the individual mGluRs have been developed, based on their degree of homology and pharmacology the cloned mGluRs can be classified into the following groups: Group 1: mGlur1a-c and mGluR5a-b. These mGluRs activate phosphoinositide hydrolysis and thus mobilize intracellular calcium. Group 2: mGluR2 and mGluR3. These mGluRs activate an inhibitory G-protein and inhibit adenylate cyclase activity. Members of Group 3 include mGluR4, mGluR6 and mGluR7, all of which are negatively coupled to adenylate cyclase. Members within each group have about 70% homology with each other, while the homology between groups is only 40%.

In order to analyse the ultrastructural distributional patterns of metabotropic glutamate receptors polyclonal antibodies were raised against C-terminal peptides of the different mGluRs. The specifity of the affinity purified antibodies were verified with immunoblotting and immunohistochemistry. Results achieved with mGluR1a and mGluR5a will be discussed in the present study. At the light microscopic level both mGluRs have a specific, characteristic distribution in the central nervous system. With immunoperoxidase method, both mGluRs are expressed always postsynaptically in association with dendritic membranes, and diffusely in the cytoplasm. When studied with immunometal method, although the localization of immunoreactivity remains postsynaptic, both mGluR immunoreactivities are always membrane associated either perisynaptically or extrasynaptically. The physiological consequences of this type of localization pattern of mGluR1 and mGluR5 remains to be elucidated.

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INTRODUCTION TO MOLECULAR NEUROGENETICS

Gulya, K.

Department of Zoology and Cell Biology, Attila József University, Szeged, Hungary

Genetic intervention is a novel and powerful means for answering basic questions about molecular events taking place in neurons, and it is also the focus of research directed towards gene therapy in several neurological diseases. Research into the molecular genetics of inherited retinal disorders, and particularly retinitis pigmentosa (RP) is in progress in our laboratory in cooperation with The Ocular Genetics Unit, Biotechnology Institute, Trinity College, Ireland, and is supported by the European Union's Copernicus Program. RP is a progressive disease involving the destruction of photoreceptor cells. As a result, night blindness develops and this is usually followed by an impairment of daytime vision. Mutations responsible for PR have now been found in two genes encoding transmembrane proteins of the rod photoreceptor outer segment disc, and a number of additional causative genes have been localized. It is likely that characterization of the majority of such genes will lead to a substantial elucidation of the molecular pathology of this hereditary disease of the neural retina.

As collaborators, we are investigating optimal systems for the delivery of gene or drug therapies into retinal tissues using replication-deficient adenovirus and adeno-associated virus. Surgical procedures enabling the introduction of sub-microliter volumes of fluid into mouse vitreous humor have now been developed. Both naturally occurring and targeted animal models are used to investigate possible methods of therapeutic intervention to alleviate or retard disease symptoms, our ultimate aim being the development of treatments for human retinal diseases. The suitability of alternative methods of delivery to the retina, such as those based on retroviral or herpes virus vectors, are under investigation.

Akadémiai Kiadó, Budapest

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FUNCTIONAL ANATOMY OF VISUAL PROCESSES IN MAN

Gulyás, B.

Division of Human Brain Research, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden

Advanced functional neuroimaging techniques in combination with computerised brain atlas systems provide us with powerful tools which help us explore the functional organisation of visual cortex in man. During the past years, in a series of brain activation experiments using various visual stimuli we have determined and localised cortical fields participating in (i) the detection of elementary visual signals, e.g. changes in luminance of colour; (ii) the processing and analysis of basic visual sub-modalities and cues, such as colour, form, motion, disparity, orientation, spatial frequency, (iii) the generation of form percepts on the basis of different visual input cues, including luminance, colour, motion, disparity, and texture, (iv) the learning and recognition of complex visual patterns, (v) the recall of visual information from visual memories (for details, see, e.g., refs. 1-8).

The experiments were performed on healthy male volunteers. In each experimental series, at least 10 subjects participated. The subjects underwent complementary scannings of their brains with a high resolution MR camera and a PET camera, using identical head fixation in both scanners (the Greitz-Bergström fixation system, refs. 9-10). In the PET camera, each subject performed four visual tasks, including experimental tasks and a reference task, while their regional cerebral blood flow (rCBF) was measured with the dynamic method of Koeppe (11), using ^{15}O-butanol as tracer. The resulting rCBF images were standardised in both size and shape using the computerised brain atlas system of the Karolinska Institute (12). 'Experimental task – reference task' subtraction images were created, which were then averaged across the subject population. Statistically significant changes in rCBF were determined (13), and cortical regions showing significant changes were localised in the brain using the standard Talairach stereotactic convention (14).

The experiments indicate that various functional networks of cortical fields participate in the processing and analysis of visual information, depending upon the input cues and task criteria. The very same field may participate in the processing and analysis of various visual sub-modalities (convergence), whereas the same visual sub- modality can be processed and analysed by a number of cortical fields (divergence). Visual detection experiments may mark the macular representations of striate cortex (V1) and at least six extrastriate visual areas in the close neighbourhood of V1 (see Figure 1), however, further anatomical studies are required to confirm the existence of these disparate visual areas. Our studies indicate, furthermore, that the visual areas in human visual cortex may be classified into two categories: (i) computational areas (area V1 and extrastriate areas in its close vicinity), which participate in the elaboration of elementary visual signals, and (ii) representational areas (areas in the occipito-parietal and occipito-temporal regions, located further away from the striate cortex), which may participate in modality-invariant (i.e. shape-, size-, colour-, etc. invariant) mental operations of visual percepts as well as in their storage in, and recall from, memory. The tentative borders of human visual cortex, together with the tentative border between computational and representation visual areas are displayed in Figure 2.

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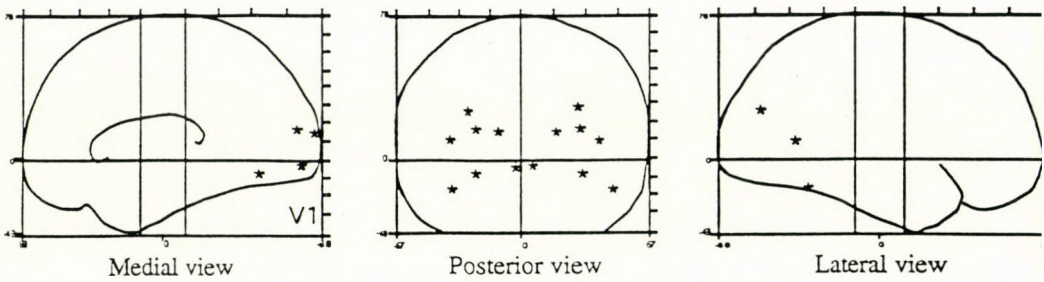

Figure 1: Proposed loci of the macular representations of striate cortex (V1) and extrastriate visual cortical areas, expressed in standard Talairach stereotactic coordinates (14).

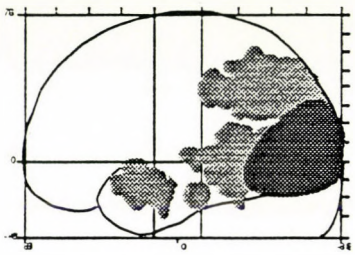

Figure 2: Tentative borders of human visual cortex. Areas, proposed to be computational, are in dark grey, areas, proposed to be representational, are in light grey.

References:

1. Gulyás, B. and Roland, P. E. Neuroreport. 2 (1991): 585-588
2. Roland, P. E., Gulyás, B. and Seitz, R. In: Squire, L. (ed.) Memory: Organization and Locus of Change. Oxford: University Press, 1991. pp. 95-113
3. Gulyás, B., Ottoson, D. and Roland, P. E. (eds.) Functional Organization of the Human Visual Cortex. Oxford: Pergamon Press, 1993. p. 391
4. Gulyás, B. and Roland, P. E. Natl. Acad. Sci. US, 91 (1994): 1239-1243
5. Roland, P. E. and Gulyás, B. Trends Neurosci. 17 (1994): 281-287
6. Gulyás, B. and Roland, P. E. Eur. J. Neurosci. 6 (1994): 1811-1827
7. Gulyás, B. et al. Proc. Natl. Acad. Sci. US, 91 (1994): 9965-9969
8. Gulyás, B. et al. Neuroreport 5 (1994): 2367-2371
9. Greitz, T. et al. Neuroradiology 19 (1980): 1-6
10. Bergström, M. et al. J. Comput. Assist. Tomogr. 5 (1981): 136-141
11. Koeppe, R. A. et al. J. Cereb. Blood Flow Metab. 5 (1985): 224-234
12. Roland, P. E. et al. Human Brain Mapping 1 (1994): 173-184
13. Roland, P. E. et al. Human Brain Mapping 1 (1993): 3-19
14. Talairach, J. et al. Atlas d'Anatomie Stéréotaxique du Télencéphale. Paris, Masson and Cie., 1967. p. 326

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EFFECT OF (-)DEPRENYL ON PEA-INDUCED ACTIVITY IN RATS

Gyarmati, Zs., Tímár, J., Barna, L. and Knoll, J.

Department of Pharmacology, Semmelweis University of Medicine, P.O.B. 370, H-1445 Budapest, Hungary

(-)Deprènyl, a selective inhibitor of B-type monoamine oxidase (MAO-B) was published to potentiate the effects of phenyl-ethyl-amine (PEA), the preferred substrate of this enzyme. However, while the stereotypy-inducing effect of PEA, a behaviour related to the stimulation of the nigrostriatal dopamine (DA)-ergic system is potentiated by (-)deprenyl pretreatement, its effect on locomotion, a behaviour related to the stimulation of the mesolimbic DA-ergic pathway, failed to change following the same pretreatment (Timár et al, 1993a).

As (-)deprenyl is belonging to the family of amphetamines, the question of its possible abuse potential has often been discussed. Recently (-)deprenyl was published not to possess any reinforcing property in drug-induced place preference conditioning (PPC) test, even applying extremly high doses (Timár et al, 1993b). The reward-motivated PPC is a type of behaviour. also believed to control (at least partly) by the mesolimbic DA-ergic system. The aim of present experiments was to investigate, whether the PEA-induced PPC can be influenced by (-)deprenyl pretreatment. The results show, that (-)deprenyl pretreatment, using either its selective MAO-B inhibitory dose (0,25mg/kg sc), or a higher one (2 mg/kg sc) did not potentiate the reinforcing capacity of PEA, providing further proof about the selective nigrostriatal action of (-)deprenyl.

References

Timár, J., Gyarmati, Zs., Tekes K., Hársing G.L. and Knoll, J. (1993a) Further proof that (-)deprenyl fails to facilitate mesolimbic dopaminergic activity. Pharmacol. Biochem. Behav. 46, 709-714.

Timár, J., Gyarmati, Zs., Knoll B., Barna, L. and Knoll, J. (1993b) (-)Deprenyl - lack of inducing place preference conditioning in rats. Eur. Neuropsychopharmacol. 3, 412.

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EFFECT OF BUSPIRONE ON BEHAVIOURAL ALTERATIONS INDUCED BY BILATERAL CAROTID OCCLUSION

Gyertyán, I., Simó, A., Bilkei-Gorzó, A., Gacsályi I. and Szemerédi, K.

Department of Pharmacology, EGIS Pharmaceuticals Ltd., P.O.Box 100, H-1475 Budapest, Hungary

5-HT_{1A} agonists were found to be neuroprotective in global and focal ischemia models (1,2). Buspirone was shown to be effective in focal ischemia (2), but it was not tested in global ischemia. One of the most widely used model of global ischemia, the transient bilateral carotid artery occlusion (BCAO) in the Mongolian gerbil, induce a hyperactive state which has been claimed as a functional correlate of hippocampal CA1 damage (3). However, studies investigating the effect of BCAO on memory and learning functions in the gerbil are lacking. The aim of our study was to examine the effect of buspirone in the BCAO model in gerbils so on hippocampal cell loss as on two behavioural responses, hypermotility and spontaneous alternation. The latter was chosen as a measure of spatial (working) memory.

Male Mongolian gerbils received vehicle or buspirone 30 mg/kg ip. 30 min before bilateral common carotid artery occlusion of 5 min. 4-5 days after the operation, gerbils were sacrified and neuronal damage in the hippocampal CA1 region was visualized by silver impregnation. In experiment 1 three days after surgery motor activity of the animals were detected for 60 minutes. In experiment 2 five days after surgery spontaneous alternation response of gerbils together with total arm entries was measured in a Y-maze for 5 min.

In experiment 1 BCAO induced significant hypermotility which was diminished by buspirone. The results of experiment 2 are shown in the Table.

Table. Effect of buspirone (30 mg/kg ip.) on Y-maze behaviour and hippocampal CA1 pyramidal cell loss induced by 5 min bilateral carotid occlusion.

	Spontaneous alternation (%)	Total arm entries	Ischemic damage score
Sham operated	60.33	40.25	–
	±3.141	±2.520	
BCAO	51.66[a]	46.63	2.75
	±1.703	±5.555	±0.164
BCAO+Buspiron	56.75	59.50[b]	1.87[c]
	±3.635	±3.268	±0.226

a: $p<0.05$, b: $p<0.001$ cf.with sham operated group, c: $p<0.05$ cf. with BCAO group

In Experiment 2 five min BCAO was not able to induce hypermotility, despite the fact that the same treatment did cause cell loss in the hippocampal CA1 region and decreased the rate of spontaneous alternation. Buspirone exerted significant neuroprotective effect against hippocampal ischemic damage, and also attenuated - though not significantly - the spontaneous alternation deficit. However, the motility of buspirone treated gerbils were significantly higher than that of the vehicle treated group. We conclude that spontaneous alternation may be a more reliable and valid behavioural correlate of hippocampal function than hypermotility which may rather reflect the state of other region(s) of the brain, which may be less sensitive to short ischemic interventions than the hippocampus.

1. Bode-Greuel KM, Klisch J, Glaser T, Traber J (1990) In: Krieglstein J, Oberpichler H (eds) Pharmacology of cerebral ischemia. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1990, pp. 485-491.
2. Bielenberg GW, Burkhardt M (1990) Stroke 21(Suppl. IV): 161-163.
3. Judge ME, Sheardown MJ, Jacobsen P, Honoré T (1991) Neurosci Lett 133:291-294.

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QUANTITATIVE ANALYSIS OF AFFECTS ORIGINATING FROM THE INTESTINAL SYSTEM

Gyetvai, B. and Bárdos, Gy.

Department of Comparative Physiology, Eötvös L. University
and
Psychophysiology Research Group, Hungarian Academy of Sciences,
Múzeum krt. 4/A, H-1088, Budapest, Hungary

The aim of the study was the quantitative analysis of the affects accompanying visceral afferent signals originating from the intestinal system. Both aversive and ingestive responses were in the focus of interest.

10 male Long-Evans rats were tested. The animals underwent two consecutive surgeries prior to testing. First, a traditional Thiry-Vella small intestinal fistula was prepared, and second, an intraoral cannula was implanted. Recovery required not more than two weeks. In most of the sessions mechanical intestinal distention was provided by a balloon inserted into the fistula and filled with either water or with air. Affective consequences of the stimulation were followed by the taste-reactivity (TR) test. 0.0004 M saccharin solution was infused into the mouth through the cannula for 30 seconds; 10 such stimuli separated by 5 minutes rests were given in each session. The reactions were video-taped and later analyzed frame-by-frame for both the ingestive and aversive behavioral elements, respectively. Volumes of 0.05, 0.09, 0.12 and 0.28 water were injected into the balloon, while the air was pumped in until the resistance of the rubber was just overridden. The balloon was inflated between the 2. and 3. trial and deflated between the 7. and 8. trial in each session, except the control experiments were no stimulation was applied at all.

The responses in both the aversive and ingestive dimensions showed a characteristic pattern; the amount of the given responses varied as the trials proceeded. Reactions followed the on- and offset of the stimulation with a significant lag the length of which depending on the stimulus intensity. The ratio of the aversive responses increased as stimulus intensity had been raised; we also recorded differences between the water and air distended balloon conditions.

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EFFECT OF PVN NOREPINEPHRINE MICROINJECTIONS ON DOPAMINE AND ACETYLCHOLINE IN THE NUCLEUS ACCUMBENS: MICRODIALYSIS IN FREELY MOVING RATS

Hajnal, A.[1], Hoebel, B. G.[2] and Lénárd, L.[1]

[1]Institute of Physiology, Pécs University, Medical School, Pécs, Hungary
[2]Department of Psychology, Princeton University, Princeton NJ, U.S.A.

There is only few data in the literature concerning the neurochemical basis of the behavioral aspects of hypothalamic hyperphagia and obesity models. Therefore norepinephrine (NE) was microinjected into the hypothalamic paraventricular nucleus (PVN) and chronic microdialysis was used to monitor extracellular dopamine (DA) and acetylcholine (ACh) in the nucleus accumbens (NAc). The PVN is a site where exogenously administered NE can act through alpha-2 receptors to elicit eating behavior and preference for carbohydrates. It was hypothesized that NE in the PVN acts on a behavior reinforcement system by altering DA/ACh balance in the NAc. NE microinjections (80 nmol in 0.3 μl), which effectively elicited feeding in satiated rats in separate test, caused a significant increase in extracellular DA (209 %) and decrease in ACh (73 %) when the same animals were tested in the absence of food. In contrast, when the food was available and ingested, ACh increased (151 %) instead of decreasing. These results suggest a functional link between the PVN and the NAc involved in the reinforcement of eating in which DA initiates and ACh stops appetitive behavior.

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SYNAPTIC CONNECTIONS AND NEUROCHEMICAL CHARACTERISTICS OF VIP-IMMUNOREACTIVE INTERNEURONS IN THE DENTATE GYRUS

Hájos, N., Acsády, L., Arabadzisz, D., Katona, I. and Freund, T.F.

Institute of Experimental Medicine, Hungarian Academy of Science, Budapest, PO Box 67, H-1450 Hungary

Vasoactive intestinal polypeptide (VIP) was shown earlier to be present in a morphologically heterogeneous subpopulation of GABAergic interneurons in the dentate gyrus, but their input and output characteristics have not been established. Using a new, specific antiserum, VIP-immunoreactive (VIP-IR) cells and axons were visualized in a Golgi-like manner. Three types of VIP-IR cells have been identified on the basis of axonal and dendritic arbor, postsynaptic targets, and colocalization with different neurochemical markers (calcium-binding proteins and cholecystokinin): 1) The axon of the first type projected to the hilus, where it formed a dense plexus of axon terminals restricted to the hilus. The postsynaptic targets of these VIP-positive cells were neurons visualised by immunostaining for substance P receptor (SPR), which is know to label different hilar interneurons. In all cells of this type VIP and calretinin (CR) were shown to coexit, while they proved to be negative for cholecystokinin (CCK). 2) VIP-IR basket cells, innervating predominantly the somata and proximal dendrites of granule cells, were found in str. granulosum and str. moleculare. In this cell type VIP colocalized with CCK, but not with CR. 3) In str. moleculare horizontal VIP-IR cells were found with dendrites and axons restricted to this layer. They established multiple contacts with non-principal cells containing VIP or substance P receptor. In 75% of these cells VIP coexisted with CR, but not with CCK.

On the basis of these observations we conclude that three different types of VIP-IR neurons are present in the dentate gyrus, and are likely to subserve different inhibitory functions, i.e.: 1) synchronization of the activity of hilar neurons or disinhibition of granule cells by specific interneuron-to-interneuron connections, 2) perisomatic inhibition of granule cells, 3) synchronization of interneurons in the str. moleculare.

Akadémiai Kiadó, Budapest

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PHYSIOLOGICALLY IDENTIFIED GABAERGIC INTERNEURONS IN AREA CA1 OF THE RAT HIPPOCAMPUS

Halasy, K.[1], Buhl, E.H.[2], Lőrinczi, Z.[3], Tamás, G.[1] and Somogyi, P.[2]

[1] Department of Zoology and Cell Biology, József Attila University, Szeged, Hungary
[2]MRC Anatomical Neuropharmacology Unit, Oxford, United Kingdom
[3]Department of Anatomy, University of Medicine and Pharmacy, Tirgu Mures, Romania

Previous studies on the rat dentate gyrus showed that distinct types of GABAergic interneurons terminate on mutually exclusive membrane domains of the postsynaptic principal cells, often associated with specific excitatory inputs. In order to determine that similar "division of labour" exists in the CA1 area, stratum pyramidale interneurons were physiologically identified and injected intracellularly with biocytin in the rat hippocampal slice preparation. The morphological features and synaptic target specificity of the filled interneurons were analysed in light and electron microscopes.

Altogether 18 fast spiking cells were identified. Depending on their dendritic and axonal distribution they were classified either as basket, or bistratified cells. Both cell types established type 2 symmetrical synapses on their postsynaptic targets. Basket cells (n=12) had a very dense axonal arbor in stratum pyramidale, and their dendrites covered each layer of area CA1. Their boutons made synaptic contacts on pyramidal somata (53%), proximal dendrites (45%) and occasionally on axon initial segments (2 %). Bistratified cells (n=6) had an axonal arbor arranged in two layers covering strata radiatum and oriens. Their dendrites avoided stratum lacunosum-moleculare. The synaptic targets of bistratified cells were predominantly dendritic shafts (79%) followed by dendritic spines (12 %) and pyramidal somata (7%).

In conclusion, the 18 neurons studied exhibit differences in the spatial distribution of their dendrites (input) and axonal target preference (output). Thus, they form two distinct subtypes of hippocampal inhibitory GABAergic interneurons in the CA1 area.

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THE β-AMYLOID$_{(1-42)}$ PEPTIDE PRODUCES SPECIFIC CHOLINOTOXICITY IN RAT BRAIN

Harkány, T., Lengyel, Z., De Jong, G. I.[1], Luiten, P. G. M.[1], Soós, K.[2], Penke, B.[2] and Gulya, K.

Department of Zoology and Cell Biology, Attila József University, H-6722 Szeged, Hungary, [1]Department of Animal Physiology, University of Groningen, 9750 AA Groningen, The Netherlands, and [2]Department of Medicinal Chemistry, Albert Szent-Györgyi Medical University, H-6720 Szeged, Hungary

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that results in a severe loss of cognitive, learning and memory functions in the elderly. In correlation with the progression of AD, extensive lesions of the basal ganglia, accompanied by pathological alterations of its transmitter systems, can be observed. One of the characteristic neuropathological features of AD is the accumulation and deposition of the β-amyloid protein in neuritic plaques and cerebrovascular deposits. In the present study we examined the neurotoxic effects of the β-amyloid$_{(1-42)}$ peptide (βAP) in an *in vivo* animal model.

βAP was injected into either the nucleus basalis magnocellularis (*nbm*) or the medial septum (*ms*) of rats. Injection into the *nbm* was followed by biochemical and receptor binding experiments after a 14-day survival time. The acetylcholine esterase (AChE, EC 3.1.1.7) and choline acetyltransferase (ChAT, EC 2.3.1.6) activities were significantly reduced in the ipsilateral frontal cortices. Muscarinic receptor (mAChR) binding studies revealed a significant decrease in the number of frontal cortical M_2AChR. Confirmation of the data with silver staining also revealed degeneration of projective fibres of the *nbm* to the frontal cortex.

After injections of the peptide to the *ms* (using the same survival time), we determined the loss of cholinergic and parvalbumin-containing neurons. Degenerating ChAT-immunoreactive neurons were identified as targets of βAP neurotoxicity, while the number of parvalbumin-containing neurons did not change significantly.

Our biochemical and histochemical results indicate that βAP exhibits specific neurotoxic effects on the central cholinergic system of rats. Our *in vivo* model system may provide a useful paradigm for study of the effects of βAP that may play an important role in the development of AD.

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REPEATED ETHANOL ADMINISTRATION OR DEHYDRATION DIFFERENTIALLY ALTERS THE LEVELS OF PARVALBUMIN AND CALBINDIN-D28K IN MOUSE CORTEX AND HIPPOCAMPUS

Harkány, T., Luiten, P.G.M.[1] and Gulya, K.

Department of Zoology and Cell Biology, Attila József University, H-6720 Szeged, Hungary and [1] Department of Animal Physiology, University of Groningen, 9750 AA Groningen, The Netherlands

One of the effects of ethanol on the physiological neuronal function is to disrupt intracellular Ca^{2+} homeostasis. Dehydration also alters the neuronal homeostasis by concentrating the ionic milieu. Calcium-binding proteins mediate or regulate the effects of Ca^{2+} ions on the intracellular metabolism. Members of this protein family, parvalbumin (PV) and calbindin (CB-D28K), are known to bind free cytosolic Ca^{2+} preferentially. In the present report, alterations in the number of neurons that express these calcium-binding proteins were studied after repeated ethanol administration or dehydration.

One group of male CFLP mice was injected intraperitoneally twice daily with 7% freshly diluted ethanol (4.1 g/kg b.w.) for 7 days. Control animals received physiological saline. Another group of animals was dehydrated for 4 days, while matching controls were kept under normal conditions. After treatment, mice were transcardially perfused, their brains were sectioned, and PV and CB-D28K immunocytochemistry was performed. The number of PV or CB-D28K-immunoreactive (ir) interneurons was quantified in the frontal and parietal cortices and hippocampus of treated and control animals.

Repeated ethanol administration significantly decreased the number of PV-ir interneurons in the frontal and parietal cortices and hippocampus. In contrast, the same treatment had reverse effects on CB-D28K-ir interneurons. The number of CB-D28K-ir neurons was increased significantly in all brain areas examined. The 4-day dehydration elevated the number of neurons that exhibited PV- or CB-D28K-ir in frontal or parietal cortical and hippocampal sections.

PV and CB-D28K are distributed in distinct neuron populations in the brain. PV and CB-D28K bind Ca^{2+} with different capacities and this may influence the tolerance of neurons to elevated Ca^{2+} levels. Only one genomic locus for both CB-D28K and PV exists on distinct chromosomes in mice. It is possible that still unidentified activating factors, Ca^{2+} or ethanol itself may alter the expression of these calcium-binding proteins.

Akadémiai Kiadó, Budapest

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ANTICONVULSIVE EFFECT OF URETHANE ON AMINOPYRIDINE-INDUCED EPILEPTIFORM ACTIVITY

Heltovics, G. , Boda, B. and Szente, M.

Department of Comparative Physiology, Attila József University, H-6726 Szeged, Hungary

The effects of urethane anaesthesia on the development and spread of ictal-like epileptiform activity induced by 3-aminopyridine, was investigated on the somatosensory cortex of rats. Under urethane anaesthesia in most animals only one abortive ictal period appeared with dose-dependent latency. Urethane completely abolished afterdischarges of high frequencies (5-10 Hz) and prevented the development of recurrent seizures in both primary and mirror foci. Urethane also eliminated ongoing epileptiform activity induced previously by 3-Ap under nembutal anaesthesia.

The antiepileptic effects of urethane on ictal-like activity may be the consequence of a presynaptic action through reduction of glutamate-mediated excitation together with a depressant effect on responsiveness of cortical neurones. The afterdischarges of high frequency could be essential to development and stabilisation of repetitive tonic events in the neocortex. These results suggest that urethane may not be an appropriate anaesthetic for the study of ictal like epileptiform phenomena in the neocortex.

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PREFRONTALLY ADMINISTERED NEUROTOXINS ALTER FEEDING BEHAVIOR IN THE RAT

Hernádi, I.[1], Karádi, Z.[2] Faludi, B.[1,2], Vígh, J.[1], Gálosi, R.[1], Fogarasy, A.[2] and Lénárd, L.[1,2]

[1]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University and [2]Institute of Physiology, Pécs University, Medical School, Pécs, H-7643 Pécs, Hungary

It is confirmed that the hypothalamus, amygdala and globus pallidus are essential in neural mechanisms controlling body weight and food and fluid intake behaviors. The prefrontal cortex (PFC) has been reported to have mutual interconnections with these forebrain structures mentioned above. Despite the abundance of literature data on PFC functions, its specific roles on regulation of feeding are poorly understood. In the present report, therefore, we aimed to provide a complex behavioral characterization of PFC lesioned animals.

Two types of neurotoxic lesions were carried out by means of glass micropipettes (tip diameter 15-25 μm). Kainic acid (KA) was microiontophoretically applied in the medio-dorsal division of PFC to damage intrinsic prefrontal neurons, whereas 6-hydroxydopamine (6-OHDA) was microiontophoretized in the same region to destroy passing cathecolaminergic (CA) projection fibers. Body weights, food and fluid intakes of both lesioned and (sham-operated or intact) control animals were daily measured. Effects of intracellular dehydration (NaCl, 1 M) and water deprivation (24h and 36h schedules) were also studied. Open field (OF) activity and scores of orientation towards visual and somesthetic stimuli were pre- and postoperatively tested and registered. To test possible changes in central taste information processing, the acquisition and retention of saccharine conditioned taste aversion (CTA) was examined in all animals.

There were no major changes recorded in body weights and food and water consumptions in either lesioned or control groups. Water consumption after dehydration treatments or deprivation schedules was similarly increased in all animals. OF activity scores of the 6-OHDA group were significantly higher than those of the other groups. Both the KA and the 6-OHDA lesioned rats displayed serious deficits in the CTA acquisition and retention tests compared to either intact or sham-operated control animals.

Although microlesions of the medio-dorsal part of PFC does not cause severe deficits in body weight control and food and water intake mechanisms, the present results suggest that the PFC is of great importance in memory storage and retrieval of CTA information. Appropriate functioning of both intrinsic neurons and passing CA fibers of the PFC appear to be necessary to avoid possibly poisonous nutrients. Furthermore, results of OF examinations and neurological tests indicate a general significance of prefrontal CA mechanisms in goal-directed, adaptive behavior of the animals.

Akadémiai Kiadó, Budapest

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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF THE EFFERENT PATHWAYS TAKING PART IN THE FEEDING BEHAVIOR OF HELIX POMATIA

Hernádi, L. and Elekes, K.

Balaton Limnological Research Institute of the Hungarian Academy of Sciences, H-8237 Tihany, Hungary

Both the lips and the posterior tentacles take part in the appetitive and consumatory phases of feeding behavior. The lips and tentacles show different movements during the two phases of feeding. The tentacles are able to make complex pattern of movement in space, such as protrusion, retraction, bending, whereas the lips are able only for local contraction and a slight relaxation.

In this study, we determined the localization of efferent cell bodies innervating the lips and tentacles in the cerebral ganglia as well as of the peripheral neurons innervating muscle and gland cells in the organs by retrograde Co^{2+} and Ni^{2+} labelling. The lips are innervated by more cerebral foci than the tentacles. Additionally, the muscle and gland cells of the lips are innervated by peripheral neurons that send processes also to the cerebral ganglia.

The comparison of the distribution of 5-HT-, DA-, CARP-, FMRFa-, and Leucokinin-immunoreactive cell bodies to the location of the efferent foci in the cerebral ganglia shows that the different foci have different transmitter dominancy. These differences between the foci suggest different functions of the foci.

In the tentacles and lips, 5-HT-, DA-, FMRFamide-, CARP-, and Leucokinin-immunoreactive fibers, but no cell bodies can be observed, demonstrating that these fibers originate from the cerebral efferent foci.

In the lips and tentacles, different types of neuro-muscular and neuro-glandular connections can be distinguished on the basis of their ultrastructure. However, the dominant types of junctions are different in the two peripheral regions.

Using immunogold electron microscopic immunocytochemistry, FMRFamide-immunoreactivity can be observed in axon varicosities containing different types of granules. It suggests a coexistence of FMRFamide with other transmitters in axon profiles forming neuro-muscular and neuro-glandular connections in the lips and tentacles.

It is assumed that the innervation of muscle and gland cells by multiple cerebral efferent foci makes possible to carry out a complex behaviour pattern of both the tentacles and the lips during feeding.

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CHARACTERIZATION OF OCTOPAMINE RECEPTOR IN OPTIC LOBES OF LOCUSTA MIGRATORIA

[1]Hiripi, L., [2]Downer, R.G.H.

[1]Balaton Limnological Research Institute of the Hungarian Academy of Sciences, H-8237 Tihany, Hungary and [2]Department of Biology, University of Waterloo, Waterloo, Ont., N2L 3G1, Canada

Presence of octopamine receptors was demonstrated in Drosophila head and locust brain, whereas tyramine receptors was demonstrated in locust brain. For the characterization of these receptors ^{3}H-octopamine and ^{3}H-tyramine were used as ligands. Displacement studies demonstrated that the binding of these ligands could be inhibited by octopamine and tyramine at nM range as well as by yohimbine at μM range.

A Drosophila cDNA for an octopamine- and tyramine-sensitive receptor has been cloned. However, for the characterization of the encoded receptor neither ^{3}H-octopamine nor ^{3}H-tyramine, but ^{3}H-yohimbine was used in the ligand binding experiments. Displacement studies demonstrated that the binding of the ligand could be inhibited by yohimbine at nM range and by octopamine or tyramine by μM range. It was suggested that yohimbine labels both octopamine and tyramine receptors.

In the optic lobes of locust the binding properties of both ^{3}H-octopamine and ^{3}H-yohimbine was investigated in order to characterize the octopamine receptor. Towards the octopamine receptor a 100 times higher affinity of ^{3}H-octopamine could be demonstrated, compared to that of yohimbine. The pharmacological properties of ^{3}H-octopamine and ^{3}H-yohimbine binding suggest that the ^{3}H-yohimbine labeled sites were not identical with the ^{3}H-octopamine binding sites and cannot be used for the characterization of octopamine receptors.

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INTRATHECALLY APPLIED α_2-ADRENOCEPTOR AGONIST (HYDROPHIL ST-91) EFFECTS ON MOTOR FUNCTIONS IN RATS

Horváth, Gy., Szikszay, M., Dobos, I. and Benedek, Gy.

Department of Physiology, Albert Szent-György Medical University, Dóm tér 10. H-6720 Szeged, Hungary

α_2-Adrenoceptor agonists are non-narcotic sedative analgesic drugs with muscle relaxant properties. There is ample evidence that supports the funcional role of the spinal cord in modulation of the antinociceptive and motor effects of α_2-adrenoceptor agonists. The motor impairment may results from a rapid diffusion of highly-lipophilic α_2-adrenoceptor agonists into the general circulation and than into the brain. Because of this diffusion, it is necessary to use a hydrophilic α_2-adrenoceptor agonist (ST-91) given intrathecally (IT) to evaluate whether the motor effects result from actions at spinal vs. supraspinal sites.

Methods: After institutional approval, chronical intrathecal catheters were inserted into male Wistar rats (n=10) at the level of L4/L5. Motor functions were examined by rod suspension, rotorod and inclined screen tests.

1. Rod suspension: The time remaining suspended from the rod until a fall onto was recorded.

2. Rotorod: The depended variable was the number of falls during a cumulative total of 3 min exposure to the rotorod.

3. Inclined screen: Strength and motor coordination were assessed by the ability of the rat to remain on a wire mesh screen inclined 60° to horizontal. Cut off time: 20 min.

Measurements were performed the day before drug administration (baseline values) and 10 min after physiological saline or ST-91 (5 µg, antinociceptive dose) IT application.

Results: ST-91 treatment produced no significant effects on motor performance compared with the physiological saline (control) adminstration. The findings are summarized in Table.

Test	parameter	control baseline	control treatment	ST-91 baseline	ST-91 treatment
Rod suspension	Mean	16.86	14.3	16.9	15.82
(sec)	SEM	6.05	2.66	5.25	3.23
Rotorod	Mean	6.6	0.4	6.4	3.0
(falls number)	SEM	1.63	0.4	1.80	2.51
Inclined screen	Mean	20	20	20	20
(min)	SEM				

Discussion: The failure to observe any effects of ST-91 treatment on motor performance contrasts to findings from previous studies. These data suggest that the depressant effect on motor function of the lipophilic α_2-adrenoceptor agonists may result from activation of supraspinal receptors.

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THE BLOOD-BRAIN BARRIER IN VITRO

Joó, F.

Laboratory of Molecular Neurobiology, Institute of Biophysics,
Biological Research Centre, H-6701 Szeged, Hungary

Ever since the discovery of Paul Ehrlich (1885) about the restricted material exchange, existing between the blood and the brain, the ultimate goal of subsequent studies has been mainly directed towards the elucidation of relative importance of different cellular compartments in the peculiar penetration barrier consisting the structural basis of the blood-brain barrier (BBB).Unlike most organ systems, the central nervous system is separated from the blood by a protective cellular barrier, manifested by the continuous lining of capillary endothelial cells, which prevents the free passage of solutes and blood contituents from the circulation to the brain parenchyma. The brain of essentially all vertebrates is characterized by capillary endothelial cells with unique morphological, physiological and biochemical characteristics. After an era of studying with endogenous or exogenous tracers the unique permeability properties of cerebral endothelial cells in vivo, the next generation, i.e the in vitro blood-brain barrier model system was introduced by us in 1973. Recent advances in our knowledge of the blood-brain barrier have in part been made by studying the properties and function of cerebral endothelial cells (CEC) with this in vitro approach. This review summarizes, in brief, the results obtained on isolated brain microvessels and on cultured cerebral endothelial cells.

Akadémiai Kiadó, Budapest

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DEVELOPMENT OF NEURAL CONNECTION BETWEEN CO-GRAFTED RAT EMBRYONIC MESENCEPHALON AND STRIATUM

Kálmán, M. and Tuba, A.

1st Dept. of Anatomy, Semmelweis University of Medicine, H-1450, Budapest, Hungary

Although the transplantation of fetal mesencephalic tissue can ameliorate experimental parkinsonism, the results have been neither satisfactory nor reproducible enough. Developing transplants need growth factors and synaptic target areas which cannot be adequately provided by the adult recipient tissues. In our previous experiments, when the rat embryonic (E15) mesencephalon was transplanted into the cortex of adult rats, the survival ratio of the transplants increased from 4/45 to 17/44 in the presence of co-grafted embryonic striatum, as a natural target area. In the present study, we demonstrate the formation of neural connections between the co-grafted mesencephalic and striatal tissues with the fluorescent tracer DiI.

Crystals of DiI were implanted into every piece of mesencephalic tissue, before grafting. In one month, the hosts were perfused with paraformaldehyde and the brains were sectioned by vibrating microtome. Having taken photographs under a fluorescent microscope we embedded the materials into epoxy resin and cut them into semithin sections. Some sections were immunostained against tyrosine hydroxylase.

The mesencephalic grafts showed bright fluorescence, with fainter straight strips running to the co-grafted striatal tissues. Some neurons of the mesencephalic transplants proved to be immunopositive against tyrosine hydroxylase.

Our experiments and other data (Constantini et al., 1994, Exp. Neurol., 127:219-31) suggest that the striatal co-graft can promote the survival of mesencephalic transplants. This adjuvant effect may be based on the production of growth factors and, as demonstrated here, on the presence of a synaptic target area.

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EFFECTS OF AMPA ANTAGONISTS ON THE INDUCTION OF HIPPOCAMPAL LONG-TERM POTENTIATION, IN VITRO

Kapus, G.[1], Tarnawa, I.[1], Világi, I.[2], Banczerowski-Pelyhe, I.[2], Ruiz, A.[3], and Durand, J. [3]

[1]Institute for Drug Research, Budapest, Hungary

[2]Department of Comparative Physiology, Eötvös University, Budapest, Hungary

[3]CNRS, Unite de Neurocybernetique Cellulaire, Marseille, France

The effects of GYKI 52466, a non-competitive AMPA receptor antagonist 2,3-benzodiazepine compound and NBQX, a competitive AMPA antagonist quinoxalinedione were studied in vitro on long-term potentiation (LTP) of synaptic responses in the CA_1 region of slices of rat hippocampus. The Schaffer collateral-commissural pathways were stimulated and field potentials were recorded from the pyramidal and radial layers.

GYKI 52466 (10-80μM) depressed dose-dependently the slope of population EPSPs and the amplitude of population spikes. When LTP was induced by high frequency stimulation, in those slices that had been incubated in 20μM GYKI 52466 the increase of the synaptic responses was more pronounced than in control slices. 80μM GYKI 52466, which almost completely depressed the field potentials, also inhibited the induction of LTP.

NBQX (0.25-1μM) also decreased both population EPSPs and spike amplitudes. In contrast to data obtained with GYKI 52466 the potentiation of synaptic responses after high frequency stimulation seemed to be smaller in the presence of NBQX than in control slices.

Our results confirm that activation of AMPA receptors is not absolutely necessary for LTP induction. The unexpected enhancement we found in the presence of GYKI 52466, but not NBQX, needs explanation and can be related to a non-specific depression of voltage-gated K^+ channels by the GYKI compound described recently, or to a possible depressant action on local inhibitory processes in CA_1 region.

Akadémiai Kiadó, Budapest

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ANALYSIS OF TEMPORAL PATTERNS OF DRINKING BEHAVIOR IN WISTAR RATS

Karádi,K., Bende,I.

Institute of Behavioral Sciences,
University Medical School of Pécs, H-7643 Pécs, Hungary

It has been generally described and accepted that during homeostatic drinking, licking rate of rats is gradually decreasing until the point of satiety. Quantitative analyses of temporal patterns left doubts about the nature of generating processes resulting drinking. We hypothesized and demonstrated that licking in bursts compared to non-burst drinking were driven by different temporal pattern generators. Drinking of four water-deprived Wistar rats were observed and monitored during 60-min. sessions for 4 consecutive days. Licking and inter-lick intervals were recorded and subsequently analyzed. After establishing criteria of "intraburst" and "interburst" licking, temporal patterns of interburst and intraburst inter-lick intervals were statistically analyzed

Results revealed that: 1. During sessions, lengths of interburst intervals showed significant increasing trends, while lengths of bursts themselves were gradually decreasing. 2. Within-burst inter-lick intervals showed the same distribution during the whole session independent to the state of satiety. Our data support the hypothesis that during homeostatic drinking there are, at least, two different lick-timing generators contributing to the observable pattern. Moreover, it can be argued that simple homeostatic feed-back models are inadequate explanations of many details of the restorative, normal drinking behavior.

Akadémiai Kiadó, Budapest

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NEUROPHYSIOLOGICAL CORRELATES OF CONDITIONED TASTE AVERSION IN THE RAT PALLIDUM

Karádi, Z.[1], Faludi, B.[1,2], Hernádi, I.[2], Deák, V.[1], Fogarasy, A.[1], Egyed, R.[1] and Lénárd, L.[1,2]

[1]Institute of Physiology, Pécs University, Medical School, H-7643 Pécs and [2]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University, H-7624 Pécs, Hungary

Neural and neurochemical mechanisms of conditioned taste aversion (CTA) are poorly understood and especially little is known of regulation of these functions in forebrain structures. In our previous studies, a particular group of globus pallidus (GP) neurons (the *chemosensitive cells*) have been shown to respond to gustatory stimuli, and pallidal neurons have also been proved to be involved in acquisition and retention of CTA.

To elucidate specific attributes of GP neurons in these functions, neurophysiological experiments were designed and performed in both alert and anesthetized CFY rats. In the former, a microwire multielectrode assembly was chronically implanted into the GP. Rats, after their recovery from the operation, were offered to drink saccharine (0.25%; 10-15 ml) and were subsequently injected intraperitoneally by LiCl (0.15 M; 20 ml/kg) to develope CTA. Aversion to saccharine was subsequently tested several times. Extracellular single neuron activity changes were chronically recorded before, during and after these behavioral interventions. In the acute preparation, animals were anesthetized by urethane (25%; 3.5 ml/kg i.p.) or ketamine (Calypsol; 35 mg/kg i.p.), and their single neuron activity was recorded by means of carbon fiber multibarreled glass microelectrodes during: 1) microelectrophoretic administration of chemicals, 2) gustatory stimulations as well as 3) before, during and after CTA conditioning.

Our pilot-studies with chronically implanted animals provided evidence for activity changes of pallidal neurons in close correlation with the development of (saccharine) CTA. In the anesthetized preparation, CTA-associated gustatory responses of GP (chemosensitive) cells were also recorded. In addition, the microelectrophoretic studies demonstrated characteristic changes of pallidal catecholamine (especilly dopamine /DA/) mechanisms in contiguity with CTA learning.

The present data, along with previous findings, indicate that GP neurons are of great importance in central processing of feeding-associated gustatory information. Specific changes of DA neurotransmission in pallidal chemosensitive cells accompany - and may causally be involved in - acquisition and retention of CTA.

Akadémiai Kiadó, Budapest

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AUDITORY CORTICAL GENERATORS OF MISMATCH-NEGATIVITY IN THE CAT AND MONKEY - A COMPARATIVE ANALYSIS

Karmos,G.[1], Javitt,D.C.[2], Ulbert,I.[1], Molnár,M.[1], Csépe,V.[1], Pincze,Zs.[1] and Schroeder,C.E.[2]

[1]Institute for Psychology of the Hungarian Academy of Sciences, H-1394 Budapest, Hungary and [2]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, U. S. A.

Mismatch-negativity (MMN) is a negative component of the scalp recorded auditory evoked potential (AEP). It is elicited by infrequent, "deviant" stimuli, presented randomly within sequences of repetitive "standard" auditory stimuli. Source localization techniques indicated an auditory cortical origin of the human MMN.

MMN appeared also in cats and monkeys as a negative component in the epidural AEPs in the latency range of 40-120 ms. To study its intracortical generators the field potentials and multiple unit activity were recorded from the auditory areas of awake cats and macaque monkeys by intracortical multielectrodes. One dimensional current source density (CSD) analysis was used to localize intracortical sinks and sources. Field potentials and MUA were separated by low- and high-pass analog filters.

Surface maps in cats indicated the spread of MMN to both AI and AII auditory areas. Intracortical records revealed that in AI area surface negativity was accompanied by an increased lamina II/III sink both in cats and monkeys. In the middle layers of the AII area of cats large amplitude local positive field was elicited by the standards in the latency range of the MMN while this positivity appeared with much smaller amplitude to the deviants. CSD analysis and the increased unit response elicited by the deviant stimuli suggested that the MMN represent a local disinhibition in the upper layers of AII area. No such intracortical pattern was found in monkeys.

The data indicate that the MMN generators are localized in the supragranular layers of the auditory cortex.

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THE ROLES OF hGAL1-30, pGAL1-29 AND A GALANIN RECEPTOR ANTAGONIST (M15) IN THE MODULATION OF ACETYLCHOLINE RELEASE FROM THE RAT BASAL FOREBRAIN

Kasa, P., Farkas, Z. and Balaspiri, L.*

Division of Alzheimer's Disease Research Laboratory, Department of Psychiatry and *Department of Medical Chemistry, Albert Szent-Györgyi Medical University, H-6720 Szeged, Hungary

The galanin (GAL)-containing peptidergic axons have been shown by immunocytochemical means to innervate the acetylcholine (ACh)-containing basal forebrain neurons. It has also been demonstrated that in human Alzheimer's disease brain samples the GAL-containing fiber system hyperinnervates the cholinergic neuronal perikarya and dendrites. Fisone et al. reported that porcine galanin (pGAL1-29) inhibited ACh release in the ventral hippocampus of the rat. The present study examined whether a similar effect occurs within the cholinergic vertical and horizontal limbs of the diagonal band nucleus. Furthermore, we have demonstrated by immunocytochemical means the GALergic innervation of the cholinergic neurons in the rat basal forebrain.

The effects of hGAL1-30, hGAL1-19, hGAL21-30, hGAL26-30, and pGAL1-29, and a GAL receptor antagonist galantide (M15) in the modulation of ACh release were investigated in the rat basal forebrain.

In double-labeling experiments (GAL immunocytochemistry and AChE histochemistry), it was shown that some of the perikarya of cholinergic and/or cholinoceptive neurons are heavily innervated by GALergic fibers, while other cholinergic neurons receive dense innervation at their dendritic surface, too.

The neuropharmacological results showed that hGAL1-30 and pGAL1-29 stimulate the basal release of ACh, although the effect of pGAL1-29 is more pronounced. The effects of both peptide on ACh release are Ca^{2+}-dependent. The amount of ACh evoked by 50 mM K^+ could not be inhibited by hGAL1-30, but pGAL1-29 inhibited the release by about 50 %. 200 µM of GAL receptor antagonist M15 completely abolished the ACh released by hGAL1-30, while the effect of pGAL1-29 was reduced by about 50%. It was interesting that the carboxy terminus of the hGAL peptide (hGAL21-30 and hGAL26-30) was as effective on the stimulation of transmitter release as was the full peptide (hGAL1-30) and this effect was also antagonized by M15. In contrast, no ACh release was evoked by the short form hGAL1-19. It is suggested that hGAL and pGAL can modulate the release of ACh from the basal forebrain cholinergic neuronal structures, but the effects of the two peptides differ in some respects.

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GAD-*lac*Z TRANSGENIC MICE AS A MODEL SYSTEM TO STUDY GENE REGULATION AND NEURONAL PLASTICITY IN THE CNS

[1]Katarova, Z., [2]Mugnaini, E., [1]Sekerkova, G., [3]Mann, J. and [1]Szabo, G.

[1]BRC; Institute of Biochemistry, 6701 Szeged, Hungary; [2]Laboratory of Neuromorphology, Univ. of Connecticut, Stross, CT, 06269-4154; [3]Beckman Res. Institute of the City of Hope, Division of Biology, CA 91010-0269, U.S.A.

The entire gene, coding for the 67 kDa form of GAD (glutamic acid decarboxylase; EC 4. 1. 1. 15) has been cloned and partially sequenced. It spans more than 70 kbp of DNA on the proximal end of the mouse Chr. 2 with at least 20 kbp of 5' regulatory region. In an attempt to analyze functionally this region, we derived transgenic mouse lines, carrying fusion genes between different portions of the GAD promoter and the marker gene *lac*Z. The expression of the transgene was analyzed by a simple histochemical reaction for the marker enzyme β -galactosidase (product of *lac*Z) on whole-mount embryos or tissue sections. The expression in the brain of all transgenics was entirely neuronal, with the exception of one only line, which showed glial expression in the cortex. It varied considerably between the lines, as a result of position effect imposed by the site of integration. Mosaic expression, influenced by the genetic background and sex of the founder transgenic was observed in embryos and adult brains, an indication that epigenetic mechanisms play an important role in the regulation of the transgene and probably the cognate gene. In different lines we detected partially or entirely orthodox and some ectopic staining. The expression domains corresponded to characteristic anatomical regions of the brain, the borders of which were clearly defined. Very little staining was detected in the brains of mice, carriers of the smallest construct. The staining pattern was dramatically improved with increasing the length of the 5' upstream region included in the construct. Moreover, the correct type of expression in late-to-develop structures was only observed in carriers of the largest construct. This clearly indicates, that the major cell type-specific enhancers are localized upstream from the basic promoter, probably arranged in an order, corresponding to their temporal activation. In most lines, the transgene was turned on early in the embryonic development, in the putative neuronal precursors, implying, that its expression is cell-autonomous. This assumption was further tested by homotopic and heterotopic transplantation in the main olfactory bulb (MOB).

In three different paradigms, the cells of the transplant not only survived a lengthy post-operative period, but also preserved their phenotypic marker- β–galactosidase, allowing the tracing of their strongly stained dendrites within the transplant and axons to distal targets. This finding would allow designing of experiments to study neuronal regeneration and plasticity after lesion and/or transplantation in many other brain regions of the of GAD-*lac*Z transgenics, similarly marked by a strong β-galactosidase expression.

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NEW INSIGHTS INTO THE ORGANIZATION OF THE NEURONAL NETWORK UNDERLYING FEEDING IN THE POND SNAIL *LYMNAEA STAGNALIS*

Kemenes, Gy.[1], Staras, K.[1], Yeoman, M.[1], Elliott, C.J.H.[2] and Benjamin, P.R.[1]

[1]Sussex Centre for Neuroscience, School of Biology, University of Sussex, BN1 9QG, Brighton, United Kingdom and [2] Deparment of Biology, University of York, YO1 5DD, York, United Kingdom

The neuronal circuitry underlying feeding in the pond snail *Lymnaea stagnalis* is an excellent model system in which to study the organizational principles of a real neuronal network consisting of individually identifiable neurones. The 3 phases of the central motor pattern of feeding in *Lymnaea* are generated in the buccal ganglia by a set of three types of interneurons, the N1, N2 and N3 cells. These form a central pattern generator that drives the motoneurones innervating the buccal muscles, the main effector organ of feeding (Benjamin and Elliott, 1989). It has also been known that interneurones outside the CPG and chemosensory stimuli can activate this central motor pattern (fictive feeding) (Rose and Benjamin, 1981; McCrohan, 1984; Kemenes *et al.*, 1986). However, little has been known about the contribution of individual CPG interneurones to the generation of the rhythm or about the mechanisms by which these cells are activated by sensory inputs or modulated by interneurones outside the CPG system.

We used laser photoinactivation and *in vitro* conditioning experiments and confocal laser microscopy to investigate the role of individual feeding interneurones in the generation and modulation of fictive feeding and the pathways and mechanisms by which tactile and chemical stimuli can activate these cells. We present electrophysiological evidence here to show that the N1 network is much less redundantly organized than the N2 network and individual N1 neurones seem to play an important role in both unconditioned and conditioned fictive feeding responses. We also demonstrate that the serotonergic modulatory interneurone CGC plays an important gating role in the activation of fictive feeding. We also present detailed maps of putative central mechanosensory neurones and show that both tactile and chemical stimuli can act in parallel on various components of the feeding network. Our new data indicate that the *Lymnaea* feeding network is much less hierarchically organized than previously thought and we discuss possible mechanisms of state-dependent activation and plasticity in this system.

References:

Benjamin, P.R. and Elliott, C.J.H. (1989) In: Neuronal and Cellular Oscillators, Ed: Jacklet, J.W. NY: Dekker, pp. 173-214.

Kemenes, G.; Elliott, C.J.H. and Benjamin, P.R. (1986) J. exp. Biol., 122, 113-138.

McCrohan, C.R. (1984) J. exp. Biol., 113, 351-366.

Rose, R.M. and Benjamin, P.R. (1981) J. exp Biol., 92, 187-201.

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THE ROLE OF SUBSTANCE P IN THE METABOLISM OF ARACHIDONIC ACID

Kis, B., Mezei, Zs., Gecse, Á., Telegdy, G.

Department of Pathophysiology Albert Szent-Györgyi Medical University of Szeged, Hungary

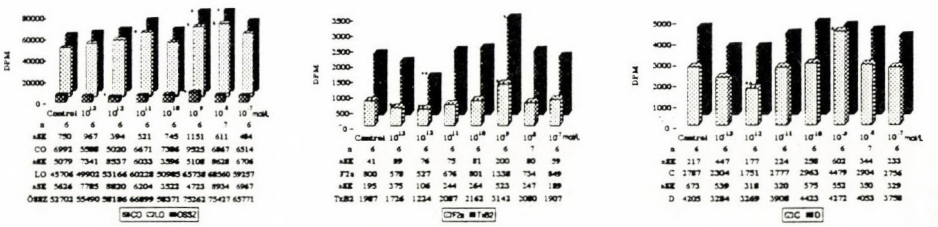
Neurogenic inflammation is initiated by the neuropeptides (substance P, calcitonin gene-related peptide, etc), which are released from the peripheral sensory nerve endings. Several receptors of neuropeptides - including tachykinin NK_1, bradykinin BK_1 and vasopressin V_1 receptor - are expressed on the surface of platelets. The substance P influences the activation of platelets via tachykinin receptor. Receptor binding of molecules on platelets might induce and/or inhibit G-protein coupled mechanisms, such as activation of phospholipases and arachidonic acid (AA) release. The platelets play significant role in several physiologic and pathologic processes. The metabolites of arachidonate cascade might play a role in the inter- and intracellular regulatory functions of platelets. The aim of the present study was to investigate the role of substance P on the arachidonate cascade in platelets. The *in vitro* experiments were carried out in rat platelets, previously labelled with 1-^{14}C-arachidonic acid. The labelled platelets were incubated in Parker medium 199 in the presence of different concentrations of substance P (10^{-13} - 10^{-7} mol/L). The arachidonate metabolites were extracted with ethyl-acetate. The 1-^{14}C-lipoxygenase products were separated with HPLC. The 1-^{14}C-metabolites both of cycloxygenase and lipoxygenase pathway were separated with OPTLC and quantitatively determined in a liquid scintillation counter. The substance P modified the arachidonate cascade of rat platelets, in several concentrations.

The effect of substance P on the

cyclooxygenase (CO) and lipoxygenase (LO)

prostaglandin F2a (F2a) and thromboxane B2 (TxB2) arachidonate metabolites

vasoconstrictor (C) and vasodilatator (D)



On the basis of our results it seems obvious that the substance P exerts some of its action through the cycloxygenase and lipoxygenase pathways of arachidonate cascade.

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DISTRIBUTION OF NEURONS CONTAINING IMMUNOREACTIVITY FOR METABOTROPIC-1α GLUTAMATE RECEPTORS IN THE RAT BASAL FOREBRAIN CHOLINERGIC COMPLEX

Kiss, J.[1], Záborszky, L.[2]

Department of Neuroendocrinology[1], Joint Research Organization of the Hungarian Academy of Sciences and the Semmelweis University of Medicine, Budapest, Hungary and Center for Molecular and Behavioral Neuroscience[2], Rutgers University, Newark NJ., USA

The actions of glutamate and aspartate neurotransmitters are mediated by two principal classes of receptors: ionotropic and metabotropic. MGluR-1α is selectively enriched within certain population of neurons in basal forebrain areas rich in cholinergic projection neurons. The aim of this study was to map precisely the distribution of neurons containing MGluR-1α glutamate receptor subtype in the rat basal forebrain and that of neurons containing both ChAT and MGluR-1α. In order to localize MGluR-1α in ChAT-positive neurons, double immunolabeling techniques were used both at the LM and EM-levels.

In double labeled experiments MGluR-1α was found to colocalize with choline acetyltransferase (ChAT) immunoreactivity (IR), however a number of MGluR-1α-IR neurons showed no ChAT immunolabeling. While in the rostral subdivisions of the BFC (MS,VDB,VP) the number of the single labeled cells was, in general, higher than that of the double labeled neurons, the absolute and relative numbers of double labeled cells showed an increased trend towards more caudal regions (hDB, GP, SI, IC). In the substantia innominata (SI), the globus pallidus (GP) and the internal capsule (IC) the proportions of the single and double labeled cells clearly show an increasing trend. There are more than twice as many double than single labeled cells in these areas.

Altough the single and double labeled cells are intermingled with each other, the MGluR-1α containing cholinergic neurons are primarily localized in more caudal portion of the basal forebrain cholinergic system.

Akadémiai Kiadó, Budapest

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SUBTYPE SPECIFICITY OF THE PRESYNAPTIC α_2-ADRENOCEPTORS MODULATING HIPPOCAMPAL NOREPINEPHRINE RELEASE IN RAT

Kiss, J. P.[1], Zsilla, G.[1], Mike, A.[1], Zelles, T.[1], Toth, E.[2], Lajtha, A.[2] and Vizi, E. S.[1]

[1]Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, P.O.B. 67. Hungary,
[2]Center for Neurochemistry, N.S. Kline Institute for Psychiatric Research, Orangeburg, New York 10962, U. S. A.

In vivo brain microdialysis and high-performance liquid chromatography with electrochemical detection were used to study the effect of different selective α_2-antagonists on hippocampal norepinephrine (NE) release in freely moving awake rat. Systemic administration (0.5 mg/kg i.p.) of either the α_{2AD}-antagonist BRL 44408 or the α_{2BC}-antagonist ARC 239 did not significantly change the basal release of NE. At a higher dose (5 mg/kg i.p.) ARC 239 was still ineffective, whereas BRL 44408 caused a significant increase of the extracellular level of NE.

Similar results were obtained from in vitro perfusion experiments. Rat hippocampal slices were loaded with ^{3}H-NE and the electrical stimulation-evoked release of ^{3}H-NE was determined. The α_2-antagonists were applied in a concentration range of 10^{-8} to 10^{-6} M. ARC 239 was ineffective, whereas BRL 44408 significantly increased the electrically induced release of ^{3}H-NE.

In agreement with the data of microdialysis and perfusion experiments, BRL 44408 displaced ^{3}H-yohimbine from hippocampal and cortical membranes of rat brain with high affinity whereas ARC 239 was less effective. The pK_i values of eight different α_2-adrenergic compounds showed a very good correlation ($r = 0.98$, slope $= 1.11$ $p < 0.0001$) in hippocampus and frontal cortex where the α_2-adrenoceptors have been characterized as α_{2D}-subtype.

Our data indicate that hippocampal NE release in rat is regulated by α_{2D}-adrenoceptors, a species variation of the human α_{2A}-subtype.

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RELATIONSHIP BETWEEN CGRP AND THE ACETYLCHOLINE RECEPTOR IN THE NEUROMUSCULAR JUNCTION

Knyihar-Csillik, E., Mohtasham, S., Nemcsok, J., Rakic, P., Vecsei, L. and Csillik, B.

Department of Clinical Neurology, Albert Szent-Gyorgyi Medical University, Szeged, Hungary (EKC, SM, LV); Section of Neurobiology, Yale University Medical School, New Haven, CT, USA (EKC, PR, BC); Bay Zoltan Institute for Biotechnology, Szeged, Hungary (JN, BC)

Calcitonin Gene-Related Peptide (CGRP) has been implied as the biodynamic factor involved in the expression and maintenance of the α-subunit of the nicotinic acetylcholine receptor. Therefore, we sought to demonstrate the microtopographical relations between CGRP and nAChR in the neuromuscular junction of the rat and the monkey, under normal conditions, after denervation and in the course of ontogeny.

CGRP was demonstrated, at light- and electron microscopic levels, either by means of Sternberger's pre-embedding technique, or with the streptavidin-biotin (ABC) system, using a polyclonal antiserum (Amersham) raised against CGRP in rabbit; this antiserum recognizes both α- and β-CGRP but does not cross-react with calcitonin. nAChR was localized either with biotinylated α-bungarotoxin or with the monoclonal antibodies harvested from mouse (mAb-27.35.74) and rat (mAb-35) hybridoma cell cultures, obtained from Dr. Bruce Chase (Omaha) and from the American Type Culture Collection (Bethesda), respectively.

CGRP outlines the terminal axon arborization in the neuromuscular junction both at the light- and the electron microscopic levels while nAChR is strictly confined to the primary post-synaptic membrane and to the necks of the junctional folds. After prolonged stimulation, CGRP is released from the terminal, suggesting its participation in the expression and maintenance of the nAChR in postjunctional (fundamental) cells. In the course of ontogeny, nAChR appears first in Golgi-bound granules around nuclei of myoblasts and myotubes. Thereafter, nAChR is translocated to the surfaces of myotubes which results in linear structures on the muscle surface membrane, successively restricted to the area underlying the developing motor end plate which initially exerts a diffuse CGRP reactivity, reminiscent of the collective innervation apparatus characterizing the autonomic ground plexus. Finally, as the axon terminal develops into the "antler" shape characterizing mature end plates, nAChR acquires the form of a pretzel_shaped subneural apparatus.

After denervation, CGRP reactivity nearly completely dissappears from the presynaptic region whereas the structural organization of nAChR undergoes a slight shrinkage. While the arrangement of CGRP and the nAChR in normal adult neuromuscular junctions is

consistent with Changeux's theory on the role of CGRP in enhancing adenylate cyclase activity in the post-synaptic cell and thus regulating of expression, synthesis and maintenance of nAChR by fundamental cells, structural correlates of such a functional linkage during ontogenetic processes or after denervation are less conspicuous.

Changeux, J-P:, Duclert, A. and Sekine, S: Calcitonin Gene-related Peptides and Neuromuscular Interactions. Ann. New York Ac. Sci. 657: 361-378. 1992

Csillik, B., Tajti, L., Kovács, T., Kukla, E., Rakic, P. and Knyihár-Csillik, E: Distribution of Calcitonin Gene-related Peptide in vertebrate neuromuscular junctions: relationship to the acetylcholine receptor. J. Histochem. Cytochem. 41: 1547-1555. 1993

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ANGULAR SENSITIVITY STUDIES IN THE BARREL CORTEX OF THE RAT

Kóródi, K., Farkas, T., Rojik, I. and Toldi, J.

Department of Comparative Physiology, József Attila University, H-6701 Szeged, Hungary

Characteristic anatomical and functional formations of the rodent primary somatosensory cortex are the so-called barrels of layer IV. These structures are especially suitable for electrophysiological studies of central responses generated by peripheric stimuli since the barrel-field, that consists of individual barrels, is the fine somatotopic projection of the whisker-pad on the animal's face. Neurones of the barrels are markedly sensitive to the direction and velocity of the deflection of the appropriate whisker.

The barrel-cortex exited by the deflection of the C1 whisker was studied by autoradiographic and CO methods. Rostral and rostrocaudal deflection of the C1 whisker caused the excitement of an expanded region of the barrel-field. Atropin treatment reduced the extent of the excited area. Dorsal and ventral deflection of the C1 whisker resulted in a special multibarrel activity-pattern of neurones in layer V. of the cortex.

The function of cholinergic modulation is known to some extent in the visual cortex but hardly anything is conceived about its role in the somatosensory cortex.

In our experiments sinus-whiskers of the rat were electromechanically stimulated, and we recorded the evoked cellular responses through glass microelectrodes. Cholinergic agents (e.g. the agonist acetyl-L-carnitine, the antagonist scopolamine) were applicated on the cells by pressure-injection through small-resistance microelectrodes attached to the recording ones. According to our results the cholinergic pharmacones tested have different effects on *on* and *off responses.* Analysing the results we found that response components with different latencies answer to cholinergic manipulation in different manner. Injecting agonists on the cells often induced the increase of response-noise ratio.

Concerning our findings we may conclude that together with the known GABAergic modulation the cholinergic system plays an important role in the control of direction sensitivity of the somatosensory cortex neurones.

Akadémiai Kiadó, Budapest

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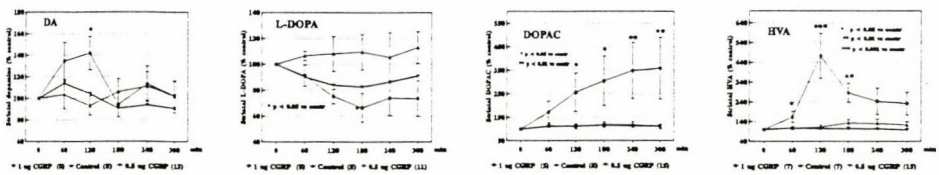
EFFECT OF I.C.V. ADMINISTRATION OF CGRP ON STRIATAL DOPAMINE RELEASE. IN VIVO MICRODIALYSIS STUDY

Kovács, A., Papp, E., Telegdy, G.

Department of Pathophysiology, A. Szent-Györgyi Medical University, Semmelweis u. 1, P.O.Box 531 H-6701 Szeged, Hungary

Calcitonin gene-related peptide (CGRP) is widely distributed throughout the central nervous system *(9)*. High densities of binding sites are present in the ventral striatum *(4,8)*. Circumstantial evidence indicates that the peptide might interact with the dopaminergic system within the brain *(4,10)*. It decreases the motor activity and causes catalepsy *(1,3)* suggesting a possible interaction with dopaminergic neurons. The data concerning role of dopamine (DA) in mediation of CGRP action are contradictory. No significant changes of striatal DA concentration were observed following smaller doses (1 ng and 100 ng) of i.c.v. injection of CGRP *(1)*. Higher dose of α-hCGRP (10 μg) produced dramatic increases in DA levels and its metabolites (DOPAC, HVA) in several brain regions studied (frontal cortex, amygdala, hippocampus and globus pallidus) *(2)*. It has been shown that dopaminergic transmissions are involved in the CGRP-induced decrease in motor activity and active avoidance response of rats *(6,7)*. These facts prompted the present in vivo microdialysis study of the effects of i.c.v. administration of CGRP on rat striatal DA, dihydroxyphenylacetic acid (DOPAC), L-DOPA and homovanillic acid (HVA) release. Dialisate samples were obtained from anesthetized (pentobarbital 35 mg/kg and urethan 1.2 g/kg) rats. For determination of DA and metabolites Hewlett-Packard HPLC system with electrochemical detection was used.

I.c.v. administration of different doses (500 ng, 1 μg) of CGRP increased the release of DA in rat striatum in a dose dependent manner. Highest level was measured at 120 min after the peptide administration **(Fig.1)**. L-DOPA, the precursore of DA gradually decreased **(Fig.2)**. The metabolite DOPAC showed a steady increase, attaining a maximum at 3, 4, 5 hrs **(Fig.3)**. HVA increased reaching a maximum at 120 min following CGRP (1 μg) administration **(Fig.4)**. The data obtained suggest that CGRP activates the dopaminergic system by utilizing the precursor of DA, L-DOPA, and increasing the output of DA, DOPAC and HVA.



References:

1) *Clementi, G. et al.* Pharmacol Biochem Behav **42**: 545-548 (1992). 2) *Drumheller, A. et al.* Ann. N. Y. Acad. Sci. **657**: 546-548 (1992). 3) *Jolicoeur, F.B. et al.* Ann. N.Y. Acad. Sci. **657**, 155-163 (1992). 4) *Inagaki, S. et al.* Brain Res. **374**: 287-298 (1986). 5) *Kawai, Y. et al.* Neuroscience **15**: 747-763 (1985). 6) *Kovács, A., Telegdy, G.* Neuropeptides Suppl. **26**:7 (1994). 7) *Kovács, A., Telegdy, G.* Physiol Behav (Accepted). 8) *Sexton, P.M. et al.* Neuroscience **19**: 1235-1245 (1986). 9) *Skofitsch, G., Jakobowitz, D.M.* Peptides **6**, 721-745 (1985). 10) *Tiller-Borcich, J.K. et al.* Neuropeptides **11**: 55-61 (1988)

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BACKWARD MASKING: A PSYCHOPHYSICAL PHENOMENON AND ITS PHYSIOLOGICAL BASIS

Gy. Kovács [1,2], R. Vogels [2], G.A. Orban [2]

[1]Department of Physiology, Albert Szent-Györgyi Medical University, H-6720, Szeged, Hungary

[2]Lab. Neuro- en Psychofysiologie, KUL, B-3000 Leuven, Belgium

It is a well known fact that the identification of a briefly presented shape is strongly impaired when it is followed by another stimulus (the mask), a phenomenon called backward masking. We observed the same phenomenon in 2 rhesus monkeys: their discrimination performance was strongly impaired for geometrical shapes, presented at 20 msec exposure time (ET) when the shape was immediately followed by another shape. To determine the neuronal correlate of this phenomenon, we measured the responses of inferior temporal neurons in the same 2 monkeys, performing a fixation task, to these briefly presented shapes which were either followed by the mask (M) or not (NM). Although the response magnitude strongly declined with decreasing ET (tested range: 20-100 msec), shape selectivity remained present even at 20 msec ET (n=54 units) in the NM condition. The surprising finding was that the majority of the units showed significant shape selectivity in the 20 msec M condition too. When using the initial 60 msec of the response, the difference between the response to the preferred and non-preferred shape was on average 0.6 and 1.0 spikes in M and NM conditions, respectively. This small, but significant difference between the selectivity in M and NM conditions, increased notably when considering the first 200 msec of the response: average response differences of 0.6 and 2.6 spikes in M and NM conditions, respectively. Thus, longer temporal integration of the response results in a marked increase in discrimination capacity in the NM condition, but not when the briefly presented shape if followed by another stimulus. This suggests that the behaviorally observed backward masking is the result of temporal integration of the neuronal responses.

Akadémiai Kiadó, Budapest

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ACTIONS OF MERCURY IONS ON STIMULUS-EVOKED POSTSYNAPTIC CURRENTS IN HELIX NEURONS

Kovács, T., Insperger, K. and Erdélyi, L.

Attila József University, Department of Comparative Physiology, H-6701 Szeged, P.O.B.533, Hungary

It is known, that toxic heavy metal ions may influence the spontaneous and evoked transmitter release both at peripheral and central synapses of vertebrate and some invertebrate animals (for review see: Cooper, G. P., Suszkiw, J. B. and Manalis, R. S. Cellular and Molecular Neurotoxicology, Raven Press, New York 1984).

We studied the actions of mercury ions on some identified and non-identified central neurons of the snail Helix pomatia L. Among identified neurons, the L and RPa2,3 L and RPl, LPa4 cells were investigated after stimulation of the left pallial nerve (0.1 ms, 15V). Spontaneous and stimulus evoked excitatory postsynaptic potentials (EPSP) and currents (EPSC) were examined under conventional current- and voltage-clamp circumstances.

Mercury ions (0.5-50 μM) had no significant effects on spontaneous transmitter release followed by electrophysiological methods. There was no measurable effect on the amplitude or frequency of spontaneous EPSPs or EPSCs in the studied neurons. This observation made our findings different from other results published on various vertebrate preparations (Manalis, R. S. and Cooper, G. P. Nature, 257: 690-691, 1975; Binah, O. et al. Eur. J. Pharm. 51: 453-457, 1978).

In contrary, the evoked transmitter release was highly influenced by mikromolar mercury ion containing medium. Low dose of mercury ions (0.2-10 μM) caused transient increase of the stimulus evoked EPSPs amplitude both in identified and non-identified neurons studied. Higher dose of mercury ions (15-50 μM) suppressed all of the studied EPSPs or EPSCs in dose-dependent and irreversible manner. This observation is comparable with other results published on various synapses of the frog and rat (Juang, M. S. Toxicol. Appl. Pharmacol. 37: 339-343, 1976, Atchison, W. D. and Narahashi, T. Neurotoxicology 3: 37-50, 1982).

We assume, that an influx of mercury ions into the presynaptic terminal of the nerve endings can influence the Ca buffering which than may facilitate and/or suppress the transmitter release process.

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DISTRIBUTION OF NEUROMEDIN U-LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE FROG

Kozicz, T. and Lázár, Gy.

Department of Anatomy, University Medical School of Pécs H-7643, Hungary

The distribution of pericarya and nerve fibers containing neuromedin U-like immunoreactivity in the brain of *Rana esculenta* was determined with an antiserum directed toward rat neuromedin U-like peptide.

In the telencephalon, immunoreactive pericarya were found only in the olfactory bulb and diagonal band. In the diencephalon, labelled pericarya were detected in the anterior and posterior preoptic area, the dorsal hypothalamus, the caudal part of the infundibulum and the posterior tuberculum. In the mesencephalon, immunoreactive cells were found only in the laminar nucleus of the torus semicircularis and the anterodorsal tegmental nucleus. In the rhombencephalon, labelled pericarya were detected in the secondary visceral nucleus, the cerebellar nucleus, the central gray, and the nucleus of the solitary tract.

Immunoreactive nerve fibers were observed in all areas of the brain that contained labelled pericarya. The densest accumulation were found in the nucleus accumbens, the dorsal part of the lateral septum, the periventricular region of the ventral thalamus, the lateral part of the infundibulum and the tegmentum mesencephali.

The distribution of neuromedin U-like immunoreactivity in the frog brain was substantially different from the distribution described for the rodent brain.

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EXPRESSION OF PKC ISOFORMS IN CEREBRAL ENDOTHELIAL CELLS

Krizbai, I.[1], Szabó, G.[2], Deli, M.A.[1], Maderspach, K.[2], Oláh, Z.[3], Lehel, C.[3] and Joó, F.[1]

[1]Institute of Biophysics and [2]Biochemistry, Biological Research Center, H-6701 Szeged, Hungary
[3]NCI, NIH, Bethesda, Maryland, USA

Protein kinase C (PKC) is a key regulatory enzyme of the signal transduction and is able to modulate large number of intracellular processes. Its presence and activation through different receptor types have been described in microvessels isolated from rat brain. Molecular cloning and biochemical studies have revealed that PKC constitutes a family of isoenzymes composed by at least four conventional (α, ßI, ßII and γ) and several related novel isoforms (δ,ε,ζ,η,θ). We have investigated the distribution of seven isoforms (α,β,γ,δ,ε,ζ,η) by using RT PCR in rat brain, freshly isolated brain microvessel fraction, primary cultures of rat brain endothelial cells, an immortalized rat brain endothelial cell line and in aortic endothelial cell cultures. Brain contained all the seven investigated isoforms. Similar expression pattern was shown in freshly purified microvessels but the presence of PK C-γ isoform could not be detected. Primary cultures of endothelial cells expressed PK C-α, -β, -δ and -ε isoenzymes, whereas the immortalized cell line expressed only PK C-α, -δ, and η. The rat aortic endothelium contained PK C-β, and -δ isoforms, only. Since the PKC isoenzymes show different sensitivity to activators such as Ca^{++} and phorbol esters the presence of different isoforms may confer a finely tuned responsivness of the brain endothelial cells to extracellular stimuly.

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MESENCEPHALIC AND BRAINSTEM CONNECTIONS OF AUDITORY SYSTEM IN THE FROG, *RANA ESCULENTA*

Kulik, A., Matesz, K.

Department of Anatomy, University Medical School of Debrecen, H-4012 Debrecen, Hungary

In our earlier experiments we have demonstrated that the frog`s primary auditory center the cochlear nucleus, has connection with the secondary auditory centers including the superior olive, torus semicircularis and nucleus isthmi. The aim of this work was to study the afferent and efferent connections of these secondary auditory centers. The *Phaseolus vulgaris* leucoagglutinin (PHA-L) was injected either into the superior olive or principal nucleus or nucleus isthmi, because this tracer was transported both in retro- and anterograde direction in frog`s nervous system. *The injection of superior olive* labelled the following structures: anterograde labelling was observed bilaterally in the cochlear nucleus, reticular formation, principal, magnocellular, and laminar nuclei of torus semicircularis, nucleus anterodorsalis tegmenti mesencephali and tectum opticum, with an ipsilateral dominance. Robust labelling could be detected in contralateral superior olive and in both lateral lemniscus and posterior thalamic nuclei, ipsilaterally. The retrogradely labelled cells were found in all the previously mentioned structures and in both nuclei anteroventralis and posterodorsalis tegmenti mesencephali bilaterally. *The injection of PHA-L into the principal nucleus of torus semicircularis* revealed anterogradely labelled structures bilaterally in all the five different subdivisions of torus semicircularis, superior olive, reticular formation and ipsilaterally in the nuclei anterodorsalis, anteroventralis, posterodorsalis, posteroventralis tegmenti mesencephali, nuclei of lateral lemniscus and posterior thalamic, nucleus isthmi, tectum opticum, contralateral cochlear nucleus. Retrogradely labelled cells were found in the contralateral cochlear nucleus, nuclei of lateral lemniscus and posterior thalamic ipsilaterally, principal, magnocellular, and laminar nuclei of torus semicircularis, reticular formation, superior olive bilaterally. *After the injection of this tracer into the nucleus isthmi* labelled terminations were bilaterally in nuclei anterodorsalis and anteroventralis tegmenti mesencephali, tectum opticum with an ipsilateral dominance. They could be seen ipsilaterally in principal, magnocellular, and laminar nuclei of torus semicircularis, superior olive, reticular formation and contralateral nucleus isthmi. Retrogradely labelled cells were in the above mentioned nuclei with the exception of contralateral tectum opticum and nucleus anteroventralis tegmenti mesencephali, but they were present in both ipsi- and contralateral cochlear nuclei.

Our results suggest that the PHA-L labelling provide a much more extensive network of auditory system of frog that has been achieved with the other methods.

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VARIOUS BURST MECHANISMS AS EXPRESSED BY PIECEWISE DIFFERENTIABLE INTERVAL MAPS

Lábos E.[1] and Tóth T.[2]

SOTE, 1st Dept. of Anatomy, MTA-SOTE-EKSz, Neurobiology, Budapest, Hungary[1] and UWCC, Dept. Physiol., Cardiff, UK[2]

Special neurons or nets in different parts of CNS in lower and higher animals with firing characterized by bimodal interspike interval distributions and also by a few further, directly recognizable or measurable properties are known since several decades. Their functional role, as well as their generation mechanisms are still often debated and not sufficiently understood. Model approaches using modified HH-equations have been proved to be suitable to simulate at least a part of their properties.

Here, a more comfortable and, at least dynamically, more transparent model-family, the interval map spike generators (IMSG) and their nets are used. Tedious mathematical details are left for full publication.

The principal categories of burst (dynamical) mechanisms are as follows: (endogenous and autonomous or autoactive attributes do not fit each other completely).

1. Non-autonomous drive - 1.1. Exogenous pacemaker - 1.2. Open loop network control, 1.3. Closed loop network drives (several versions), 1.4. Added control, 1.5. Parameter-control (threshold, speed, equilibrium control, etc.);

2. Endogenous, autonomous drive - 2.1. Spikes separable from a pacemaker, rescricted to a single unit, 2.2. Some non separable cases, 2.1.1. Virtual equilibrium control, 2.1.2. Control of spike time course;

3. Mixed mechanisms, combinations. Else.

The **output parameters** of the burst firing are rather adjustable (spike numbers and their shapes in bursts, changing frequency in bursts, inter-burst behaviour or shape and elapsed time, statistical properties or degree of regularity , etc.).

Biological and theoretical interests coincide only partially.

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CONTROL OF ION AND WATER HOMEOSTASIS IN THE BRAIN BY GLIAL/NEURONAL INTERACTION

Latzkovits, L.

Institute of Experimental Surgery, Albert Szent-Györgyi Medical University, Szeged, Hungary

A great number of data published over the two last decades has shown that ion and water homeostasis in the brain is controlled by the cell to cell communication of astroglia and neuron. Neuronal firing continuously distorts the ion equilibrium in the neuronal micro-environment: as a consequence of neuronal activity, K^+ accumulates (20-80 mM), while Ca^{++} concentration decreases (0.9-0.5 mM) in the extracellular cleft surrounding neuron. These changes activate the entire transport system of astroglia to correct all the alterations and to reestablish ion equilibrium. The time course of glial activation is well comparable with neuronal firing. Therefore, the glial buffering of ion composition in the neuronal micro-environment requires a sophisticated signalization system functioning to synchronize neuronal and glial activity. The existence of a multifaceted signalization system coordinating neuronal and glial functions is well documented by the fact that glia bears a certain replica of the specific receptors present on neuronal membrane. Indeed, an abundance of first messenger molecules, such as, neurotransmitters leaking out from the synapses, hormones, neuropeptide growth factors, are carrying messages from neuron to glia and vice versa. The cellular control of ion and water homeostasis in the brain is actually accomplished by these first messenger molecules. For the study of this control system, primary cultures of dissociated brain cells provide an efficient model.

Over the last years, in my laboratory we focused our sudies on the effect of arginine vasopressin (AVP) and atriopeptin (AP) upon the cation and water homeostasis in cultured astroglial cells. Both peptides are synthetized and released in the brain from neurones in special areas. They are disributed, however, over the entire extraxellular space in the brain by volume transmission. In good agreement with in vivo studies reported in the literature, our findings support the hypothesis that these two peptides exert a major control on the intracellular water content of astroglial cells: AVP increases, while AP decreases the volume of astroglia. That phenomenon may play a role in the regulation of volume transmission.

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EFFECTS OF MORNING AND EVENING ADMINISTRATION OF ALPHA ADRENERGIC AGONISTS ON SLEEP

Lelkes, Z.

Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary

The brain levels of neurotransmitters and the sensitivity of their receptors display circadian fluctuations. Circadian variations have been described in the effects on sleep of various monoaminergic agonists, such as apomorphine, a dopaminergic (DA) agonist, and ritanserine, a serotoninergic ($5HT_2$) agonist. Similar variations were found in the effects of other drugs acting on the monoaminergic systems, e.g. amitriptyline, a noradrenaline (NA) and 5HT reuptake blocker, and nomifensine, a NA and DA uptake blocker. It is possible that NA sensitivity also displays a circadian fluctuation which may influence the effects on sleep of drugs acting on the NA system. We studied the sleep effects of an $alpha_1$ agonist, methoxamine and an $alpha_2$ agonist, dexmedetomidine, administered in the morning or in the evening.

Male Wistar rats with implanted golden jewellery screws for EEG recording were kept in a 12 h light - 12 h dark cycle, the same conditions were maintained during the experiment. At light and dark onset, saline was administered intraperitoneally on the control day, and methoxamine (4 mg/kg) or dexmedetomidine (1 or 5 μg/kg) on the experimental day. The sleep-wake activity was recorded for 12 h after the injection.

After both morning and evening administration of 4 mg/kg methoxamine a suppression of REM sleep (REMS) was observed. The amount of non-REM sleep (NREMS) was decreased only after morning administration of the drug. The higher (5 μg/kg) dose of dexmedetomidine elicited a marked suppression of REMS both in the morning and in the evening. NREMS was enhanced only in the evening. The lower (1 μg/kg) dose dexmedetomidine suppressed REMS only in the evening. NREMS was not effected by 1 μg/kg dexmedetomidine. Our results indicate that there is a circadian variation in the sleep effects of alpha adrenergic agonists.

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NICOTINIC AGONISTS MODULATE THE RELEASE OF SEROTONIN IN RAT HIPPOCAMPUS

Lendvai B.[1], Sershen H.[2],* Baranyi M.[1], Lajtha A.[2] * and Vizi E.S.[1]

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary [1]
The Nathan S. Kline Institute; for Psychiatric Research, Orangeburg, NY, USA[2]

In the present study we investigated the effect of different nicotinic agonists (dimethyl-phenylpiperazinium-iodide (DMPP), nicotine, cytisine, (-)lobeline, and (-)epibatidine) and antagonists (mecamylamine and dihydro-ß-erythroidine) on the release of [^{3}H]5-HT from hippocampal slices. The nicotinic agonists DMPP, lobeline and electrical field stimulation released [^{3}H]5-HT from the hippocampus; other nicotinic agonists, such as nicotine, cytisine, and epibatidine had no effect. The effect of DMPP and lobeline was $[Ca^{2+}]_O$-independent and tetrodotoxin-insensitive. Unlike lobeline-induced release of [^{3}H]5-HT, the effect of DMPP was antagonized by mecamylamine (20 μM). The action of DMPP did not show desensitization, at least for 30 minutes. Although DMPP decreased the electrically evoked release of [^{3}H]5-HT, this effect was probably not mediated via nAChRs since DMPP added just before the stimulation did not cause significant reduction in the evoked release of [^{3}H]5-HT. In Ca^{2+}-free medium DMPP was still able to release tritium from the hippocampus, while the stimulation-evoked release of [^{3}H]5-HT was abolished. Neither N-type Ca^{2+} channel blockers, cadmium, or ω-conotoxin GVIA, nor L-type blocker nifedipine could modulate the effect of DMPP. Because of the dual character of the action of DMPP and lobeline, we investigated the effect of an ion channel modulator, flufenamic acid (FFA), on the release of [^{3}H]5-HT. FFA, at a concentration of 100 μM, inhibited the effect of DMPP and lobeline to release 5-HT from hippocampal slices. 50 μM FFA decreased the effect of DMPP and it had no effect on the action of lobeline. Therefore, it is proposed that the effect of DMPP and lobeline to enhance the release of [^{3}H]5-HT from the hippocampus was most likely mediated via nAChRs in part, while another source of the 5-HT-releasing property of DMPP and lobeline may be the activation of ion channels.

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THE RELEASE AND MODULATION OF ENDOGENOUS ATP AND [^{3}H]ACh IN RAT SUPERIOR CERVICAL GANGLION

Liang, S.D. and Vizi, E.S.
Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

It is generally accepted that acetylcholine (ACh) is the transmitter in the ganglion. The release of endogenous ATP, measured by the luciferin-luciferase assay, and the release of [^{3}H]ACh from the isolated superior cervical ganglion (SCG) of the rat loaded with [^{3}H]choline were studied simultaneously. The activity of ecto-ATPase was also measured. The electrical stimulation (2-10-30 Hz, 300 shocks, 1 ms) released endogenous ATP in a frequency-dependent manner. The evoked release of both endogenous ATP and [^{3}H]ACh was $[Ca^{2+}]_o$ dependent. The Na^+ channel blocker TTX (1 μM) inhibited the stimulation-evoked release of endogenous ATP and of [^{3}H]ACh. Ten days after decentralization of the SCG the stimulation-evoked release of ATP and [^{3}H]ACh was completely abolished and the uptake of [^{3}H]choline was significantly reduced but not inhibited. Our neurochemical data, the presence of ectoATPase (K_m=475$\pm$24 μM and v_{max}=3.50$\pm$0.18 nmol/min/mg tissue), the enzyme able to terminate the effect of ATP, and the observation of Evans et al (Nature 1992, 357: 503-505) that ATP is able to produce current via P_{2x}-purinoceptors of postsynaptic location, strongly indicate that ATP of presynaptic origin is a fast excitatory transmitter in the ganglion. The evoked release of both endogenous ATP and [^{3}H]ACh was $[Ca^{2+}]_o$- dependent. Since the release of both ATP and ACh was subjected to presynaptic modulation via presynaptic A_1-adenosine, nicotinic and muscarinic receptors, it is also concluded that SCG is an integrative center: the transmission can be presynapticlly modulated by different endogenous ligands.

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CEREBROVASCULAR, NEURONAL AND BEHAVIORAL CHANGES DURING AGING AND ALZHEIMER'S DISEASE

Luiten, P. G. M.[1], Nyakas, C.[2], Bohus, B.[1], Majtényi, C.[3] and De Jong, G. I.[1]

[1]Dept. of Animal Physiology, University of Groningen, Netherlands; [2]Central Research Division, Haynal University and [3]Dept. of Neuropathology, Nat. Inst. of Nervous and Mental Disease, Budapest, Hungary

Abnormal brain function as often encountered during aging may already find its origin in the early developmental period of life. This can be demonstrated in rat by the effects of perinatal hypoxia, which delays the ingrowth of cholinergic (and other) fiber systems in cortex and hippocampus during early postnatal development. This transient growth retardation is a Ca^{2+} dependent process that can be antagonized by application of the L-type Ca^{2+} channel blocker nimodipine. Anatomical experiments further indicate that delayed presynaptic cholinergic fiber growth may result in presistent alteration in the expression of postsynaptic muscarinic receptor proteins. The hypoxia-induced developmental injury was also reflected in a lifespanning behavioral dysfunction as demonstrated in a range of cognitive test conditions. Notably in the aging period perinatal brain damage became manifest in strikingly abnormal cognitive but also social behaviors.

In general during aging, neuronal and behavioral changes are often associated with a decrease control of intracellular Ca^{2+} regulation. E.g. aging neurons in the hippocampus show calcium dependent increase of afterhyperpolarization, there is a partial decrease of intracellular calcium binding proteins and most behaviors are impaired, all of which can be counteracted by blockade of calcium influx.

Derangement of Ca^{2+} homeostasis during aging not only affects neuronal mechanisms, but also influences cerebrovascular integrity. In the course of life, the microvascular wall, being the anatomical basis of the blood-brain barrier, is progressively affected by the aging process. The basement membrane of the microvascular wall is thickened, there is perivascular deposition of collagen fibrils, and hypertrophy of perivascular astrocytic endfeet, while the endothelial cell shows abnormal cellular structure. This microvascular breakdown during aging was considerably delayed by chronic treatment with the Ca^{2+} antagonist nimodipine. Interestingly the microvascular pathology also occur with high incidence in the aging human brain. Preliminary data suggest that microvascular pathology may even be increased in Alzheimer patients. Recent experiments further indicate a potentially important role of intracellular Ca^{2+} in the mechanisms related to the induction of neuronal damage by bamyloid deposition. This mechanism of neuronal degeneration in aging and Alzheimer's disease is subject of current investigation. Some risk factors and potential neuroprotective impact of Ca^{2+} antagonists will be discussed.

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FINE-LOCALIZATION OF KAPPA-OPIOID RECEPTORS IN THE MAMMALIAN AND AVIAN BRAIN TISSUE. LIGHT- AND ELECTRON-MICROSCOPIC IMMUNOHISTOCHEMISTRY

Maderspach,K., Cserpán,E., @Takács,J., @Csillag,A., #Schmidt,P., *Schröder,H.

Inst. Biochemistry, Biological Res. Ctr., Hungarian Acad. Sci., H-6701 Szeged, Hungary;@1st Department of Anatomy, Semmelweis University Medical Scool, Budapest; University of Cologne, *Dept. Anatomy and #Inst. of Forensic Medicine, Cologne, Germany

Anatomical distribution of transmitter receptors in the CNS can be studied by ligand autoradiography or immuohistochemistry. The mapping of opioid receptors was performed primarily by autoradiography. Applying radioligands of relative mu, delta or kappa-type selectivity, a marked difference in the labelling of cortical and subcortical structures have been found, however, essential overlapping was also detected. This method is inadequate for receptor-localization at cellular or subcellular level.

In the case of opioid receptors the difficulty in the immunohistochemistry is the preparation of the specific antibody. Some antibodies have been established until now against mu or delta opioid receptors were not used for mapping, exept the anti-ß-endorphine anti-idyotype antibody raised by Gramsch et al. (1). They have localized receptors on the soma and proximal dendrites of neurons in the rat brain at light microscopical level.

Our laboratory prapared a monoclonal antibody against the frog kappa-opioid reeceptor with wide species specificity and high selectivity (2). It was used for immunolocalization of kappa-receptors on cultured embryonic chick, rat and human nervous cells (3) and histological sections of human (4), chick and rat brain. Neurons were labelled on their soma and dendrites. Glial immunoreactivity was detected on astro-2 type cells. Electron-microscopic studies revealed immunostaining on the plasma-membrane of glial cells and neurons, the latter was post-synaptic and exra-synaptic. Intracellular immunoreactivity have been rendered to ribosomes, polyribosomes, endoplasmic reticulum membrane and dendritic microtubules. Golgi and nuclei were devoid of label. We suppose that the antibody detects also the just synthetising receptor which is transported along the dendritic microtubules to the place of integration and function in the membrane. The fine localization established here is in accordance with that of described for other transmitter receptors.

1. Gramsch et al. *J.Biol.Chem.* **263**(1988)5853-5859
2. Maderspach et al. *J.Neurochem.* **56**(1991)1897-1904
3. Maderspach and Németh *Neurosci.* **57**(1993)459-465
4. Schmidt et al. *Brain Res.* **654**(1994)223-233

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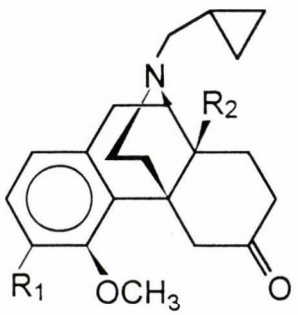
BIOCHEMICAL CHARACTERIZATION OF NEW μ SELECTIVE OPIOID ANTAGONISTS (MORPHINAN ANALOGUES)

Márki, Á.[1], Ötvös, F.[1], Tóth, G.[1], Schmidhammer, H.[2] and Borsodi, A.[1]

Institute of Biochemistry and Isotope Laboratory, [1]Biological Research Center, Szeged, Hungary and Institute of Pharmaceutical Chemistry, [2]University of Innsbruck, Innsbruck, Austria

The three major opioid receptor types (mu, delta and kappa) exhibit different ligand selectivity profiles, localization and mediate different physiological functions. The use of specific compounds is crucial for understanding the mechanism of opioid action at the level of the endogeneous system, neurochemical processes in various mental diseases and pain states, and of direct benefit in improved therapy. In the last few years new antagonists (N-cyclopropylmethyl-3-R_1-4-methoxy-14-R_2-morphinan-6-ones) with high selectivity for the mu receptors have been developed (Fig. 1.).



Compound **1**: R_1= H, R_2= OMe
Compound **2**: R_1= OH, R_2= OMe
Compound **3**: R_1= OH, R_2= OEt

All of the compounds have been tested by in vitro binding assays in rat brain membranes. All these ligands bind to mu opioid receptors with high affinity (K_i values in nanomolar range). The compounds **2** and **3** have relatively higher potency toward delta and kappa receptors, than compound **1** (cyprodim) which probably due to the presence of the 3-OH group. Cyprodime has recently been radiolabelled with tritium resulting in high specific radioactivity (36.1 Ci/mmol). These new ligands will provide excellent tools for the further characterization of mu opioid receptors at the molecular level.

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ORGANIZATION OF THE GLOSSOPHARYNGEAL-VAGUS AND ACCESSORY NUCLEI IN THE FROG, *Rana esculenta*

Matesz, C., Szekely, G.
Department of Anatomy, University Medical School of Debrecen, H-4012 Debrecen, Hungary

The motoneurons of the glossopharyngeal (nIX), vagal (nX) and accessory (nXI) cranial nerves form a ventrolateral column in the caudal part of the frog brainstem. This column of neurons is often referred to as the ambiguus nucleus. With the aid of the cobalt labelling method we have investigated the central representation of the peripheral targets to find any musculo (viscero) topic organization within the nucleus. In order to facilitate the separation of the different components of this nuclear complex the dendritic arborization pattern and the morphological parameters of the perikarya were studied.

Applying the cobaltic-lysin to the common root of the IX-X-XI nerve revealed that most of the cells are organized into a longitudinal column. In the rostral compact part of the nuclear complex the small cells are located dorsally laying in a groove formed by the ventrally located medium-sized cells. In the caudal loose formation of the nucleus the two different cell types may intermingle. Towards the obex the number of small cells gradually decreases and a new type of cell, having a large perikaryon, appears. Dorsally and dorsomedially to the continuous cell column 15-20 cells intermingle with the cells of the reticular formation. On the basis of their localization and sizes we regard them as the primordial form of the dorsal nucleus of the vagus nerve (Xd) in mammals. Labelling of the branches of vagal nerve revealed that the neurons of the gastric nerve contain most of the small cells. The cardiac and gastric nerves originate also from this primordial Xd nucleus. The laryngeal nerve contain predominantly large neurons most of them are located dorsally to the large cucullaris neurons. The latter neurons can be separated from the laryngeal neurons, on account of their different dendritic arbor. The reconstruction of the dendritic tree revealed that the cardiac, gastric and pulmonary neurons have similar dendritic arborization pattern with a weak dorsomedial and a broom-like ventrolateral dendritic tree. The dendritic arborization of the Xd neurons occurs mainly within the gray matter between the hypoglossal and ambiguus nucleus. Most of the dendrites of laryngeal neurons are oriented in a dorsal direction, the cucullaris dendrites have a mediolateral orientation.

Our results revealed that there exists a viscero (musculo) topic organization within the IX-X-XI nuclear complex of the frog. On the basis of the morphology and peripheral targets of the neurons we can conclude that the cells of the frog rostral parasympathetic outflow are organizied in a similar manner as we have described for the lizard and the rat. The dorsal nucleus of the vagus (Xd) in mammals has been found in a primordial form in the frog. The large motoneurons supplying the laryngeal muscle are in a similar position and show similar morphological characteristics as in the rat. The geometry of the dendritic tree of the laryngeal and the accessory neurons so profoundly differ from one another that it makes possible an unambiguous distinction between the two groups of neurons.

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EXPRESSION OF THE RAF PROTOONCOGENE IN THE SPINAL CORD AND DORSAL ROOT GANGLIA OF THE RAT AND GUINEA PIG

Mihály, A.,[1] Priestley, J.V., [2] Rapp, U.R. [3]

Department of Anatomy, Albert Szent-Györgyi Medical University, Szeged, Hungary [1], Department of Physiology, UMDS, St. Thomas's Campus, London, UK [2], National Cancer Institute, Lab. of Viral Carcinogenesis, Frederick, MD, USA [3]

The raf protooncogenes are the cellular counterpart of the v-raf oncogene, originally discovered in murine sarcoma cell lines. The products of the raf genes are cytoplasmic serine-threonine protein kinases which belong to the src superfamily and share structural similarities with protein kinase C isoenzymes. Few data are at hand about the possible substrates of raf kinases - possibly they phosphorylate Jun proteins, MAP kinases and MAP kinase kinases. Keeping in mind the regulatory influence of growth factor receptors on the phosphorylation of raf kinases, the aim of the present study was to detect raf proteins in sensory ganglion cells and the spinal cord of adult male albino rats and guinea pigs. The animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, transverse sections were cut from each segment of the spinal cord with vibratome, and stained with monoclonal and polyclonal anti-raf-peptide sera. The L1-5 dorsal root ganglia were cut with cryostat and stained with double labelling technique: the polyclonal raf antibody was mixed with monoclonal RT 97 neurofilament antibody and biotinylated Griffonia simplicifolia isolectin B4. The avidin-biotin detection systems and TRITC or FITC conjugated secondary antibodies were used. Some sections from the lumbar intumescence were subjected to double labelling, too. Both large and small neurons displayed raf-protein-like immunoreactivity (RPI) in lumbar sensory ganglia. In the spinal cord, ventral horn motor neurons contained strong RPI. Scattered, medium-sized cells were stained in lamina I. Lamina II and III were devoid of immunostaining, which was conspicuous, because we observed strong lectin staining of dorsal root fibers on the same section. Our studies proved the presence of cytoplasmic raf kinases in large and medium-sized neurons of the spinal cord and the cell bodies of sensory ganglion cells. However, we failed to detect RPI in the central processes of primary sensory neurons. This localization pattern raises the possibility, that raf kinases may participate in growth factor mediated processes only in the cell body of these cells.

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HETEROGENEITY OF PRESYNAPTIC NICOTINIC ACETYLCHOLINE RECEPTORS

Mike,A.[1], Sershen,H.[2], Balla,A.[1], Lajtha, A.[2] and Vizi,E.S.[1]

[1]Dept. of Pharmacology, Institute of Experimental Medicine, Hung. Acad. Sci., Budapest, Hungary.
[2]Center for Neurochemistry, Nathan Kline Institute, Orangeburg, New York, USA

On the basis of the results obtained by expression studies, the subunit composition of nicotinic acetylcholine receptors (nAChRs) in different tissue preparations can be deduced by assaying the rank order of potency of different agonists and antagonists. This study attempted to characterise the subunit composition of nAChRs participating in pre- and postsynaptic mechanisms of neural transmission, and to study the role of voltage-sensitive calcium channels in the nAChR-mediated events. For these experiments we studied the effects of a number of nicotinic agonists, including the potent novel analgetic epibatidine, in three different preparations: The guinea pig ileum-myenteric plexus and vas deferens and rat hippocampal slices. We recorded the contraction of the ileal longitudinal muscle to study the postsynaptic effects, and monitored the release of norepinephrine using the superfusion technique to study the presynaptic effects in the latter two preparations. The rank order of potency of agonists suggests that the predominant type of nAChRs present in the presynaptic noradrenergic nerve terminals of the rat vas deferens and hippocampus, as well as those present on other nerve terminals are composed of $\alpha 3\beta 2$ subunits, whereas in the guinea-pig myenteric plexus the somatodendritic nAChRs are composed of $\alpha 4\beta 2$ subunits.

Akadémiai Kiadó, Budapest

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DIFFERENTIAL RESPIRATORY MODULATION OF SYMPATHETIC A- AND C-REFLEXES IN ANAESTHETIZED CATS

Mitsányi, A., Fedina, L.*, Pelczer, K.*

Department of Physiology, Semmelweis University Medical School, P.O.B. 259, H-1444 Budapest and *National Insitiute of Neurosurgery, P.O.B. 25, H-1426 Budapest, Hungary

The classic pattern of a somato-sympathetic reflex picked up in a sympathetic postganglionic efferent nerve in response to electrical stimulation of a spinal nerve consisted of an initial burst of discharges and a subsequent "silent period" (Sell et al. 1958). Later on, it has been shown that in certain conditions stimulation of A+C fibre afferents elicits a second burst of longer latency, too (Fedina et al. 1966). The two distinct volleys have been termed sympathetic A-reflex and C-reflex, respectively. In our experiments, however, instead of the two volleys often a C-reflex alone could also be observed, although both A- and C-fibre afferents had been excited. In order to investigate the cause of this differential extinction of a somato-sympathetic reflex component, experiments were performed on cats anaesthetized with various mixtures of chloralose and urethane (33-50 and 133-800 mg/kg, respectively) and immobilized with pipecuronium bromide (60 μg/kg). Artificial ventilation was adjusted to maintain end-tidal CO_2 at about 4%. Electrical activities of renal and vertebral sympathetic postganglionic efferents, ECG, arterial blood pressure, ventilation, and capnogram were continuously recorded. Somato-sympathetic reflexes were elicited by repetitive electrical stimulations (series of supramaximal single impulses or short trains of stimuli [3-5 impulses at 50-100 Hz]) of the tibial, brachial, superficial peroneal nerves and the nerves innervating the gastrocnemius-soleus muscles. The results have shown that a ventilation-locked suppression of A-reflexes along with a maintainance of C ones prevails in the evoked activity of the renal sympathetics. Stimulation of afferents of various origins, however, leads to further differentiation in the ventilatory modulation of sympathetic outputs of different destinations. The undoubted participation of second order blood pressure wave-induced activation of circulatory baro-receptors does not seem to be exclusive in the inhibition of somato-sympathetic reflex components.

REFERENCES

Sell, R., Erdélyi, A., Schaefer, H.: Untersuchungen über den Einfluss peripherer Nervenreizung auf die sympathische Aktivität. *Pflügers Arch.* **267**: 566-581 (1958).

Fedina, L., Katunskii, A.Ya., Khayutin, V.M., Mitsányi, A.: Responses of renal sympathetic nerves to stimulation of afferent A and C fibres of tibial and mesenterial nerves. *Acta Physiol. Hung.* **29**: 157-176 (1966).

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DIMENSIONAL ANALYSIS OF THE EEG AND EVENT-RELATED POTENTIALS IN STROKE PATIENTS

Molnár, M.[1], Karmos, G.[1], Gács, Gy.[2] and Skinner, J. E.[3]

[1]Institute for Psychology, Hungarian Acad. Sci., Budapest, [2]Péterffy S. Hospital, Budapest, [3]Totts Gap Med. Res. Laboratories, Bangor, USA

The origin of the endogenous event-related potentials is still not clear. It is generally presumed that both the hippocampal formation and the temporoparietal cortex play an important role in this event. The objective of the present study was to calculate the point-correlation dimension of the EEG and auditory evoked potentials in patients with circumscized lesions caused by stroke. By the application of these measures inferences can be drawn as to the functional state of the cortical areas mapped by the above methods.

The case of a 64 year old female is presented who suffered stroke in the area of the right lentiform nucleus and internal capsule caused by embolism from the heart. A moderate hypodensity was shown by CT scan in this region.

The EEG and auditory event-related potentials were recorded using 22 scalp electrodes. Compared to normal individuals, a lower dimensional area was spotted in the right parietal region suggesting the malfunctioning of this region. No P3 event-related component was seen in the right temporoparietal area. These observations suggest that 1) the application of the methods of chaos theory may provide valuable data on the functional state of the nervous system and 2) other areas than previously suggested may play an important role in the generation of the P3 event-related component.

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INBORN SOCIAL COMPETENCE: THE PHENOMENON AND ITS MECHANISM

Nagy, E. and Molnár, P.

Albert Szent-Györgyi Medical University, Department of Medical Psychology, Szeged, Hungary

The problem: While examining the phenomenon of ***newborn imitation, human imprinting*** it appeared, that certain newborns - besides being capable to imitate, also initiate gestures "waiting for" an answer (i.e. the examiner's imitation). We tried to establish the reliability and to map the mechanism of the above ***"provocation phenomenon".***

Methods: In two experiments the subjects were 28 (Experiment 1.) and 16 (Experiment 2.) infants, respectively, ranging in age from 3,5 hours to 40 hours. Each infants was shown 5 gestures in a random order and all potential responses, including free interactions were videorecorded and the records were later rated by 32 independent observers. During Experiment 1. the infant' heart rate changes were also measured using disposable chest electrodes.

Results:

1./ In Experiment 1. from the 28 infants 5, while in Experiment 2. from the 16 infants 6 initiated provocative actions. The independent observers could reliably differentiate the 11 provocative actions from the imitative ones. ($p < 0.0001$).

2./ The provocative actions (in Exp. 1.) were usually accompanied by strong heart rate deceleration. The group of these newborns can be well distinguished from the rest -i.e. the only imitators- by measuring average heart rate: the "provocative" subjects were characterized by significantly lower average heart rate.

Conclusion: The above described provocation phenomenon is a new challenge for our present conception of human nature. It shows, that competence -originally described in Erik Erikson's theory- is present from birth, in the form of ***inborn social competence***.

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DISTRIBUTION OF EXCITATORY AMINO ACID RECEPTORS IN THE RAT SPINAL CORD

Nagy, I.

Department of Anatomy, University Medical School of Debrecen, 4012 Debrecen, Hungary

A cobalt uptake method has been used to study the morphology and distribution of spinal cord neurons expressing α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, or N-methyl-D-aspartic acid (NMDA) excitatory amino acid receptors in the lumbar enlargement of the rat spinal cord. The technique involved perfusion of hemisected spinal cords of 14 day old rat pups in vitro with excitatory amino acid receptor ligands in the presence of $CoCl_2$. Cobalt has been shown to enter cells through ligand-gated ion channels in place of Ca^{++}. Cells which accumulated cobalt ions following activation by ionotrophic excitatory amino acid receptors were visualised histochemically. The cobalt uptake generated receptor specific labelling of cells, as the NMDA receptor antagonist D-(-)-2-amino-(5)-phosphonovaleric acid (D-AP-5) (20μM) blocked the NMDA, but not kainate induced cobalt uptake. The kainate induced cobalt labelling was reduced by the non-selective excitatory amino acid receptor antagonist, kynurenic acid (4mM). Passive opening of the voltage-gated Ca^{++}-channels by KCl (50mM) did not result in cobalt uptake indicating that cobalt enters the cells through ligand-gated Ca^{++}-channels.

AMPA (500μM), kainate (500μM) or NMDA (500μM) each induced cobalt uptake with characteristic patterns and distributions of neuronal staining. Overall, kainate induced cobalt uptake in the greatest number of neuronal perikarya while NMDA induced uptake was the lowest. α- Amino-3-hydroxy-5-5methyl-4-isoxazole propionic acid and kainate, but not NMDA superfusion, resulted in cobalt labelling of glial cells.

Our results show that the cobalt uptake technique is a useful way to study the morphology and distribution of cells expressing receptors with ligand-gated Ca^{++} channels.

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THE CELLULAR AND SUBCELLULAR LOCALIZATION OF TWO METABOTROPIC GLUTAMATE RECEPTORS (mGluR1a AND mGluR5a) IN THE RAT CEREBELLAR CORTEX

Négyessy, L.[1], Vidnyánszky, Z.[1], Görcs, T.[1], Kuhn, R.[2], Knöpfel, T.[2] and Hámori, J.[1]

[1]First Department of Anatomy, Neurobiology Laboratory, Semmelweis University Medical School, 1450 Budapest, Hungary. [2]Department of Molecular and Cellular Biology, CNS Research, Ciba, CH-4002 Basel, Switzerland

The cellular and subcellular localization of the two inositol phosphate second messenger linked metabotropic glutamate receptors (mGluR), mGluR1a and mGluR5a were identified in the rat cerebellar cortex on both light, and electron microscopic levels using preembedding immunoperoxidase and/or immunogold techniques.

The light microscopic observations revealed different distribution of the two receptors. mGluR1a characteristically resulted in strong neuropil labelling in the molecular layer. In contrast, mGluR5a immunoreactivity (IR) was found to label perikarya and dendritic trees of a population of interneurons.

Electron microscopically, the majority of mGluR1a IR were found to localize in dendrites and dendritic spines of the calbindin immunopositive Purkinje cells on double labelled sections. Furthermore, using preembedding immunogold method, we found that in the neuropil both mGluR-IRs were in association with the cell membrane, i.e. at the periphery of the postsynaptic specializations as well as extrasynaptically.

Our findings give further morphological evidence of the involvement of different mGluRs within circuits of the cerebellar cortex.

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CORTICOSTEROID RECEPTORS, BRAIN DEVELOPMENT AND BEHAVIOR

Nyakas, C., Felszeghy, K. and Gáspár, E.

Central Research Laboratory, Haynal University of Health Sciences, H-1389 Budapest, P.O.B. 112, Hungary

Brain corticosteroid receptors are known regulatory factors of pituitary-adrenal function and adaptive behavior. Since neonatal hormonal manipulations may cause alteration in adult behavior, we studied the connection between long-term changes in brain receptor parameters and the modified behavior of adult rats following neonatal dexamethasone (DEX) and ACTH peptide treatments.

Changes in binding characteristics of corticosteroid receptors were examined in different brain areas in rats treated sc. with 1 μg/g DEX or ACTH 4-9 analogue (ORG2766) on postnatal days 1, 3 and 5. [3H]-corticosterone (CORT) binding capacity (Bmax) and affinity (Kd) were determined by using saturation analysis at 3 weeks age and at adult ages of 3 and 16 months in hippocampus, striatum, amygdala and pituitary gland. Mineralocorticoid type receptors (MR) and glucocorticoid receptors (GR) were measured separately with single point analysis applying a selective glucocorticoid ligand RU 28362. Psychomotor activity was tested in adult age in three situations: water immersion test (Porsolt), open- and colsed-field tests.

The behavioral results showed that DEX treatment resulted in behavioral depression in the Porsolt's test as well as other activity tests. The effect of ACTH peptide was an opposite in nature. Neurochemical results showed that DEX decreased the number of CORT binding sites in all structures investigated. Furthermore, DEX permanently down-regulated GR, while left intact MR development. Contrary to that, the ACTH peptide up-regulated MR but did not influence GR development.

In conclusion: 1) Both neonatal hormone treatments induced long-term and selective modulation of development of one or another type of CORT receptors. 2) The behavioral and receptorial findings appear to be functionally interrelated.

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INVOLVEMENT OF GHRH IN THE SLEEP PROMOTING-ACTIVITY OF INTERLEUKIN-1β

Obál, F. Jr.[1,2], Fang, J.[2], Payne, L. C.[3] and Krueger, J. M.[2]

[1]Department of Physiology, A. Szent-Györgyi Medical University, Szeged, Hungary; [2]Department of Physiology and Biophysics, University of Tennessee, Memphis, USA; [3]Department of Physiology, University of Alabama, Birmingham, USA

The aim of the experiments was to study the interaction between two sleep factors, the hypothalamic neurocrine, growth hormone-releasing hormone (GHRH), and the cytokine, interleukin-1β (IL1), in the promotion of sleep.

Previous experiments showed that icv or systemic administration of GHRH enhances non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) in rats and rabbits, whereas inhibition of endogenous GHRH by means of a competitive antagonist or antibodies suppresses sleep. The sleep-promoting activity of GHRH was confirmed in human subjects. GHRH is regarded as a physiological sleep-promoting substance. Increases in sleep have been reported in response to IL1 in various species. Only small doses of icv IL1 enhance sleep in the rat; high doses suppress sleep. IL1 may contribute to physiological sleep regulation, and IL1 is also a major mediator of the somnolence accompanying infectious diseases. Various endocrine actions have been attributed to IL1, including the stimulation of growth hormone (GH) secretion. We report here two experiments describing the interaction between IL1 and GHR:

1. IL1-induced GH secretion was studied in rats after pretreatment with antibodies to GHRH (GHRH-ab). Blood samples were obtained from freely moving rats via a chronic intracardial catheter. Somnogenic doses of IL1 (1 or 2.5 ng) elicited a significant GH release 15-30 min postinjection. High doses of IL1 (10 or 25 ng) inhibited GH secretion. GHRH-ab suppressed IL1-induced GH secretion.

2. The sleep response and the febrile response (brain temperature recording) to IL1 were studied in rats pretreated with GHRH-ab or control IgG. In the rats pretreated with control IgG, IL1 enhanced the NREMS duration and sleep intensity (EEG slow wave activity) for 4-6 hours and caused fever for 6 hours. GHRH-ab abolished the IL1-induced enhancements in sleep duration, and attenuated the increases in slow wave activity. GHRH-ab also attenuated the IL1-induced fever; this effect was attributed to a suppression of somatostatin following the immunoneutralization of GHRH.

The results suggest that GHRH is involved in the mediation of the GH release and enhanced sleep elicited by IL1.

References:

Payne, L. C., Obál, F. Jr, and Krueger, J. M.: Stimulation and inhibition of growth hormone secretion by interleukin-1β: the involvement of GHRH. Neuroendocrinology, 56(1992) 118-123

Obál, F. Jr, Fang, J., Payne, L. C. and Krueger, J. M.: Growth hormone-releasing hormone (GHRH) mediates the sleep promoting activity of interleukin-1 (IL1) in rats. Neuroendocrinology, in press (1995)

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THE ROLE OF THE THALAMOCORTICAL FEEDBACK IN THE CORTICAL SYNCHRONIZATION

Orzó L., Vidnyánszky Z.

SOTE, 1st Dept. of Anatomy, MTA-SOTE-EKSZ, Neurobiology, Budapest, Hungary

There are several theories which can utilize the synchronous discharge of the subpopulations of cortical cells. This type of synchronization can bind different representations of the same object (binding problem, Singer), it can help to anticipate the motion of an object, or it can facilitate to organize the developing cortical interconnection patterns (Singer). They could measure synchonous (40 Hz oscillaton) activity, with zero time lag, from the cat visual cortex. They expected that the cortical synchronization originates solely from the cortical interactions. It has been recently shown that the cortical activity synchronizes the thalamical spike discharge (Sillito). We tried to demonstrate, that the synchronous activity in the cortex and the thalamus can derive not only from the cortical interactions but it can originate from the corticothalamic feedback loop as well. We made a simple model of the thalamocortical loop. We used a cellular neural network CNN (Roska) as a modeling frame. The analog computation and local connectivity made the CNN a useful frame to model the subthreshold and spiking activity of the cells and the topographic maping. The spiking activity of the cells were accomplished by UPG (Labos), a simple itterative alghorithm, which generate spikes by intervall mapping. We tried to emulate the in vivo really existing interconnection pattern of the cortex and of the thalamus. For the synchronization of this type of units a end strong excitation is necessary. This brief excitation can be achived only by a direct excitation and a lightly delayed inhibition. This activation pattern can be accomplished by the direct corticothalamic feedback on the relay cells and a delayed inhibition through the LGN interneurons. The brief excitation is sampling the sensory input and makes the thalamic output more synchronous and increases the cortical synchronization.

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SUBCORTICAL INNERVATION OF DIFFERENT VIP-CONTAINING INTERNEURON TYPES IN THE HIPPOCAMPUS

Cs. Papp, E., Acsády, L., Hájos, N. and Freund, T. F.

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest POB 67, H-1450, Hungary

VIP-immunoreactive interneurons (IN) were shown earlier to form three anatomically and neurochemically well characterized cell populations in the hippocampus. Two of these types establish synaptic contacts selectively with other GABAergic interneurons of the hippocampus. Neurons of the third type innervate pyramidal cell bodies and proximal dendrites, and are therefore called basket cells. The medial septal (MS) and median raphe (MR) afferents are known to innervate interneurons in the hippocampus selectively. The median raphe afferents distinguish even among types of inhibitory cells, they innervate interneurons involved - either directly (calbindin) or indirectly (calretinin) - in dendritic inhibition of pyramidal cells. Here we examined whether VIP-containing interneurons involved in inhibition or disinhibition of pyramidal cells were among the targets of these subcortical pathways, and whether either pathway would distinguish among the three VIP-positive cell types.

The anterograde tracer PHAL was injected into either the MS or the MR and the innervation of different VIP-containing cells was examined using immunocytochemical double-staining. In agreement with our earlier results GABAergic MS fibres established multiple contacts with all types of interneurons examined, including the three VIP-containing cell types. MR axons also innervated all types of VIP cells, but formed only single or double contacts with most of their targets. MR afferents more often innervated dendrites than somata of their postsynaptic targets, unlike the MS input.

Our results demonstrated that neither subcortical pathways distinguish among the functionally different VIP-containing interneuron types, and equally modulate perisomatic as well as dendritic inhibition of pyramidal cells. However, the direction of modulation by MR axons may be different, basket cells are likely to have $5HT_3$ receptors allowing an excitatory effect of serotonin, whereas interneurons exerting dendritic inhibition may be inhibited by serotonin via $5HT_{1A}$ receptors.

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PLASTIC CHANGES IN THE ARCUATE NUCLEUS: ESTRADIOL EFFECT IS ACCOMPANIED BY INCREASED EXO-ENDOCYTOTIC ACTIVITY OF NEURONAL MEMBRANES

Parducz, A.[1], Szilagyi, T.[1], Hoyk, S.[1] and Garcia-Segura, L.M.[2]

[1]Institute of Biophysics, Biological Research Center, Szeged, Hungary
[2]Instituto Cajal, CSIC, Madrid, Spain

Recent studies have indicated that the organizational effects of gonadal steroids on neuronal connectivity is not limited to developmental stages. In the hypothalamic arcuate nucleus the number of axo-somatic synapses fluctuates during the ovarian cycle and experiments on ovariectomized animals clearly show that estradiol plays a decisive role in this phenomenon. It was found that 24 hours after estrogen treatment there is a transient and significant decrease in the number of GABAergic axo-somatic synapses and simultaneous increase in glial covering of perikaryal membranes. The synaptic changes in arcuate nucleus are accompanied by dramatic modifications in the ultrastructure of neuronal plasma membrane as well, and our hypothesis is that the hormonal induction of synaptic plasticity may be, at least in part, the result of an effect of estradiol on the distribution/internalization of adhesion or recognition molecules in neuronal membranes. The coated vesicles are thought to be the cell organelles which may transport these molecules to and from the membranes, therefore we performed experiments to determine if there is a change in the number of coated vesicles and coated pits during development, sexual differentation and following estrogen treatment i.e. during the synaptic remodelling.

The present results clearly show that in estradiol treated animals the number of coated pits and vesicles is increased. In freeze-fracture replicas the increased number of exo-endocytotic pits are also signalling a more active transport or turnover of perikaryal membrane constituents.

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SYNTHESIS AND INVESTIGATION OF ß-AMYLOID PEPTIDES

Penke, B.[1], Törő, I.[1], Soós, K.[1], Varga, J.[1] and Baranyi, A.[2]

Department of Medical Chemistry, [1]Szent-György Medical University, H-6720 Szeged, Hungary and [2]Department of Animal Physiology, Attila József University, H-6725 Szeged, Hungary

Alzheimer's disease is the most frequent form of senile dementia. Neurotoxic ß-amyloid peptides might trigger the development of the disease. In our laboratory human ß-amyloid polypeptide 1-40 and 1-42, two analogs ([Gln]^{22} 1-42, [Glu]^{15} 1-42) and rat ß-amyloid 1-42 were synthesized. Two other fragments of the amyloid precursor protein (APP) with 47 and 68 amino acid residue (APP 609-655 and APP 588-655) were also synthesized for protease experiments. As natural ß-amyloid peptide show a very low water solubility, we have synthesized 4 new analogues containing hydrophilic amino acids. These analogues do not aggregate and show an excellent water solubility.

Each ß-amyloid peptides proved to be neurotoxic in brain tissue culture. In vivo, on cat brain cortical neurons it was proved that the synthetic fragments from the ß- (32-35) to the ß-amyloid 1-42 bind to the neuronal NMDA-receptors and cause a Ca^{2+}-influx into the cell. The Ile-Gly-Leu-Met-NH_2 tetrapeptide is the shortest neurotoxic ß-amyloid peptide, it might be the active center of the molecule. Permanent Ca^{2+}-influx causes death of neurons by known biochemical mechanisms. Neuron death releases a high concentration of the otherwise neuroprotective protein APP into the extracellular space. The relatively low protease activity in the brain of elderly people may results in a high concentration of water-insoluble and neurotoxic ß-amyloid 1-40 to 1-43 peptides which precipitate forming diffuse plaques. A permanent release of the neurotoxic amyloid peptides from the plaques might be responsible for the maintenance of neuronal death. (Our protease experiments with the 47 and 68 amino acid APP fragments support this hypothesis).

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SPECIFIC GLYCOPROTEINS IN THE RAT OLFACTORY BULB

Pestean, A., Krizbai, I., Parducz, Á., Joó, F. and Wolff, J.

Institute of Biophysics, Biological Research Centre, H-6701 Szeged, Hungary, and
*Zentrum Anatomie, Georg August Universitat, Kreuzbergring 36, Göttingen, Germany

The structure and function of proteins are largely determined by posttranslational modifications such as glycosylation. The carbohydrate chains attached to a given protein in many cases are tissue specific. Previously it has been shown that the rat olfactory bulb is specifically stained by the Ulex europaeus agglutinin I (UEA I), which recognizes the Fuc (α1-2)Gal(β1-4)GlcNAc- structure (or the blood determinant H type 2).

By using electron microscopy we could demonstrate that the UEA I reactivity is restricted to the axonal membrane of the primary olfactory neurons. In order to determine to which protein is attached the oligosaccharide recognized by UEA I, we have performed Western blotting experiments using UEA I and TPA (Tetragonolobus purpureus agglutinin) lectins, as well as anti-N-CAM antibodies and a monoclonal antibody (2B8) which recognizes a glycoform of a neural cell adhesion molecule in rats and stains specifically the accessory olfactory bulb.

On blots, the UEA I recognizes a single protein band with an electrophoretic mobility of approximately 215 kDa which overlaps with a band obtained with the anti-N-CAM polyclonal antibody and with the 2B8 monoclonal antibody.

Our data raises the possibility that the protein carrying the H type 2 determinant is a particular glycoform of the neural cell adhesion molecule (N-CAM), confined to the olfactory system in rats.

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CHARACTERISTICS OF AUDITORY EVOKED POTENTIALS IN ALERT AND ANESTHETIZED MACAQUE MONKEYS

Pincze Zs., Ulbert I., Csonka P., Karmos G.

Institute for Psychology of the Hungarian Academy of Sciences, H-1394 Budapest, Hungary

One of the fundamental organizational principles of the mammalian auditory cortex is the tonotopic representation of the stimulus. The auditory cortex of the macaque monkey is located in a hidden position similarly to the human auditory cortex. In the present study, auditory evoked potentials (AEP) to tone stimuli were recorded from chronically implanted epidural electrodes in two macaque monkeys in awake and anesthetized conditions. Furthermore, AEPs were mapped in six intact pentobarbital anesthetized monkeys using 15 needle-electrodes - inserted into one side of the scalp - and contralateral ear reference. Tone stimuli were delivered to the contralateral ear. We used four different frequencies (500 Hz, 1 kHz, 2 kHz, 4 kHz), the other parameters of the tones were identical (loudness=70 dB peSPL, duration=200 ms, rise and fall time=5 ms, ISI=1 s). The state of the animal was monitored by EEG. Colour maps were made from the averaged AEPs. Although the responses to the different frequency tone stimuli did not show conspicuous differences in their distribution, obvious difference was seen comparing the maps. An early positive component with posterior amplitude maximum was distinguished from the large amplitude 17-22 ms peak latency positive component which had a fronto-central amplitude maximum. Its scalp distribution depended mostly on the frequency. In the case of 500 Hz it showed its maximum amplitude near the frontal pole, while the maximum of this component elicited by 4 kHz was shifted caudally to the central area.

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THE TERMINATION PATTERN OF AXONS ARISING FROM THE A5 NORADRENERGIC CELL GROUP IN THE RAT BRAINSTEM AND SPINAL CORD

Polgár, E., Sherdel, F., Berki, Á. Cs. and Antal, M.

Department of Anatomy, University Medical School of Debrecen, H-4012 Debrecen, Hungary

The efferent connections of the A5 noradrenergic cell group which play an important but still poorly defined role in sensory and motor functions as well as autonomic regulation were studied in the rat brainstem and spinal cord by using the anterograde neural tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L). After iontophoretic injection of PHA-L into the caudal portion of the A5 nucleus, labelled fibres and terminals were found in several areas of the brainstem and spinal cord. After arising from the A5 cell group, many labelled axons crossed the midline at various levels of the brainstem, while others coursed ipsilateral to the injection site. Both crossed and uncrossed axons formed ascending and descending fibre tracts that emitted several collaterals to various regions of the brainstem and spinal cord. Ascending fibres extended as rostral as the caudalmost regions of the diencephalon, whereas the most caudally projecting axons reached the level of the thoracic spinal cord. In the diencephalon abundant projections were seen in the zona inzerta, and area of Forel mostly contralateral to the injection site. At the level of the brainstem, most of the terminals were revealed in somatomotor and visceromotor nuclei of cranial nerves (principal motor nuclei of oculomotor, trigeminal, abducens, facial nerves; nucleus ambiguus; accessory motor nuclei of the trigeminal, abducens, facial nerves; superior salivatory nucleus; lacrimal nucleus of the facial nerve) but some fibres projected also to the lateral parabrachial area, pretectal nuclei, interstitial nucleus in the mesencephalon as well as the nucleus of the Probst bundle and the vestibular nuclei in the pons. In the spinal cord, labelled fibres descended in the ipsilateral ventral, and contralateral ventral and dorso-lateral funiculi and terminated in the ventral gray matter (laminae VII-IX) and the intermedio-lateral cell column.

The termination pattern of labelled fibres suggests that the A5 cell group has the potential to exert a significant influence on various somatomotor and autonomic functions both in the brainstem and spinal cord, but presumably has a less substantial, if any, direct effect on sensory information processing.

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PROCESSING OF LUMINANCE, TEXTURE AND KINETIC BOUNDARIES IN THE MONKEY INFERO-TEMPORAL CORTEX

Sáry, Gy.[1], Vogels, R.[2], Kovács, Gy.[1] and Orban, G. A.[2]

Department of Physiology, Albert Szent-Györgyi Medical University, H-6725 Szeged, Hungary[1],
Laboratorium voor Neuro-en Psychofysiologie, K.U. Leuven, B-3000 Leuven, Belgium [2]

1. We recorded from neurons responsive to gratings in the inferior temporal cortex of macaque monkeys. One of the monkeys performed an orientation discrimination task while the other maintained fixation during stimulus presentation. Stimuli consisted of gratings based on discontinuities in luminance, relative motion and texture.

2. IT cells responded well to gratings defined solely by relative motion, implying either direct or indirect motion input into IT, an area which is part of the ventral visual cortical pathway.

3. Response strength in general did not depend on the cue used to define the gratings. Latency values observed for the two static grating types (luminance and texture defined gratings) were similar, but significantly shorter than those measured for the kinetic gratings.

4. Stimulus orientation had a significant effect in 27%, 27%, and 9% of the cells tested with luminance, kinetic and texture defined gratings, respectively.

5. Only a small proportion of cells were orientation sensitive for more than one defining cue. The average preferred orientation for luminance and kinetic gratings matched, while the tuning with was similar for the two cues.

6. Our results indicate that IT cells may contribute to cue-invariant coding of boundaries and edges. The relevance of these results to visual perception is discussed.

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USE OF GAD-*lac*Z TRANSGENIC MICE IN HOMOTOPIC OLFACTORY BULB TRANSPLANTATION- *lac*Z-POSITIVE CELLS OF THE GRAFT IDENTIFIED BY X-Gal STAINING

Sekerkova, G.[1], Katarova, Z.[1], Joo, F.[2] and Szabo, G.[1]

[1]Institute of Biochemistry and [2]Institute of Biophysics, BRC, Szeged, Hungary

We have derived nine transgenic lines, carrying the marker gene *lac* Z under promoter control of the mouse gene, coding for L-glutamic acid decarboxylase(GAD), the biosynthetic enzyme for GABA. Preliminary experiments suggested, that the β-galactosidase, detected by X-gal histochemistry in the central nervous system (CNS) of mice, carrying the transgene, behaves as an intrinsic cellular marker even when expressed at ectopic sites. This promted us to use the GAD-*lac* transgenic mice in different transplantation paradigms, as models to study the neuronal survival and plasticity in the CNS. This presentation is focused on the results from a homotopic transplantation of embryonic (E16-E17) olfactory bulbs (OB), derived from thransgenic line *TgGAD*1 into unilaterally bulbectomized neonatal (*B57Bl6*♀*xCBA*♂)F1 mice. In the donor OBs, β-galactosidase was ectopically expressed in the tufted (TC) and mitral (MC) cells. Three months following transplantation, saggital sections from the transplanted and control sides were processed for β-galactosidase histochemistry and olfactory marker protein (OMP) immunohistochemistry. The transplanted tissue was clearly distinguished by its disorganised and randomly distributed OMP- positive *de novo* formed glomeruli-like structures. The large output neurons (TC, MC) found throughout the whole transplant were clearly identifiable by the impressive X-gal staining, which compares to Golgi impregnation. The arborization of their processes varied from cell to cell. Besides the neurons with long dendrites, the terminals of which ramify in more distal glomeruli, we found cells with very short dendrites, arborizing within the closest glomerulus. Ocasionally, their axons, projecting to the pyriform cortex could be traced. The high metabolic activity of the transplanted tissue was illustrated by cytochrome oxidase histochemistry . This clearly demonstrates a tendency by the large output neurons towards a restoration of the perturbed connections with their natural targets.
In conclusion, we have used a novel approach, which allows us to follow the fate of intrinsically marked neurons months after transplantation to a homotopic site. The potentials of this method are practically unlimitted, given the fact, that the product of the X-gal reaction could be visualised under EM and the large spectrum of anatomical sites, expressing β-gal in the different lines.

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PLASTICITY IN THE X-RAY IRRADIATED RAT HIPPOCAMPUS

Seress, L., Czéh, B. and Horváth, Zs.

Department of Physiology, University of Medical School Pécs, H-7643 Pécs, Hungary

Wistar rats of different ages were X-ray irradiated (600 rad). For histologic study Nissl, Timm and Golgi staining were used. Animals were sacrificed at different postnatal ages between 30 days and 3 months.

Radiosensitivity of neurons depends on their postnatal developmental stage. Neonatal X-ray irradiation induces a selective reduction of granule cells by 85%, whereas irradiation at day 5, 10, 14 results a reduction by 60, 45 and 35%, respectively. Number of pyramidal neurons in CA1-3 areas were never reduced. However, in the case when 85% of granule cells were destroyed the number of hilar neurons were also reduced by half.

Timm staining of the hippocampus of the irradiated anilmas revealed a capacity for plastic changes, because mossy fiber terminals of 20% of granule cells occupied half of the stratum lucidum, whereas mossy terminals of 50% of granule cells appeared to fill the entire width of stratum lucidum. In those rats which were irradiated at birth, the apical dendrites of CA3 pyramidal cells formed a few or no thorny excrescences. Most of the dendritic surface was smooth, displayed no spines and received no synapses.

Granule cells of the irradiated dentate gyrus did not display more symmetric axosomatic synapses, than normal animals do, but frequency of the asymmetric axosomatic synapses was increased. Many of them were found on somal spines, which are rare in normal animals.

Kindling in X-ray irradiated animals induced sprouting of mossy fibers in the stratum moleculare of the dentate gyrus.

Present results indicate that several plastic changes occure in the irradiated rat hippocampus and the axonal growth may be limited by the parent neuron and not decisively by the available postsynaptic surface of the target area.

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STRUCTURAL ANALYSIS ON EXTRACELLULAR RECORDS OF CEREBELLAR PURKINJE CELLS

Simon, A., Laczkó, J., Csótai, J. and Simon, L.

1st Dept. of Anatomy, Semmelweis Med. Univ., Budapest, Hungary

Spontaneous extracellular activity patterns of cerebellar Purkinje cells (Pc-s) of adult cats were analyzed on the base of a proposed (5) nonlinear, phase-sensitive compound spike generating mechanism.

In addition to the common type evaluation (interspike interval and peri-CFR histogram, etc.) first the Markovian structure of the interval sequence was analyzed, then compared to the local average frequency.

The revealed alternating sections of basically different types of the spiking pattern indicate the fluctuating inhibitory control of Pc by basket and outer stellate cells.

From "irregular" spike sequence periods (2) of negative Markovian feature, trials were made to separate two rhythmic patterns, resulted from the (generally) double spike generating process, referring independent dendritic regions of the Pc (5).

Dendritic records giving birth to the extracellular activity patterns (1, 3) were also analyzed and matched against the above described extracellular features. The possible basic phenomena of sensorimotor adaptive processes were then analyzed on the tachogram and phase-shift diagram (4) of the independent, basically rhythmic processes in the surrounding of the climbing fiber response.

References:

1, Bell, C. C. and Grimm, R. J.: Discharge properties of Purkinje cells recorded on single and double microelectrodes. J. Neurophysiol. 32 (1969) 1044-1055

2, Braitenberg, V., Gambardella, G., Ghigo, G. and Vota, U: Observations on spike sequences from spontaneously active Purkinje cells in the frog. Kybernetic 2/5 (1965) 197-205

3, Martinez, F. E., Crill, W. E. and Kenedy, T. T.: Electrogenesis of the cerebellar Purkinje cell responses in cats. J. Neurophysiol. 34 (1971) 348-356

4, Simon, L.: Modification by climbing fiber response of spike generators of deteriorated Purkinje cells. In: Szentágothai, J., Hámori, J. and Vizi, E S., Eds. Neuron Concept Today. Akadémiai Kiadó; Budapest. 1977: 241-248

5, Simon, L.: Complex integrative properties of the cerebellar Purkinje cell suggest to postulate an asymmetric organization in the dendritic input. In: Székely, Gy. et al. eds. Neural Communication and Control. Akadémiai Kiadó, Budapest, 1981. 249-254

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ALTERNATIVE CUES FOR THE CEREBELLAR RESEARCH

Simon, L., Simon, A. and Laczkó, J.

1st Dept. of Anatomy, Semmelweis Med. Univ., Budapest, Hungary

New preferences for cerebellar research and modeling are proposed to replace the..

Generally accepted dubious ideas, like..

a, the cortico-ponto-cerebellar pathway conveys informations only about ongoing motor commands,

b, the mossy fiber input should build up a patch-like or focal, obviously clearcut map-like somatotopic organization on the cerebellar cortex,

c, the input context on the dendritic tree of the Purkinje cell (Pc) is symmetric (or probabilistic),

d, the dendritic tree of the Pc is a morpho-physiologically consistent body, giving place to linear integration,

e, the local connection of the Pc early axon arborization is (at least transfolially) symmetric,

f, sensorimotor adaptive/learning processes should be based either on the Marr-, or on the Albus-hypothesis and

g, the only chronic function of the interior olivary input to the Pc is the gradual rearrangement of synaptic efficacy at parallel fiber Pc-dendritic contact for a new sensimotor task/skill.

Proposal A – Emerging new context of the functional organization of cerebellar input- and intracortical connectivity, like..

a, the dendritic tree of the Pc generally separates to two main compartments (4), by function-related morphologic parameters,

b, the generally double, nonlinear and phase-sensitive spike generating integration (6, 8) is inherently sensitive to slight differences in the input context of the two main dendritic compartments, and

c, the sagittal, but more the transfolial asymmetries (7, 9) revealed in the local connections for the Purkinje cell, "eo ipso" (via inhibitory and Pc to Pc interconnections) perform a basically asymmetric neighbourhood of Pc,..

.. all argue upon systematic analysis of mossy fiber input organization as for a gradually changing meshwork with substantial overlap among proprioceptive sensory channels and also the cortico-ponto-cerebellar input system.

C, A new conceptual model of the cortico-ponto-cerebellar pathway (12):

All along the history of the cerebellar research it was the "skeleton in the cupboard" that the vast majority of the mossy fiber input comes from cerebral cortex regions (via pontine nuclei) other than just from the motor areas.

As a possible paradigm for the future research, one can assume the entire brain function being based on dynamic macro-patterns of reflex-like processes.

In that case the cerebellum can also serve as the general adaptive processor of "higher" nervous functions.

D, Alternative cues for research and modeling (3, 5):

a, Cerebellar paradigm may be related to brain functions beyond adaptive motor control. The significance of the entire ponto-cerebellar pathway should be the subject of cerebellar research (12)

b, The mossy fiber input organization should be analysed mostly for overlapping (10) of the different posture-, and motion-referring channels.

c, A rather continuous gradual changing in the input context, different for the (generally) two Pc dendritic compartment is to be revealed.

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d, Statistical morphometric analysis should be focused on dendritic main compartments, updating multicompartmental neuron modeling (4).

e, Asymmetries in the local axonal arborization of Pc specified for subdivisions should be taken into consideration by modeling. (7, 9),

f, Permanent Heterosynaptic Modification (PHM) concurrently uses both the Marr (2) and the Albus (1) paradigm.

The strategy of consecutive modifications (11) seems to be the key element for a new understanding of the cerebellum.

g, A role of the climbing fiber response (CFR) could mean not only to chronically build up, but also to promptly actualize/load (12) formerly elaborated Body-Environment-Model (BEM)-relared (10) sensimotor programs/routines.

References:

1, Albus, J. S.: A theory of cerebellar function. Math. Biosci. 10 (1971) 25-61

2, Marr, D.: A theory of the cerebellar cortex. J. Physiol. 202 (1969) 437-470

3, Pellionisz, A. and Llinás, R.: Analysis of simple and complex spike generator mechanism of dendritic trees: a computer model of Purkinje cell. In: Szentágothai, J. et al. Eds. Neuron Concept Today. Budapest: Akadémiai Kiadó (1977) 195-202

4, Sarkisian, V. A., Simon, L. and Martos, J.: Dendritic space-compartment (DSC)-oriented asymmetries in the local connections of cerebellar Purkinje cell [2302P]. II World Congr. of Neurosci. 1987; Budapest

5, De Schutter, E. and Bower, J. M.: An active membrane model of the cerebellar Purkinje cell: I. Stimulation of current clamps in slice. J. Neurophysiol. 71/1 (1994) 401-419

6, Simon, L.: Modification by climbing fiber response of spike generators of deteriorated Purkinje cells. In: Szentágothai, J., Hámori, J. and Vizi, E. S., Eds. Neuron Concept Today. Budapest: Akadémiai Kiadó (1977) 241-248

7, Simon, L., Rosina, A. and Provini, L.: Intracellular HRP staining of cerebellar Purkinje cell (Pc) and attached interneuron in the cat. Acta Physiol. Acad. Sci. Hung. 56 (1980) 132

8, Simon, L.: Complex integrative properties of the cerebellar Purkinje cell suggest to postulate an asymmetric organization in the dendritic input. In: Székely, Gy. et al., Eds. Neural Communication and Control. Budapest: Akadémiai Kiadó (1981) 249-254 (Adv. Physiol. Sci.; V.30.).

9, Simon, L.: Cerebellar cortical anisotropies and asymmetries (in Hungarian) Ann. Meeting of Neurophysiol. Section of Hung. Physiol. Soc. 1985, Budapest

10, Simon, L.: Sensimotor System of Orientation Control (SOC) based on dynamic body/environment models (BEMs) built up by cerebellar cortex. II. World Congr. of Neuroscienc/IBRO; (1987) Budapest

11, Simon, L., Sarkisian, V. H., Simon, A., Fotakis, S. T., Albus and Marr: Heterosynaptic modification and resetting action of climbing fiber response (CFR) in Cerebellar Purkinje Cells (PCs) XVII. Ann. Meet. of Eur. Neuroscience Assoc., Vienna, P 1812 (1994)

12, Simon, L.: New proposals for cerebellar studies. 24th Ann. Meeting of Soc. for Neuroscience, P.711.8, Miami (1994)

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COMPUTER AIDED TECHNIQUE FOR COMBINED VISUALISATION OF IMMUNOLABELLINGS FROM ADJACENT SERIAL SECTIONS

Somogyi, J.[1], Szél, Á.[2], Röhlich, P.[2]

1st[1] and 2nd[2] Departments of Anatomy, Semmelweis University Medical School, H-1094 Budapest, Tűzoltó. u. 58, Hungary

In principle, the combination of different histological staining techniques or staining patterns can be based on two types of methods. In an optimal case, the procedures are compatible with each other and can be carried out on the same section. In most cases, however, especially when more than two procedures are needed this is impossible or the procedures have to be compromised to an extent that they loos their specificity and sensitivity, significantly. Alternatively, the single staining procedures are carried out on different sections and the resulting staining patterns are combined with an appropriate imaging technique.

A computer aided technique was devised to combine and analyze different histological staining patterns. Wholemounted guinea pig retina was stained with antibodies specific to green- (COS-1) and blue-sensitive (OS-2) visual pigments. The IgGs code named as COS-1 and OS-2 were conjugated with FITC (green) and TRITC (red), respectively. Pairs of black and white photographs were taken from selected fields using the appropriate fluorescent filter sets. The prints were digitized with a scanner and stored in industrial standard TIF file format on an IBM compatible PC. The files were imported into ADOBE Photoshop™ version 2.5.1. The 8bit (256) grey levels were converted into 8bit monochrome green and red colours according to the fluorescence. The corresponding monochrome images were combined into composite (24bit) RGB colour format. In the composite image the double labelled cones appeared to be yellowish according to the extent of the double labelling. The relative intensity of the two labellings was analyzed and used as criteria for mapping.

The method proved to be appropriate for the identification and mapping of cones expressing more than one colour pigments.

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THE ROLE OF Ca^{2+} CHANNELS IN THE INITIATION OF ENDOGENOUS ATP RELEASE FROM RAT HABENULA

Sperlágh, B., Jurányi, Zs. and Vizi, E.S.

Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary

It has been established that purinergic neurotransmission plays functional role in the medial habenula of rat. In our previous study we showed that ATP is released stimulation-dependently and is metabolized by the ectoATPase enzyme in this tissue. In this study we addressed the question whether depolarization-induced Ca^{2+} influx via different Ca^{2+} channels is involved in the electrical stimulation-evoked release of endogenous ATP from rat habenula, measured by the luciferin-luciferase assay.

The L-type Ca^{2+} channel blocker, nifedipine (0.1-1 μM) and the N-type Ca^{2+} channel blocker ω-conotoxin GVIA (0.01-0.1 μM) reduced dose-dependently the stimulation-evoked release of ATP. Similarly, the inorganic Ca^{2+} antagonist, Cd^{2+} (0.02 mM) exhibited inhibitory effect on the outflow.

On the other hand, removal of the Ca^{2+} from the superfusing media did not inhibit, but increased the evoked-release of ATP from the habenula, and this effect was inversely related to the Ca^{2+} concentration in the media. Neither the presence of Ca^{2+} chelators, nor the change in Mg^{2+} concentration in the media did exert significant effect on the Ca^{2+}-independent release of ATP in the absence of $[Ca^{2+}]_o$. Tetrodotoxin (1 μM) - while inhibiting the majority of the release of ATP under normal condition -, only weakly reduced that of under Ca^{2+} free conditions. Although a small shift in the proportion of endogenous ATP/ ADP levels in the effluent could be observed by creatine phosphokinase assay, the breakdown of extracellular ATP was not slowed down under Ca-free conditions, shown by high performance liquid chromatography combined with ultraviolet detection.

In conclusion, it seems likely that both N and L type Ca^{2+} channels are involved in the initiation of electrical-stimulation-evoked ATP release in the rat habenula in case of normal extracellular environment. In the absence of $[Ca^{2+}]_o$, an additional, Ca^{2+}-independent release of ATP occurs, which is not associated with action-potential propagation and is probably related to non-specific changes in the membrane stability.

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RECEPTOR TYPE SPECIFICITY AND IRREVERSIBILITY OF OPIOID AGONIST LIGANDS ON RAT BRAIN MEMBRANE PREPARATIONS

Szabó, É., Monory, K., Schmidhammer, H. and Borsodi, A.

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; *Institute of Organic and Pharmaceutical Chemistry, University of Innsbruck, Innsbruck, Austria

In our experiments we have examined *in vitro* opioid receptor binding and irreversibility of eleven new morphinan derivatives on rat brain membranes. Based on the displacement experiments using different receptor type selective radiolabelled ligands we have found most of them to bind to the *mu* type of opioid receptor with the highest affinity. Their agonist/antagonist feature was characterised establishing the so called Na-index (i.e. the ratio of the K_d values achieved in the presence and absence of 100 mM Na^+). Thus we have found nine of them to be agonist and the remaining two to be partial agonist-antagonist. The degree of the irreversible binding was characterized by the percentage of the specific binding remaining after four washing steps. It was determined that two compounds (N-methyl-3,14-methoxy-morphinan-6-phenylhydrazone and N-methyl-3-methoxy-14-ethoxy-morphinan-6-phenylhydrazone) bind to the receptor in a partially irreversible manner.

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SUBDIVISIONS OF THE SONGBIRD HIPPOCAMPAL FORMATION

Székely, A.D.[1,2] and Krebs, J.R.[1]

[1] Departments of Pharmacology and Zoology, University of Oxford,Oxford OX1 3PS, United Kingdom and
[2] 1st Department of Anatomy, Semmelweis University of Medicine, Budapest H-1450, Hungary

The avian hippocampal formation (HP) is considered to be homologous to the mammalian hippocampus on the basis of topography, developmental origin and its role in processing spatial memory. However, the morphological organisation of the avian HP is very different from that of mammals and components similar to the subdivisions of the mammalian structure are not readily recognisable.

In order to characterise subdivisions within the avian HP and to draw parallels between these subregions and those of the mammalian hippocampus we studied the neural composition and the efferent connectivity of HP in passerine birds. Following Calbindin immunocytochemistry, three spatially distinct populations of immunopositive neurones were found in the dorsolateral (DL), dorsomedial (DM) and ventral (V) aspects of HP. Iontophoresis of *Phaseolus vulgaris leucoagglutinin* revelead three consistently different projection patterns arising from the same subregions.

Generally, there is a medial-to-lateral topographical organisation of hippocampal efferents in relation to the septal complex. The DL region could be paralleled to the subiculum of mammals with its main projections to the basal ganglia, the limbic archistriatum, the lateral septum, and the paraxial meso-diencephalic centres. The V subdivision is likely to be homologous to the Ammon's horn of mammals with its commissural projections to the contralateral HP. Based on its purely intrinsic connectivity, the DM region could be a good candidate for an equivalent of the dentate gyrus.

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THE FUNCTIONAL MORPHOLOGY OF THE NEURON

Székely G.

Department of Anatomy, University Medical School of Debrecen, H-4012 Debrecen, Hungary

According to the findings obtained from embryological experiments, coordinated limb movements can be controlled only by spinal cord segments which differentiate at the level of the limbs in amphibia. Histological studies revealed conspicuous morphological differences between the dendritic arborization of motoneurons in the limb section and in the thoracic section of the spinal cord. In order to study the different morphological characteristics of motoneurons, the total extent of the dendritic arbor was computer reconstructed from serial light microscopic sections of neurons which previously received intracellular injection of cobalt salts. The length and surface area of the dendrites were calculated from the data in the computer file. In a series of electron microscopic investigations the number of synapses was determined in samples of the dendritic arbor using serial ultrathin sections. From these data it was estimated that the length of the dendritic arbor of individual neurons varied between $3\text{-}5x10^4$ micrometer, and each dendritic arbor was covered by $3\text{-}6x10^4$ synapses. More than two-thirds of the synapses impinged upon distal dendrites. Their efficacy in impulse transmission was studied in model experiments which indicated that due to the gradual decrease of the dendrite diameter, the distal synapses disclosed a high degree of efficiency. This observation suggests that the total extent of the dendritic arbor is involved in impulse processing in the integrative action of the spinal cord.

In order to pursue further this problem, motoneurons of individual jaw moving muscles were labelled with cobalt and their dendritic arbor was reconstructed from serial sections. The geometry of the dendritic arbor of neurons innervating jaw closer and jaw opener muscles, respectively, was compared. A total of 32 parameters were measured, 17 of which were concered with length measrements of different parts of the dendrites, and 15 parameters contained information about the arborization pattern. The application of multivariant statistics to the quantitative data separated the "closer" neurons from the "opener" neurons with a good approximation. Taking into account the extermelly large number of synapses and the extensive divergence and convergence in the centre, it is suggested that the motoneurons are hooked up on a statistical basis to the premotor centre in the medulla. The characteristically different dendritic geometry of "closer" and "opener" neurons provided a preferential selectivity in the establishment of the appropriate synaptic connections. Further sudies are in progress to clear up the nature of the premotor structures in the spinal cord and the medulla.

Akadémiai Kiadó, Budapest

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INDUCTION OF C-FOS PROTEIN IMMUNOREACTIVITY IN ACUTE NEOCORTICAL EPILEPTIC FOCI OF THE RAT. CHARACTERIZATION OF THE ACTIVATED NEURONAL POPULATION WITH DOUBLE LABELLING

Szente,M.,[1] Boda, B.,[2] Dubravcsik, Zs.,[3] Király, E.,[3] and Mihály, A.[3]

Department of Comparative Physiology,[1] Attila József University, Department of Neurology[2] and Department of Anatomy,[3] Albert Szent-Györgyi Medical University, Szeged, Hungary

The cellular c-fos protein quickly translocates to the cell nucleus, associates with c-jun protein and the heterodimers bind to the AP-1 site, thus regulating transcription of the DNA. The activation of transcription can be followed with the immunohistochemical detection of the c-fos protein, since only stimulated cells express c-fos immunoreactivity. Diverse stimuli lead to c-fos expression: light stimulus in the retina, ischemia, surgical lesions, NMDA treatment, growth factors and convulsant drugs in the forebrain. Despite the ample descriptions of neuronal and glial c-fos activation, very few studies have been devoted to the characterization of the activated neuronal populations. The present experiments aimed the description and immunocytochemical characterization of the activated neuronal population. Focal neocortical seizures were induced with the topical application of 20 mM solution of 4-aminopyridine in adult anesthetized Wistar rats. The EEG has been recorded for 1 h from the surface of the skull on both sides. The animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Coronal plane vibratome sections were cut and incubated in the mixture of polyclonal (rabbit) c-fos antibody and monoclonal anti-calbindin. Fluorescent secondary antibodies or avidin-biotin systems were used for detection. The diffusion of 4-aminopyridine has been recorded with autoradiography using [H^3]-4-aminopyridine and paraffin embedded coronal brain sections. The focal treatment resulted in vertical diffusion of the drug; no lateral diffusion was observed. Similarly, the contralateral hemisphere was completely devoid of radioactivity. The drug was absorbed probably by capillaries and the choroid plexus of the lateral ventricle. C-fos immunoreactivity was seen in the whole cortical mantle of the treated hemisphere: neocortex and allocortex (prepiriform areas) were equally stained. Scattered immunostained nuclei were seen in the contralateral neocortex in every layers. The reticular nucleus of the thalamus, the claustrum, the amygdaloid nuclei and the medial habenula contained c-fos immunostaining. Calbindin-containing neurons in the neocortex, prepiriform cortex and basolateral amygdaloid nucleus expressed c-fos protein. The calbindin and c-fos expressing neurons of the cerebral cortex were medium-sized non-pyramidal cells, having only a few but long dendrite-like processes. These preliminary results prove that the local chemical stimulation of the neocortex is activating large neuronal populations in the cortical mantle. Some of these cells should be inhibitory. The activated subcortical neuronal circuits may have excitatory connections with the cerebral cortex; and these synaptic connections have to be very effective, probably, they induce gene expression and long term changes. Obviously, we need more immunohistochemical data from further colocalization experiments, in order to delineate the neuronal circuits which are sensitive to convulsant stimulation.

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GABA- AND GLUTAMATE ACTIVATED ION CURRENTS ARE MODULATED BY INTERLEUKIN-10 ON THE NEURONS OF *LYMNAEA STAGNALIS* L

Szűcs, A., Győri, J., Rubakhin, S. S., *Stefano, G. B., **Hughes, T. K. and S.-Rózsa, K.

Balaton Limnological Research Institute, Tihany P.O. Box 35; *State Univ. of NY, College of Old Westbury, Long Island, New York, 11568, USA; **Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550, USA

There is increasing evidence of bidirectional interaction between the immune and central nervous system. Interleukin-10 (IL-10) is one of the most important cytokines involved in the regulation of immune cell responses and production of other cytokines. Despite the intensive studies of role of IL-10 in connecting the immune, endocrine and nervous system its effect on ionic channels of central neurons has not been described. The present experiments were carried out to demonstrate whether IL-10 can modify ionic currents activated by GABA and glutamate on molluscan neurons.

Experiments were performed on isolated, internally perfused neurons of *Lymnaea stagnalis* L. using concentration clamp techique.

The GABA-induced inward current was reduced with low concentration of IL-10 while it was augmented at high concentration. The time-to-peak and inactivation time constant decreased during IL-10 application. The IL-10 effect was significant and reversible. The glutamate-induced inward current was less affected by IL-10 than that activated by GABA.

Our results showed that modulatory effect caused by IL-10 on the GABA-activated current might involve binding of IL-10 molecules to the allosteric site of $GABA_A$ receptors or conformational changes in channel protein.

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SENSORY INNERVATION OF THE SUPRATENTORIAL DURA BY CALCITONIN GENE RELATED PEPTIDE (CGRP) AXONS: STRUCTURAL, FUNCTIONAL AND CLINICAL ASPECTES

Tajti, J., Knyihár-Csillik, E., Sári, Gy., Mohtasham, S. and Vécsei, L.

Department of Clinical Neurology (JT, EKC, SM, LV) and Physiology (GS), Albert Szent-Györgyi University Medical School Szeged, Hungary. Section of Neurobiology, Yale University Medical School, New Haven, CT USA (EKC) and Bay Zoltán Insitute for Biotechnology, Szeged, Hungary (EKC)

Of the primary headaches, including migraine, cluster and tension types, the prevalence of the migraine is relatively high (7.8 %). Since its exact pathomechanism is still unknown, there is no causal therapy available to efficiently treat migraine attacks.

The vascular theory of migraine headache, as proposed by Wolff (1941), and the neurogenic theory (Lance, 1982) are amalgamated by Moskowitz presently prevailing "trigemino-vascular" theory (1984). Supporting this theory, it has been shown that, in human subjects, the level of CGRP undergoes characteristic alterations (increase following decrease) during migraine attacks (Goadsby et al, 1990; Vécsei, 1994). In animal experiments, electric stimulation of the Gasserian ganglion was found to induce release of vasodilator neuropeptides, like CGRP, substance P and vasoactive intestinal polypeptide, into blood vessels of the cerebral dura (Buzzi et al, 1991). We sought to demonstrate structural correlates of these functional alterations by means of immuno histochemical experiments.

We have shown that a short-lasting (5 min) electrical stimulation of the Gasserian ganglion results in a conspicuous swelling (up to 800%!) of the CGRP-imunoreactive perivascular club-like sensory nerve terminals in the supratentorial cerebral dura mater. On the other hand, a long-lasting (30 min) electrical stimulation induced disintegration and rupture of the same sensory nerve terminals, most probably inducing release of CGRP into blood vessels of the dura.

In conclusion, our experimental results seem to furnish a morphological basis for the trigemino-vascular system during migraine attacks and support the role of CGRP in migraine headache.

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QUANTITATIVE IMMUNOCYTOCHEMICAL STUDY OF mGluR1a RECEPTOR IN THE PURKINJE CELLS OF MOUSE CEREBELLUM TREATED WITH METHYL AZOXY METHANOL (MAM)

Takács, J.[1], Gombos, G.[2], Görcs T.J.[1], Becker, T.[2], Knöpfel, T.[3], Kuhn, R.[3], De Barry, J.[2] and Hámori, J.[1]

(1) Laboratory of Neurobiology, 1st Department of Anatomy, Semmelweis University Medical School, H-1094 Budapest, Hungary,
(2) Laboratory of Cellular Neurobiology, Centre of Neurochemie, C.N.R.S. , Strasbourg, France
(3) Laboratory of Molecular and Cellular Biology,CNS Research, Ciba-Geigy, CH-1042 Basel, Switzerland

Metabotropic glutamate receptor mGluR1a plays a crucial role in excitatory (glutamatergic) neurotransmission. This receptor is characteristically present in the dendritic spines of Purkinje cells receiving the massive parallel fiber input. Early postnatal - PN days 5/6 - treatment of developing mice with methyl azoxy methanol (MAM) results in the partial loss of granule cells decreasing also the number of parallel fibers.

In the present study we compare the morphological changes and the presence of the mGluR1a receptor in the dendritic spines of Purkinje cells in MAM treated and control mice 30 days PN. The thickness of the molecular layer decreased by about 30 % and was 132.2 $\pm$ 6.5 and 195.2 $\pm$ 6.8 µm in the treated and in the control cerebella, respectively. The linear density of Purkinje cells did not change (treated: 3.38 $\pm$ 0.16, control: 3.28 $\pm$ 0.08 cells/100 µm). The molecular layer area and volume belonging to 1 Purkinje cell was significantly reduced in the MAM treated animals, 5,100 $\pm$ 322 µm^2 and 76,494 $\pm$ 4,828 µm^3 versus 7,379 $\pm$ 94 µm^2 and 110,068 $\pm$ 1,407 µm^3 in the control cerebella respectively. The numerical density of the Purkinje dendritic spines in the molecular layer proved to be the same (treated: 153.15 $\pm$ 36.12 x 10^6 spines /mm^3, control: 143.72 $\pm$ 12.27 x 10^6 spines/mm^3). The number of spines belonging to 1 Purkinje cell, however, was reduced by about 25 % ; 11,636 $\pm$ 2,41 and 15,916 $\pm$ 1,560 spines/Purkinje cell in the MAM treated and in the control cerebella, respectively The proportion of the "vacant" synaptic spines was the same (about 30 %) in both experimental group However, in the MAM treated mice large, climbing fiber-like axon terminals appeared which built up synapses with 4-6 dendritic spines, replacing the missing parallel fiber afferents.

The presence of mGluR1a receptor was detected by DAB immunocytochemical method on postfixed ultrathin sections. The proportion of the immunopositive spines labelled with electron dense precipitate was the same (about 80 % of all Purkinje spines) both in the treated and in the control cerebella. Although the innervation deficit can be characterised by several morphological changes, the expression of the mGluR1α receptor was not influenced by the reduced glutamatergic parallel fiber input.

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DIFFERENTIAL EFFECTS OF PERINATAL EXPOSURE TO MATERNAL ACTH EXCESS ON THE BEHAVIORAL DEVELOPMENT AND AGING IN RAT

Tamásy, V., Bokor, I. and Balatincz, J.

Department of Physiology, University Medical School of Pécs,
H-7643 Pécs, Hungary

CFY female rats were adrenalectomized at age of 2 months. Commencing on the 15th postoperative day estrous cycle was checked and sperm positivity of vaginal smear was regarded as fetal day 0 (ADX-F_0). To test the offsprings' suckling capability and the amount of maternal milk production, 5-, 10- and 15 day-old pups (M-ADX) were separated from their mothers for 4 hrs in the morning and then reunited and allowed to suckle. Normal pups gained body weight at the end of both the first and second hour postreunion, while M-ADX pups gained only during the first hour and lost weight in the second hour of testing. When the pups were exchanged between normal and ADX mothers, opposite results were obtained, indicating that the reduced gain in M-ADX rats was not due to impaired suckling capability, or insufficient sensory stimulation for milk secretion but to an altered milk production of ADX mothers. Behavioral tests of prepubertal (35-38 day-old) pups revealed lasting effects of early ACTH exposure on the exploratory activity, emotionality, concomitantly with an improvement of avoidance learning. Between 12 and 18 months of age (mean life-span of CFY rats: 21.5 ± 2.7 months; *Tamásy et al. 1994*), significantly impaired maze learning scores and accelerated deterioration of spatial memory indicated a precocious aging of brain function in perinatally ACTH-exposed rats. It is suggested that not only the development of the central nervous system but also the onset and time-course of aging can be determined by the hormonal environment existing during the critical periods of perinatal life.

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THE ROLE OF AMPA RECEPTORS IN MEDIATION OF SPINAL REFLEXES, IN CATS

Tarnawa, I.[1], Farkas, S.[2], and Berzsenyi, P.[2]

[1]Institute for Drug Research, H-1325 Budapest, P.O.Box 82., and [2]Chemical Works of Gedeon Richter, Ltd., Pharmacological Research Centre, H-1475 Budapest, P.O. Box 27, U. S. A.

To elucidate the role of AMPA-type glutamate receptors in various spinal segmental reflexes, we compared the effects of the non-competitive 2,3-benzodiazepine AMPA antagonist and some of its new derivatives on the monosynaptic patellar reflex, and on the polysynaptic flexor reflex, in chloralose anesthetized cats. Effects on flexor reflexes were investigated by electrically stimulating one hind paw and recording responses from the anterior tibial muscle, myographically. For patellar reflex measurements, responses were evoked by tapping the patellar tendon by using an electronically controlled hammer, and the contractions of the thigh muscle were recorded via a force-displacement transducer.

A strong, dose-dependent inhibition by intravenously, or orally applied GYKI 52466 was observed in the patellar reflex model. Flexor reflexes were depressed by similar doses of intravenously applied GYKI 52466 (ED50s: 1.0 and 0.9 mg/kg, respectively). Some novel analogues of the compound are even more potent blockers of the flexor reflex, some causing complete inhibition well below 1.0 mg/kg. In contrast to what was found by Block and Schwarz (Eur. J. Pharmacol. 256: 149-153, 1994), in rats, the flexor reflex antagonism by the 2,3-benzodiazepine compounds was not influenced by the benzodiazepine antagonist Ro 15-1788. The competitive NMDA antagonist, LY 233053 caused a partial (<50%) blockade of the flexor responses, the dose-response curve showed a saturation at around 10 mg/kg i.v.

We concluded that the contribution of AMPA receptors in mediation of flexor reflexes is more significant than that of NMDA receptors, and central-type benzodiazepine receptors are not involved in the flexor reflex inhibitory action of the 2,3-benzodiazepines, in cats.

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GABA-IMMUNOREACTIVE ELEMENTS IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM OF THE EARTHWORM (*LUMBRICUS TERRESTRIS*)

Telkes, I.[1], Csoknya, M.[1], Hámori, J.[1], Elekes, K.[2]

[1]Department of Zoology, JP University, Pécs, Hungary, [2]Department of Experimental Zoology, Balaton Limnological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

The distribution, anatomy and fine structure of GABA-immunoreactive (GABA-IR) elements were studied in the central and peripheral nervous system of the earthworm (*Lumbricus terrestris*), by applying light and electronmicroscopical immunocytochemistry.

In the cerebral ganglion there are 43 GABA-like cells, which are 1.98% of the total nerve cell number. According to their localization GABA-IR neurons can be divided into 7 groups. Three groups (altogether 28 cell) are localized in dorsal position. One group is localized in the lateral part of the brain (4 cells). There are 2 ventral groups, one of them is in medial (4 cells) and another one (3 cells) in lateral position. The nerve cells of the 7th group (4 cells) are situated in the circumpharyngeal connectives.

The axons of most GABA-IR cells, belonging to the ventromedial, external and internal dorsolateral cell groups, richly arborize in the ventral part of the neuropil. Other processes as well as the axons of the lateral GABA-IR neurons can be traced in the circumpharyngeal connectives.

In the subesophageal ganglion there are 47 GABA-IR cells (3,33 % of the total cell number). These neurons are arranged to symmetrically localized groups. Three groups are situated in medial and four ones in lateral position. In the rostral and caudal parts of the ganglion two groups (4 and 3 cells in each) can be seen ventromedially. One medial (4 cells) and one lateral group (3 cells) is situated at the origin of the circumpharyngeal connectives. Other lateral immunoreactive cell groups can be found at the origin of the I. (6 cells), at the I.-II.(19 cells), and at the II.-III. (8 cells) segmental nerves. The axons of these labelled neurons run into the central neuropil giving both contra- and ipsilateral processes.

Altogether 54 neurons (4,02 %) of a ventral cord ganglion show GABA-immunopositive reaction. The localization of the cell groups is different in the rostral and caudal sections of the ganglion. In the rostral part of the ganglion, there are 4 cell groups localized ventromedial (3 cells), ventrolateral (5 cells), lateral (9 cells) and dorsolateral (5 cells) positions. In the caudal part of the ganglion medial (7 cells), mediolateral (17 cells), lateral (5 cells) and dorsolateral (3 cells) GABA-IR cell groups can be found. Axons of the GABA-IR cells arborize in the central neuropil. Those cells situated at the origin of the segmental nerves send their processes into the segmental nerves.

The stomatogastric nervous system also contains GABA-IR neurons which are found in the stomatogastric ganglia and in the enteral plexus. The plexus situated on the surface of the muscle layer contains both nerve cells and fibres. All over along the digestive tract many small bipolar GABA-ergic neurons are localized among the epithelial cells.

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CALPAIN AS A SWITCH ENZYME: ITS EFFECT ON MAP2 AND PKA

Tompa, P. and Friedrich, P.

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, H-1518 Budapest, P.O. Box 7, Hungary

Rearrangement of the neuronal cytoskeleton constitutes an intimate step in the morphological transitions that underlies plasticity of neuronal information processing and transfer. Initial step of this rearrangement is inevitably the destabilization of the cytoskeletal structure which may be initiated by the cleavage of microtubule-associated protein 2 (MAP2) by calcium-activated neutral protease (calpain). Biochemical and cell biological data point to the phosphorylation level and/or pattern of MAP2 as the main factor in the control of this process. Our aim was to reveal fine details of the calpain-mediated degradation of MAP2, with particular emphasis on understanding i, the role of calcium in the activation of calpain; ii, the effect of phosphorylation by various protein kinases of map2 on its sensitivity to calpain and iii, the protective effect of MAP2 on the degradation of the regulatory subunit of PKA by calpain.

We have presented kinetically sound evidence that native calpain is an inactive proenzyme which has to undergo limited autoproteolysis for activation. This requires a relatively high calcium concentration (100 μM) and results in a truncated enzyme that already displays full activity at about 1 μM calcium. As a consequence, calpain behaves as a molecular switch which remains in the on position long after the high-calcium signal that turned it on has subsided. This feature may be important in controlling proteolysis of MAP2, which is extremely sensitive to this protease. This proteolytic process, however, can also be controlled by the phosphorylation level of MAP2 as phosphorylation by PKA or PKC significantly protects MAP2 from calpain, but a substantially lower phosphorylation by CaMK II has no effect. In accordance with these observations, dephosphorylation of MAP2 by alkaline phosphatase significantly sensitizes the protein to calpain. We have also found that MAP2 protects the regulatory subunit of its endogenous kinase, PKA, from calpain. All these observations can be interpreted within the framework of the destabilization of the microtubule lattice brought about by changes in MAP2 phosphorylation level.

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CALCIUM SIGNALS IN CULTURED TYPE-1 ASTROGLIA DERIVED FROM NEWBORN RAT BRAIN

Torday, Cs., Fónagy, A. and Latzkovits, L.

Institute of Experimental Surgery, Albert Szent-Györgyi Medical University, Szeged, Hungary

A great number of evidence shows that parallel with neuronal firing in the central nervous system various potential waves are generated in the astroglia syntitium which engulfs the neuronal network. Amongst them, Ca^{++} waves, periodical, fast and transient increases of intracellular free Ca^{++} concentration $[Ca^{++}]_i$, which propagate from cell to cell via gap junctions, have been reported to bear outstanding importance.

The transient increase of cytoplasmic calcium can be induced by various neurotransmitters leaking out from the synaptic cleft following firing or by any first messengers acting on the astroglia membrane. It represents either a release of free calcium from intracellular pools or uptake through activated ion channels.

Cultured Type-1 astroglial cells express an abundance of various membran receptors for many neuroactive substances like astrocytes *in situ*. They provide an excellent opportunity to analyze signal transduction mechanisms. In our present study effect of various neuroactive substances on the $[Ca^{++}]_i$ in cultured Type-1 astrocytes loaded by Indo-1 fluorescent calcium indicator were studied applying a Hitachi-2000 fluorescence spectrophotometer. For detailes of methodology, see: R.Y.Tsien et al., J. Cell Biol. 94:325-334, 1982. Rat type-1 astrocytes grown on glass coverslip showed characteristic elevation of $[Ca^{++}]_i$ in response to different neuroactive substances (Ca^{++} signals), such as, noradrenaline (10^{-5} M), serotonine (10^{-5} M), ATP (10^{-4} M), glutamate (10^{-4} M), vasopressin (10^{-7} M), endothelin-1 (10^{-9} M).

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DIFFERENT FUNCTIONAL ROLE FOR PERISOMATIC AND DENDRITIC INHIBITION IN THE HIPPOCAMPUS

Tóth, K.[1,2], Miles, R.[2], Gulyás, A. I.[1,2], Hájos, N.[1] and Freund, T. F.[1]

[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, H-1450 Hungary; [2]Department of Biotechnology, Pasteur Institute, Paris, France

Focal stimulation, or dual recordings of CA3 inhibitory cells and postsynaptic pyramidal cells with subsequent light and electron microscopy was used to investigate functional differences between inhibitory cells innervating pyramidal cell dendrites or somata.

Axonal morphology was revealed for 21 CA3 inhibitory cells, 13 of them arborized in the perisomatic region of pyramidal cells. The axons of the other 8 neurons terminated on pyramidal cell dendrites. The number of synaptic terminals mediating the electrically characterised perisomatic interaction varied between 3 and 8 in the four pairs where one postsynaptic pyramidal cell was unambiguously identified. Dendritic IPSPs were smaller and slower than somatic IPSPs. For two dendritic IPSPs we identified 5 and 12 synaptic contacts.

Sodium-dependent action potentials could be suppressed or delayed by the activation of inhibitory cells innervating the perisomatic region of pyramidal cells, it was most effective when timed to coincide with the depolarising afterpotential. In contrast, dendritic Ca-dependent electrogenesis was prevented with activation of inhibitory fibers terminating on pyramidal cell dendrites.

We conclude that in the hippocampus different groups of inhibitory neurons are responsible for the control of electrogenesis in somata and dendrites of target pyramidal cells.

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MAPPING OF BuChE-POSITIVE STRUCTURES IN RAT BRAIN

Tóth, L.[1], Penke, B.[1], Bódi, I.[2] and Kreutzberg, G. W.[3]

Departments of Medical Chemistry[1] and Pathology[2], Albert Szent-Györgyi University Medical School of Szeged, Hungary and Max Planck Institute for Psychiatry, Dept. of Neuromorphology[3], München-Martinsried, Germany

It appeared in previous histochemical investigations that the BuChE (E. C. 3.1.1.8) is mainly localized to capillaries and glial cells in the central nervous system (CNS). This enzyme also can be found in visual system, thalamus, and certain neuronal elements of brain stem, whereas its role in the CNS is not well-known.

We investigated the BuChE activity in CNS of rat as a mapping format.

Coronal and sagittal serial sections were taken from male Wistar rat brain after transcardial perfusion. The incubation was performed according to Kása-Csillik method using BuThChJ as substrate, 10^{-4} M BW284 C51 was applied for inhibition of AChE. Lewis-Shute method was used for cytochemical localization of the enzyme.

Conspicuous enzyme activity was detected in some neuronal structure of the brain stem; e. g. nucleus of n. XII, dorsal nucleus of n. X, nuclei of spinal tract of n. V, inferior olive, nucleus Edinger-Westphal, etc. The nuclei of thalamus almost without exceptions showed enzyme activity. Purkinje cells of Larsell X lobe cerebellum were positive. Very striking axonal axtivity is localized to colliculus superior, area pretectalis, and ventral commissura of hippocampus.

Ultrastructurally, the enzyme was seen in perikaryons, rER system, Golgi apparatus, subsurface cisterns, and dendrite tubules. Sometimes the axolemma was also positive.

BuChE can be localized to endothel cells of blood-brain barrier as well as areas out of blood-brain barrier. Different capillarity of gray and white matter was clearly detectable, for instance in the magnocellular nuclei of hypothalamus that is rich in capillaries.

All types of astrocytes, oligodendrocytes showed prominent positivity at light microscopy, which is localized to rER, sER, Golgi apparatus.

It is known, besides its esterase activity, that BuChE also has peptidase features, therefore it can play role in breaking down of neuropeptides, prohormons and APP.

The role of BuChE in blood-brain barrier is not exclusive.

Besides AChE, it can be important in regulation of ACh level.

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RECEPTOR-MEDIATED PROMOTION OF NEURONAL DIFFERENTIATION AND DOWN-REGULATION OF OPIOID-RECEPTOR CONCENTRATION BY KAPPA-OPIOID AGONISTS

Tóth, P., Cserpán, E., Bajenaru, L. and Maderspach, K.

Inst. Biochemistry, Biological Res. Ctr., Hungarian Acad. Sci., H-6701 Szeged, Hungary

The external and internal signals play regulatory role in early development and differentiation of the nervous system. Recent data suggest that endogenous opioids could also function as developmental signals.

The primary cell culture provides an excellent tool to study this question, since morphological and biochemical changes can be followed under controlled conditions. We examined the effect of chronic treatments with opioid agonists and antagonists on primary cultures estabilished from 8-day-old rat cerebellum (consisting of granular neurons and glial cells), as well as from 7-day-old chick embryo forebrains (mainly neurons). The opioid agonists, as bremazocine (10^{-7}, 10^{-8} M, mainly κ-specific), U50,488 (10^{-6}, κ_1-specific) and antagonists, as norbinaltorfimine (10^{-7}, 10^{-8} M, κ-selective) and naloxon (10^{-5} M, non-selective) were added to the cultures alone or in combination with agonists, between the cultivation days 1-4. Controls received vehicle (Hank's solution, 50μl). Similar chronic treatment was performed by the monoclonal antibody KA8 (mAb KA8) specifically recognizing the κ opioid receptor (1). The changes were followed by radioligand-binding ([^{3}H] naloxone, Kd 0.6nM (2), the 4th cultivation day) and double immunohystochemistry (mAb KA8 and GFAP, as well as neurofilament 200kDa).

K-type agonist bremazocine and also mAb KA8 exerted trophic effect on neuronal morphological differentiation in both cultures. In line with this the expression of neurofilament 200kDa, examined in chick cultures, increased (3), and the division of rat astroglial cells type 2, (which-in contrast with type 1-posesses κ opioid receptors) was repressed. The specific radioligand-binding, measured after the removal of the chronic drug, showed down-regulation of the opioid receptors which was dependent on the treatment duration and drug concentration. The changes mentioned above were recovered by naloxone and the κ-specific antagonists norbinaltorfimine.

Our results support that endogeneous κ-opioid agonists (which were modelled by bremazocine and mAb KA8 in our experiments) may regulate - among others - the neuronal and glial differentiation through specific receptors.

References: (1) Maderspach et al., J. Neurochem. 1991, 56, 1897.,
(2) Maderspach et al., Brain Res. 1988, 441, 41.
(3) Maderspach-Németh., Neurosci. 1993, 57, 459.

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CHANGES OF AUDITORY EVOKED POTENTIAL IN SELECTIVE ATTENTION SITUATION IN MACAQUE MONKEYS

Ulbert, I.[1], Mehta, A.D.[2], Schroeder, C.E.[2], Karmos, G.[1]

[1]Inst. for Psychology of the Hungarian Acad. of Sci., H-1394 Budapest, Hungary and
[2]Depts. Neurosci. Albert Einstein Coll. Med., Bronx, NY-10461,U. S. A.

We studied the neural mechanisms of selective attention in monkeys using an intermodal conditioning paradigm. After a long training period the monkey was implanted with guide tube matrices. Through this matrix we were able to target the primary auditory cortex, and the surrounding region with a long shaft multichannel electrode. The surgical preparation was assisted by MRI scan in order to identify the auditory cortex.

Monkeys were required to depress a switch to initiate the task. During this periods, a stream of auditory stimuli occurred at random ISI. A stream of visual stimuli was presented at the same ISI range counterphase. In each stream a deviant stimulus occurred randomly on 20% of the trials. The monkey was cued to respond to the deviant stimulus in one modality for each trial block, and modality was altered across blocks. Laminar profile of evoked potential (EP), current source density (CSD) and multiunit activity (MUA) were sampled using the linear array multielectrodes during penetrations in auditory cortices.

Our findings indicate that attention modulates both the early and late activities in the auditory cortex.

Early modulation happens mostly in the middle layers. MUA shows positive and negative changes, suggestive of subcortical modulation of throughput. Later modulation occurs more distributed over both superficial and lower layers.

There are some evidences, that the effects are linked to the tonotopic sensitivity, though further studies are necessary to prove these statistically.

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MEMBRANE EFFECTS OF FRX-OH TYPE PEPTIDES ON IDENTIFIED *HELIX* NEURONS

Vehovszky, Á. and Kiss, T.

Balaton Limnological Research Institute of the Hungarian Academy of Sciences, Tihany, HUNGARY H-8237

FRX-OH peptides with Phe-Arg sequence at their N terminals were initially isolated from the ganglia of *Helix pomatia* by Minakata et al (1993). In our experiments membrane effect of the members of the FRX-OH family, FRTF, FRTFE, FRTFQ, FRTFQK was studied on identified neurons in the suboesophageal ganglia of *Helix pomatia*.

Peptides applied locally onto the surface of neurons evoked either excitatory or inhibitory effects on the spontaneous activity, or a corresponding inward or outward current using voltage clamp. Although there was no basic difference in the responses of the same neuron evoked by different peptides, the hexapeptide FRTFQK had the strongest effect on the neurons.

Bath application of peptides had a biphasic effect on the Ca currents activated by voltage pulses in Ca-saline. A transient increase of the peak amplitude of the Ca current was followed by a long-lasting decrease.

Serotonin (5HT) or acetylcholine (Ach)-induced responses of identified neurons were also studied in the presence of FRTFQ and FRTFQK. Both peptides decreased the transmitter-evoked responses of neurons shifting their reversal potential to a more negative value.

Dose-response curves of the transmitter responses in the presence of peptides and receptor antagonist (mianserin for 5HT) suggest that the peptides exert have their effect on the transmitter receptors in a non-specific, non-competitive way.

The multiple effect of the peptides studied on the neurons of *Helix pomatia* suggest a modulatory role of the FRX-OH peptide family in the central regulatory processes.

Reference:

Minakata H., Ikeda, T., Fujita, T., Kiss, T., Hiripi, Ll., Muneoka, Y. and Nomoto, K. 1993 Neuropeptides isolated from *Helix pomatia*. Part 2. FMRF-amide related peptides, S-lamide peptides, FR-peptides and others. In Peptide chemistry 1992. *Edited by* N. Yanaihara. ESCOM Science Publishers B. V. Leiden, The Netherlands. pp. 579-582

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HYPOTHALAMIC [^{3}H]-NALOXONE BINDING DURING SEXUAL MATURATION IN FEMALE RATS

Vértes, Zs., Környei, J. L., *Kovács, S. and Vértes, M.

Institute of Physiology and *Institute of Pathophysiology,
University Medical School, Pécs, Hungary

The experiments were aimed to study the possible changes of the hypothalamic [^{3}H]-naloxone (NAL) binding during sexual development.

CFY female rats of different age were used. In experiments on adults the animals were ovariectomized. Passing 10 days they were injected with 10μg/100 g b.w. of oestradiol at different time before killing. The animals were killed by decapitation. [^{3}H]-NAL binding was assessed by in vitro saturation analyses in the membrane and nuclear fractions of homogenates of oestrogen sensitive hypothalamic regions (preoptic area, anterior hypothalamus, medio-basal hypothalamus, eminentia mediana). For controls the whole cerebrum was used. Samples, in triplicates were incubated with increasing concentrations of [^{3}H]-NAL with or without a 600 fold excess of unlabelled NAL at 25 °C for 30 min. The unbound hormone was removed by filtration through Whatmann GF/A filter discs. Binding parameters were estimated by non-linear curve fitting according to the least squares method aided by a computer program developed in our laboratory.

Two types of membrane associated [^{3}H]-NAL binding sites, a high affinity (Kd ~1-5 nM) and low capacity and lower affinity (Kd ~15-20 nM) with higher capacity were found in both hypothalamic and cerebral tissues of developing and adult animals. The binding capacities uniformly increased by the age. No other changes in the binding parameters were observed.

Beside these "classical" opioid binding sites, in the nuclear fraction a high affinity (Kd ~1-3 nM) [^{3}H]-NAL binding was also found. The profile of this binding changed by the age. In immature rats, at 11, 21, 26-30 days of age, the Scatchard plots of saturation data were linear, the Hill coefficient 1. However that 30 days old animals in which the vaginas were already opened, the Scatchard analysis of [^{3}H]-NAL binding gives a curvilinear component, with Hill coeff. 2. In the cerebrum, [^{3}H]-NAL binding with linear Scatchard plot can be observed up to 21 days of age, later only the curvilinear form is present.

In adults, after ovariectomy, the pattern of [^{3}H]-NAL binding is similar to those observed in immature rats before vagina opening. As a result of oestradiol treatment, this type of binding sites with linear Scatchard plot disappears and returns again at least 22 hrs after Oe injection. Meanwhile the form with curvilinear Scatchard plot takes over.

This results may suggest, that the oestradiol induced change in the hypothalamic nuclear opioid binding could be, at least in part, a factor that plays a role in the onset of puberty.

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CHARACTERIZATION OF CORTICOTHALAMIC PROJECTIONS BASED UPON THEIR TARGET CELLS AND POSTSYNAPTIC RECEPTORS

Vidnyánszky, Z., Görcs, T.J., and Hámori J.
First Department of Anatomy, Neurobiology Laboratory, Semmelweis University Medical School, 1450 Budapest, Hungary

The synaptic organization of corticothalamic projections from different visual cortical areas was analysed in the dorsal lateral geniculate nucleus (dCGL) and lateral posterior nucleus (LP) of the rat and cat. *Phaseolus vulgaris* leucoagglutinin anterograde tract tracing method was used for the labelling of corticothalamic terminals. Postsynaptic targets were characterized by postembedding GABA immunocytochemistry and the postsynaptic receptors of the cortical terminals were analysed by preembedding immunoperoxidase and immunogold receptor immunocytochemistry.

It was found in cats, that thalamocortical projections from different visual cortical areas in the same target regions (from area 17 and 18 in the dCGL) and the cortical projections from the same area in different visual thalamic nuclei (from area 17 in dCGL and LP) exhibit specific, differential synaptic organization pattern, ie. they innervate differently the two types of thalamic neurons, the principal cells and the interneurons.

It was also demonstrated that in rats the corticothalamic projection from area 17 forms two morfologically distinct types of synaptic endings (small and giant boutons), which also exhibit a clear difference in their types of postsynaptic glutamate receptors. The small terminals - constituting the majority of corticothalamic boutons and found both in dCGL and LP - establish asymmetrical synapses with mGluR1a-immunopositive dendrites with immunometal particles concentrated at the periphery of their postsynaptic membranes. In contrast, synapses formed by giant boutons in the LP were always mGluR1a-immunonegative.

These results indicate, that similarly to the parallel, functionally and morphologically distinct pathways and channels existing in the retinothalamocortical system, there are parallel pathways and channels in the corticothalamic feedback too.

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EFFECTS OF GLUTAMATE ANTAGONISTS ON EPSPs IN CORTICAL SLICES OF RATS OF DIFFERENT AGES

Világi, I., Taranawa, I. and Banczerowski-Pelyhe, I.

Dept of Comp Physiol.,Eötvös University, H-1088, Budapest, Hungary

Excitatory amino acid transmitters play an important role in the direction of cortical development and in the maintenance of cortical activity. There is a critical period during the postnatal development, when the excitability of the cortex is very high and the glutamatergic processes could be activated very easily. On the basis of our previous experiments this period could be observed between the second and third week in the somatosensory cortex of rats.

The goal of the present study was to investigate the role of different types of excitatory amino acid receptors in the electrically evoked synaptic responses of the cortical slices of rats. In our intracellular experiments the effects of NMDA antagonist APV and the non-NMDA antagonist GYKI 53655 were analyzed. The 400 μM slices were taken from the somatosensory cortex of 2-week-old and adult rats. The drugs were bath applied in different concetration to determine the effective doses. The changes of the amplitude and the undercurve area of EPSPs evoked by stimulation of the white matter below the site of recording were determined.

Comparing the passive neuronal membrane parameters in the 2-week-old and the adult rats slight differences could be observed. The repetitive firing characteristics were rather similar. The thresholds of EPSPs and spikes were not significantly different in the two age-group, however the timecourse, amplitude, duration and latency of EPSPs differed significantly.

2 μM APV decreased, 16 μM APV seemed to inhibit maximally the amplitude of EPSPs in both age-groups, although the maximal inhibition was 70% vs 50% in the slices from young and adult rats. In the case of the 2.5-10 μM GYKI 53655 the percentage of inhibition did not differ significantly.

Our results show that cortical slices from young animals are more sensitive to the inhibitory action of APV than those obtained from adult animals, while there is not so marked difference in the effect of non-NMDA antagonist. This suggest, that NMDA receptor mediated processes play a very important role in the excitatory transmission during the development of the cortex.

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A COMPUTER MODEL OF THE BINOCULAR VISION IN THE CEREBRAL CORTEX

Virág, T. and Fehér, O.

Department of Comparative Physiology, József Attila University,
H-6726 P.O.B. 533, Szeged, Hungary

The investigation of basic mechanisms of binocular vision has clearly proven that in view of stereopsis the topology of images projected from an object to the retina is of decisive importance. Usually the two images are not projected to identical places of the retina. In this horizontal difference is coded the depth of visual space. Many types of the so called binocular cells, involved in this coding process, have been characterized experimentally. Our model deals with three types of the neurons described in Poggio and Talbot's experiments. These are the following: 1./ tuned excitatory neurons which do not make out difference between near and far objects and 2./ "near" vis. "far" neurons which give different responses to objects lying nearer or farther than the point of intersection of the visual axes. The "near" neurons discharge to near ,"far" neurons discharge to far objects as related to the point of intersection. These cells have also orientation selectivity and give maximal response at disparities, characteristic for them.

Our model consists of a network of simulated neurons, in which the function is determined by the connectivity. This network processes the input coming from 252 retinal elements on either side. The input signals feed simple and complex orientation-, direction-, and excentricity selective neurons. The complex cells innervate the binocular neurons on retinotopic basis. This relatively simple system, not exceeding 2000 cells is able to analyze simple spatial patterns, discriminate near and far objects, being capable of incorporating any other function of the visual system in organic unity with those, simulated in its present state.

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SYNAPTIC AND NON-SYNAPTIC INTERACTIONS: PRESYNAPTIC MODULATION OF CHEMICAL TRANSMISSION

Vizi, E. S.

Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, P. O. B. 67, Hungary

Our way of thinking about the transmission of information between neurons is still dominated by the knowledge gained from anatomical studies on patterns of neuronal wiring. Within a given neuron, electrical signals are initiated and transmitted as waves of electrical potential change produced by increase in the permeability of the membrane to different ions. The propagated electrical signal reaching the axon terminal end usually in depolarization and causes the release of chemical substances, provided that $[Ca_o^{2+}]$ is available.

In addition to the synaptic communication there is a further possibility for interneuronal communication: it is a non-synaptic interaction between neurons at the presynaptic level (cf. Vizi, 1979., Vizi et al., 1991., Vizi and Lábos, 1992). One neuron can communicate with many others without making synaptic contact: i.e. there is a non-synaptic chemical "cross-talk" between neurons (Vizi, 1979, 1984). This would be a transitional form of communication between discrete classical neurotransmission and the relatively non-specific neuroendocrine secretion (through circulation).

It became clear that chemical neurotransmission may be a far more complicated event than previously assumed: the release of transmitters/modulators can be presynaptically modulated through auto-, homo-, and heteroreceptors. Transmitters/modulators can be released from sites other than axon terminals and from varicosities situated synaptically or non-synaptically. Regional disparities in the relationship of the inputs to different regions (receptor mismatch) of the brain make possible the regional non-synaptic/synaptic control of chemical neurotransmission in the neuronal network.

Akadémiai Kiadó, Budapest

References

Vizi, E.S. Presynaptic modulation of neurochemical transmission. *Progr. in Neurobiol.* 12:181-290 (1979)

Vizi, E.S. Non-synaptic interactions between neurons: modulation of neurochemical transmission. Pharmacological and clinical aspects. *John Wiley and Sons*, Chichester, New York, (1984)

Vizi, E.S., Kiss, J., Elenkov, I.J. Presynaptic modulaton of cholinergic and noradrenergic neurotransmission: interaction between them. *News Physiol. Sci.* 6:119-123 (1991)

Vizi, E.S., Lábos, E. Nonsynaptic interactions at presynaptic level. *Progr. in Neurobiol.* 37:145-163 (1991)

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EFFECTS OF NATRIURETIC PEPTIDE FAMILY ON ANALGESIC ACTION OF MORPHINE IN MICE

Vízi, Z., Babarczy, E. and Telegdy, G.

Department of Pathophysiology, Albert Szent-Györgyi Medical University, H-6701 Szeged, Hungary

The natriuretic peptide family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). Previously Azarov et al. (1992) investigated the effects of ANP on the development of analgesic, tolerance and dependence action of morphine. It was found that these peptide was able to diminish the development of acute and chronic morphine tolerance centrally administrated ANP.

As a continuation of the former experiments we have studied and compared the antinociceptive effects of the member of natriuretic peptide family in male CFLP mice. The analgesic effect was measured by a tail-flick test. The different doses of peptides were administered into the lateral brain ventricule and morphine (5 mg/kg) was injected subcutaneously. These peptides themselves had no analgesic effect, however, they depressed the acut nociceptive effect of a single subcutaneous dose of morphine. The most effective dose of ANP and CNP was 20 ng, while BNP affected in 0.2 ng. Each peptide blocked the development of acute tolerance to morphine, ANP and CNP in 200 ng dose, while BNP in 20 pg dose produced the most prominent block. All three peptides influenced chronic morphine tolerance: it was blocked by 20 or 200 ng ANP, 0,2, 2 or 200 ng BNP, and also by CNP, but this peptide displayed a significant effect only in the 0.2 ng dose. The effects of the natriuretic peptides were investigated on the development of naloxone-precipitated withdrawal syndromes. Neither naloxone-induced jumps nor changes in body weight and temperature were affected by central administration of natriuretic peptides.

The amino acid sequences of the three peptides are known to be highly homologous, with a common ring frame formed by a disulfide linkage, which explains why this action of ANP displays a similar profile to those of BNP and CNP.

This work was supported by grants of OTKA (T-6084, T/3, 1354) and by Ministry of Social and Welfare of Hungary (T-11,549/93) and FEFA (1008/1).

Neurobiology 3 (1), p. 148, 1995

SEROTONIN- AND CATECHOLAMINERGIC NEURONS IN THE DEVELOPING NERVOUS SYSTEM OF GASTROPODS

[1,2]Voronezhskaya, Elena E., [1]Hiripi, L. and [1]Elekes, K.

[1]Balaton Limnological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary and [2]Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

Little is known about the development of transmitter phenotype of neurons in gastropods. We investigated the embryonic and postembryonic development of serotonin-like immunoreactive (5-HTLI) and catecholaminergic (CA) neurons, these latters visualized both by dopamine [DA] and tyrosine-hydroxylase [TH] immunocytochemistry and by glyoxylate-induced fluorescence), in the pond snail, *Lymnaea stagnalis*. The embryonic development of 5-HTLI and CA neurons was additionally compared in different lymnaeid and planorbid species. In both transmitter systems, the first pair of transmitter specific neurons appear during the early embryonic development (E30%) outside the future CNS, in the region of the mantle wall. These cells cease to express their transmitter phenotype close to hatching, hence we named them Transient Serotonergic (TS1) and Transient Catecholaminergic (TC1) neurons. All planorbids studied (*Planorbis planorbis*, *Planorbarius corneus*, *Helisoma trivolvis*) differ from lymnaeids (*Lymnaea stagnalis*, *Physa fontinalis*) in that that they have a pair of TS1 neurons, whereas lymnaeids have TC1 neurons.

In both transmitter systems the labelled neurons show a gradual increase in number and degree of axonal arborization during the embryo- and postembryogeneses. Although CA neurons can easily be demonstrated in the embryonic nervous system, using glyoxylate-induced fluorescence, they can be visualized only in a low number immunocytochemically, when applying anti-TH antibodies. The majority of CA neurons, including the giant DAergic (RPeD1) neuron, appear in hatchlings. According to HPLC assay, 5-HT and DA are present in *Lymnaea* embryos, and the concentration of both monoamines increases in the course of development, parallel to the maturation of the CNS. We suggest that a part of CA neurons undergo developmental changes related to the mechanism of transmitter synthesis and/or the chemical nature of actual transmitter substance.

Supported by OTKA Grant No. 6284.

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Neurobiology 3 (1), p. 149, 1995

ERP CORRELATES OF CHANGING BETWEEN TWO DIFFERENT REFERENCE STIMULI

Winkler, I.[1], Karmos, G.[1] and Näätänen, R.[2]

[1]Department of Psychophysiology, Institute for psychology, Hungarian Academy of Sciences, H-1394 Budapest P.O.Box 398, Hungary and Cognitive Psychophysiology Unit, Department of Psychology, University of Helsinki, Finland

The mismatch negativity (MMN) is a frontocentrally negative event-related brain potential (ERP) component typically peaking between 100 and 200 ms from stimulus onset. It is elicited by infrequent changes in repetitive series of auditory stimuli. Infrequent stimuli *per se* do not evoke this component. The MMN can be elicited even when the subject disregards the auditory stimulation by performing a task not related to it. On this basis, MMN was suggested to reflect a neural mismatch process comparing the infrequent deviant sound to the trace(s) of the previous frequent (standard) sounds.

The present study addressed three questions related to the generation of the MMN: 1) How does a change of the standard stimulus occur; 2) Can the memory underlying the MMN contain two different standard stimuli in parallel; 3) Can one deviant stimulus elicit two MMNs with respect to two different standards?

Subjects were sitting in a comfortable chair performing a computer-controlled visual tracking task while EEG was recorded from the Fpz, Fz, Cz, Pz, Oz (10-20 system) and 6 equidistant locations (LM, L2, L1, R1, R2, RM) along the coronal line connecting the two mastoids via FZ. The horizontal EOG was monitored at the other canthus of the left eye. 19 blocks of 400 simple tone-bursts presented at a stimulus onset asynchrony interval of 800 ms were delivered to the subject's right ear by headphones. Stimulus intensity was 80 dB (NHL), frequency 1000 Hz, rise and fall times 10 ms, each. Auditory stimulation consisted of short trains starting with 6 stimuli of 450 ms duration (long standard). These stimuli were equiprobably followed by 0, 2, 4, or 6 stimuli of 150 ms duration (short standard). Each train ended with a 300-ms long stimulus (deviant). The -100-600 ms interval of the responses was digitized (lowpass 40 Hz, sampling rate 250 Hz) and averaged separately according to the position of the stimulus in the train and the number of short standard within the train. Responses exceeding 100 μV (minimum to maximum) on any EEG or EOG channel were rejected from the analysis.

The MMN to deviants peaked between 140 and 170 ms from the point were the deviant and the standard stimulus started to differ (ie., a 150 and a 300-ms long stimulus start to differ from each other at about 150 ms from the stimulus onset). Results showed that the emergence of a new standard stimulus is gradual: increasing the number of short standards within the train increased the amplitude of the MMN elicited with respect to the short standard while at the same time decreasing the amplitude of the MMN in reference of the long one. Deviants following 2 or 4 short standards elicited 2 consecutive MMNs (one at about 300 ms and another at 450 ms from stimulus onset). Thus two standards can be active in the memory reflected by MMN in parallel and the deviant can elicit two consecutive MMNs.

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A NEW NEURONAL TREE RECONSTRUCTION SYSTEM THAT SUFFICIENTLY ESTIMATE THE THREE-DIMENSIONAL GEOMETRY OF NEURONS FROM THEIR PLANAR PROJECTIONS

Wolf, E.[1], Birinyi, A.[1] and Pomahazi, S.[2]

Department of Anatomy, [1]University Medical School of Debrecen, H-4012 Debrecen, Hungary and Center for Informatics and Computing, [2]Kossuth University of Debrecen, H-4012 Debrecen, Hungary

This work was an attempt to develop and test a relatively fast and sufficiently accurate neuronal tree reconstruction system, which combine the two seemingly contradictory factors: speed and precision. The accuracy of our 3DNEURON program was tested by comparing it with a very precise but time consuming method of reconstruction (NEUTRACE).

Comparison was performed by reconstructing eighteen dendritic arbors of frog spinal motoneurons from serial sections with both methods and comparing several morphological parameters of the two reconstructions. In 3DNEURON the planar projection of the dendritic arbors was drawn with the aid of a microscope equipped with a drawing tube and the X,Y coordinates were fed into an IBM-compatible PC through a graphic tablet. Dendritic coordinates along the (focus or Z) axis perpendicular to the plane of drawing were estimated by cubic polynomials of a modified AKIMA interpolation. The interpolation was based on the lengths of projected dendrites and the coordinates of points where dendrites entered the next section. Focus axis coordinates of these points could automatically be calculated from the serial numbers and thicknesses of sections. 3DNEURON was tested by comparison of the distributions of characteristic points of dendrites, segment lengths and branching angles. A modified product moment analysis on dendritic arbors was also performed to reveal and compare the overall geometry of dendrites.

It was concluded that 3DNEURON is a fast reconstruction system without any systematical error of interpolation that can correctly supply the most morphological parameters and visualize the natural arborizations.

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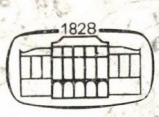

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Neurobiology 3 (2), pp. 151–164, 1995

SPECIES-SPECIFICITY OF GLIAL VIMENTIN AS REVEALED BY IMMUNOCYTOCHEMICAL STUDIES WITH THE VIM 3B4 AND V9 MONOCLONAL ANTIBODIES

Gereben, B.[1], Gerics, B.[2], Gálfi, P.[1],
Rudas, P.[1], Hajós, F.[2] and Jancsik, V.[2]

[1]Department of Physiology and Biochemistry, [2]Department of Anatomy and Histology, University of Veterinary Science, Budapest, HUNGARY

Summary: Two monoclonal antibodies directed against vimentin, Vim 3B4 and V9 could distinguish between vimentins originating from certain species, when tested on cell lines (Bohn et al, 1992). Our comparative immunohistochemical studies in the rat and chicken brain with the same antibodies suggest the coexistence of two vimentin forms in the glial cells of both species. One of these forms bearing the epitope present in the respective non-glial cell lines is present in astrocytes and Bergmann glia independently of the ontogenic state of the animal. The other epitope appeared also mutually in both species, albeit its expression was more restricted. These patterns suggest that in these two species, the expression of the different vimentin forms might be differently regulated.

Key words: intermediate filaments, ontogenesis, monoclonal antibodies, species-specificity, immunohistochemistry

Correspondence should be addressed to: Dr. Balázs Gereben
Department of Physiology and Biochemistry, University of Veterinary Science, H-1400 Budapest, P.O.B. 2, Hungary

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INTRODUCTION

Vimentin, one of the type III intermediate filament subunits is expressed in a wide variety of cells of mesenchymal origin (Franke et al., 1978), frequently together with other intermediate filament subunits. Within the ectoderm-derived central nervous system (CNS) it is expressed in a developmentally regulated way in cells developing along the astrocytic pathway. Typically, vimentin expressed during the early phase of the differentiation of these cells is replaced later on by glial fibrillary acidic protein (GFAP). This "vimentin-GFAP transition" (Dahl, 1981) occurs at the time of unset of myelination, and of withdrawal of radial glia. Only Bergmann glial fibres and some astrocytes express vimentin in the adult rat (Pixley and de Vellis, 1984). Similarly, in the adult chick cerebellum, Bergmann glia and some astrocytes in the granule cell layer are permanent vimentin-positive structures (Roeling and Feirabend, 1988).

Due to the highly conservative nature of the amino acid sequence of this protein, vimentin antibodies generally bind to vimentin in different types of cells of species of considerably different phylogenetic ages (Franke et al., 1978). Nevertheless, two commercially available monoclonal antibodies (clones Vim 3B4 and V9) have recently been reported to show somewhat restricted immunofluorescence recognition patterns when checked on several cell lines of primate, mammalian, and avian origin (Bohn et al. 1992). Among the cell lines tested, Vim 3B4 reacted with vimentin in all cells except for those of rodent origin, while V9 did not recognize vimentin in chicken fibroblasts and in all murine cell lines.

The question arises whether glial cells display the species-specificity of these antibodies, with respect to the ontogeny as well. To answer this question, brains of domestic chicken (*Gallus domesticus*) and rat were chosen as model, since the above data suggested that the recognition by the two antibodies was mutually exclusive in these two species.

MATERIALS AND METHODS

Immunoperoxidase technique

Adult and 7-day-old albino rats (LATI, Hungary) of either sex and adult and 1-day-old chicken (Bábolna TH, Hungary) were anaesthetised with ether. The animals were then transcardially perfused with a physiological salt solution followed by Zamboni's fixative (0.1 M phosphate buffer, 15% saturated picric acid, 4% paraformaldehyde and 0.25% glutaraldehyde, pH 7.4). After removal from the skulls, cerebella were further incubated for 2 h in the same fixative, then 60 μm thick sagittal sections were cut on the vibratome (Vibroslice, Campden Instruments).

The free floating sections were washed several times with 0.9% NaCl-containing 0.05 M Tris-HCl, pH 7.4 (TBS). Endogenous peroxidase activity was exhausted by treating the sections with 3% H_2O_2 for 5 min at room temperature. From here on all steps were performed at 4 °C under continuous gentle shaking and included a TBS rinse interposed between the changes of immunoreagents. Successive incubations were as follows: 20% normal goat serum for 7 h followed by an incubation in Vim 3B4 or V9 antibodies (diluted 5x and 4x, respectively, according to the manufacturer's instruction) for 48 h. Primary antibodies were then removed and the sections were processed by the biotin/avidin/peroxidase technique (Vectastain® ABC- Immunoperoxidase Kit, Vector Laboratories, USA). As a chromogen 3,3′-diaminobenzidine tetra hydrochloride (DAB, Sigma) was used to visualize the immunoperoxidase complex.

Indirect immunofluorescence technique

Adult and 1-day-old chickens were deeply anaesthetised with 30 mg/kg body weight pentobarbitone sodium (Nembutal). For preparing cryostate sections, the cerebella and telencephalons were dissected out and were snap-frozen in dry ice-aceton. In a cryostate (Reichert-Jung Frigocut 2800 E) 20 μm thick sections were cut from the middle third of the telencephalon and from the cerebellum, in the coronal and sagittal planes, respectively. Sections were fixed on glass slides in acetone at -20 °C for 10 minutes. Acetone was allowed to evaporate. Sections were incubated with Vim 3B4 (Boehringer Mannheim GmbH), using dilutions of 1:5, in a moist chamber for 45 minutes at room temperature, then rinsed three times in PBS. The incubation with sheep- anti-mouse Ig-fluorescein $F(ab')_2$ fragment (Boehringer Mannheim GmbH) was carried out in a moist chamber, as proposed in the manufacturer's instructions. After rinsing in PBS, the sections

were mounted with glycerol:PBS (1:2) containing 0.1% 1,4-phenyldiamine (Sigma). Preparations were examined using a fluorescent microscope (Leitz Diaplan). For the microphotos "Kodak Ektachrom 320 T Professional" film was used.

Controls

Incubations were made by omitting the primary antiserum. In these cases the tissue was incubated with the conjugates only. Controls showed no reaction at all either with the fluorescence or the peroxidase conjugate.

RESULTS

In the telencephalon of 1-day-old chicken, Vim 3B4 clearly stained astrocytes (*Fig. 1*) which appeared as evenly distributed stellate-like structures. The glia limitans covering the pial surface was particularly intensely stained (*Fig. 2*) as seen in areas where blood vessels penetrate the telencephalon from the surface. In the adult chicken, immunostained fibres were observed in the cerebrum, while the cerebellum showed a staining of the Bergmann glia fibres (not shown).

A more detailed comparative study on the localisation of vimentin was performed by applying the immunoperoxidase technique to the cerebella of young and adult rats and chicken. After immunostaining for Vim 3B4, the 1-day-old chicken cerebellum showed a marked staining of Bergmann glia fibres, astrocytes, and pericapillary glia cells. This staining pattern was observed unaltered in the adult (*Figs. 3, 4 and 5)*. In the rat, Vim 3B4 immunoreactivity was obviously different at different ages: while in the 7-day-old cerebellum Bergmann glia, astrocytes, and vessels were all stained (*Fig. 6*), no immunoreaction occurred in the Bergmann glia of the adult (*Fig. 7*).

After immunostaining for V9, when applied to the chicken cerebellum, astrocytes were not stained, independently of the age of the animal. However, a faint staining of Bergmann glia fibres was observed; even this weak staining was restricted to the top of the folia *(not shown)*. In the rat cerebellum, a strong immunoreactivity of the Bergmann glial fibres was observed, at both 7-day-old and adult ages, as shown in *Figs. 8 and 9.* The persistence of V9 immunoreactivity in some of the astrocytes in the adult rat cerebellum was still remarkable (*Fig. 10*).

It is worth to note that V9 immunoreactivity was absent in the blood vessels of the 1-day-old chicken, and of the 7-day-old rat. However, a prominent

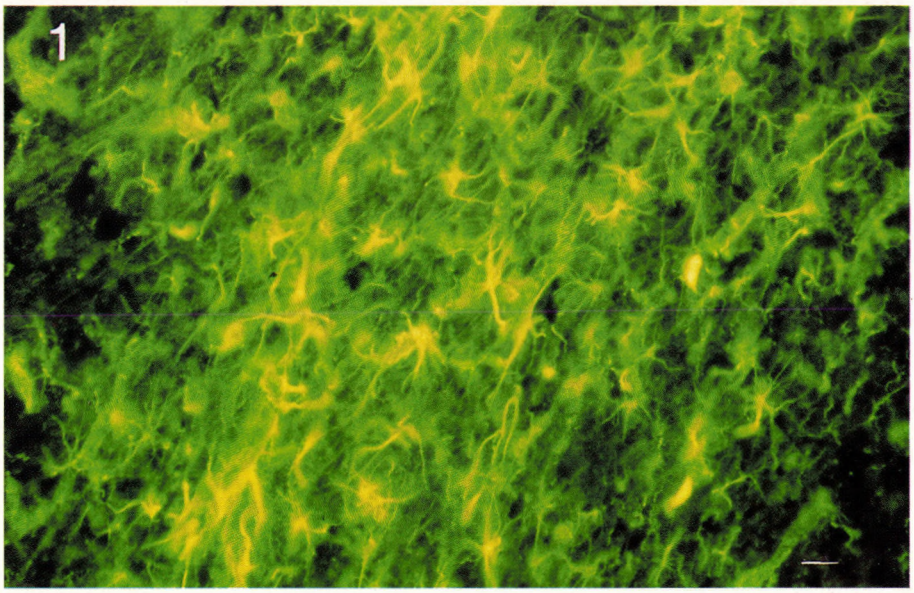

Fig. 1: Vim 3B4 positive astrocytes in the telencephalon of a 1-day-old chicken. Scale bar: 15 μm

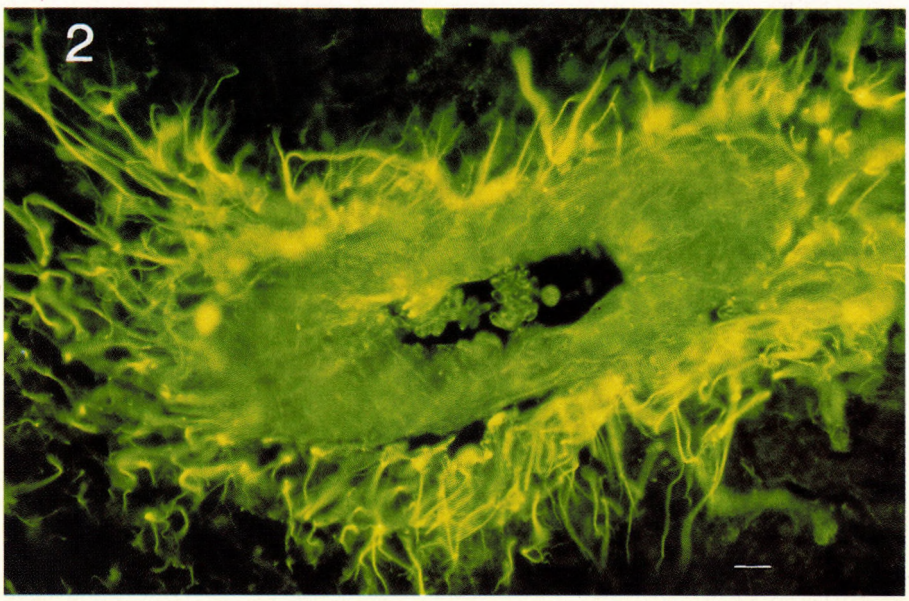

Fig. 2: Vim 3B4 positive glia limitans around a blood vessel in the telencephalon of a 1-day-old chicken. Scale bar: 15 μm

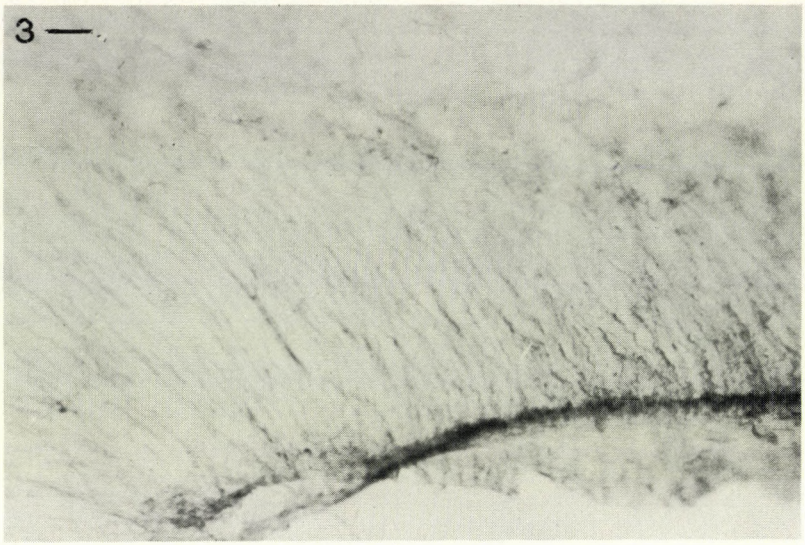

Fig. 3: Vim 3B4 positive Bergmann glia fibres in the cerebellum of a 1-day-old chicken. Scale bar: 50 μm

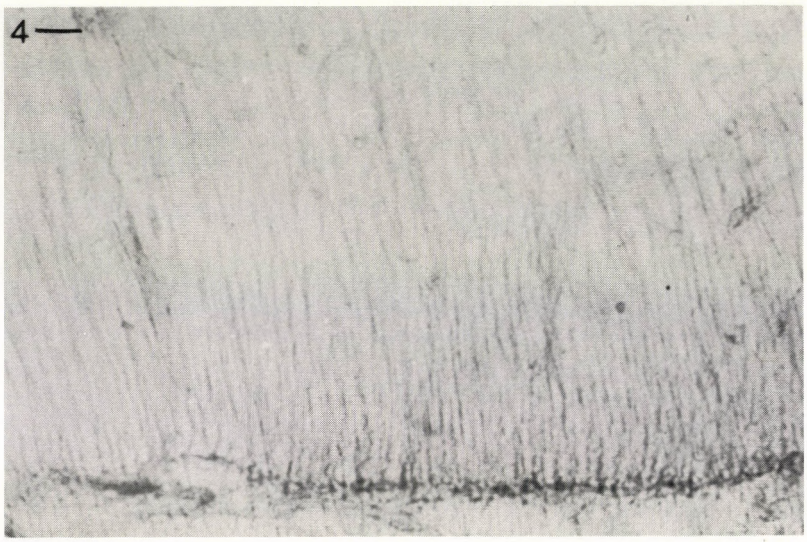

Fig. 4: Vim 3B4 positive Bergmann glia fibres in the cerebellum of an adult chicken. Scale bar: 50 μm

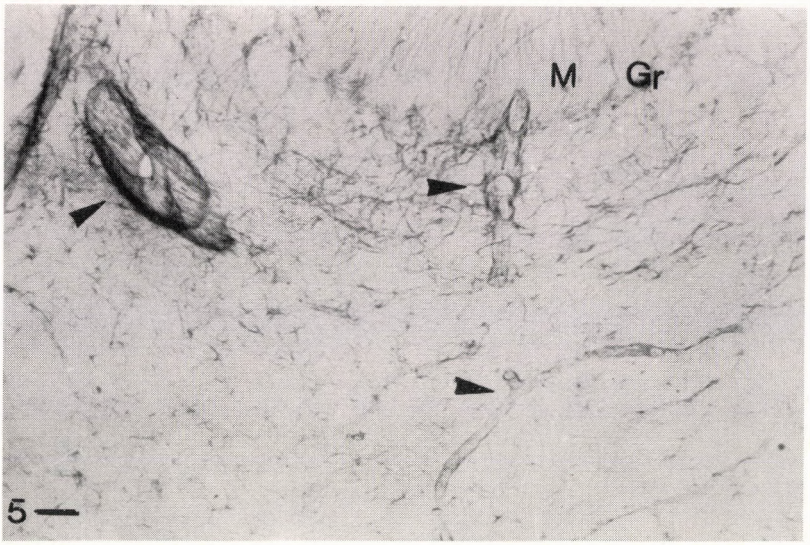

Fig. 5: Blood vessels (arrowheads) marked by Vim 3B4 in the white matter of the adult chicken cerebellum. Gr=granular layer; M=molecular layer. Scale bar: 50 μm

immunostaining was observed in the adults in both species (for the rat see *Fig. 10*), using the V9 antibody. On the contrary, Vim 3B4 marked blood vessels in both age groups in the chicken (cf. *Fig. 5*), and in the rat, also. A summary of the results obtained with Vim 3B4 and V9 is given in Table 1.

Table 1

Immunostaining of cerebellar structures by Vim 3B4 and V9.

	Bergmann glia		astrocytes		blood vessels	
	Vim 3B4	V9	Vim 3B4	V9	Vim 3B4	V9
1-day-old chicken	+	±	+	–	+	–
adult chicken	+	±	+	–	+	+
7-day-old rat	+	+	+	+	+	–
adult rat	–	+	–	+	+	+

+ : intense, specific staining - : no staining ± : weak staining

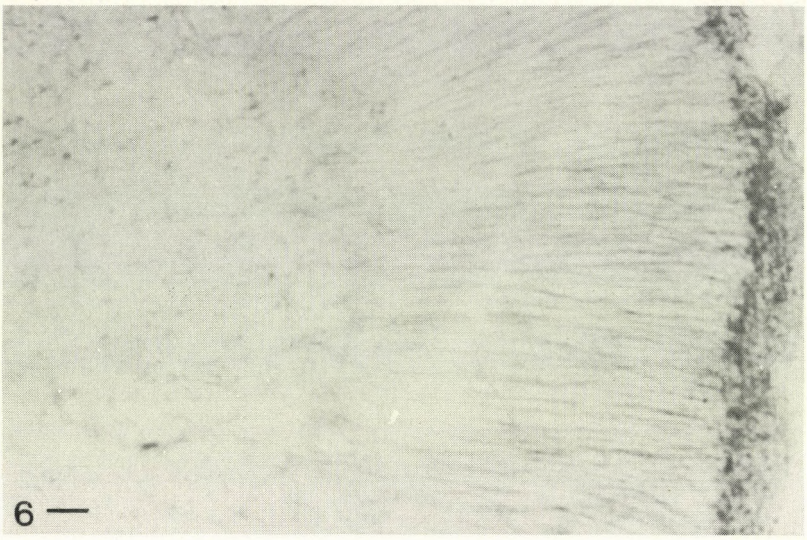

Fig. 6: Vim 3B4 positive Bergmann glia fibres in the cerebellum of a 7-day-old rat. Scale bar: 50 μm

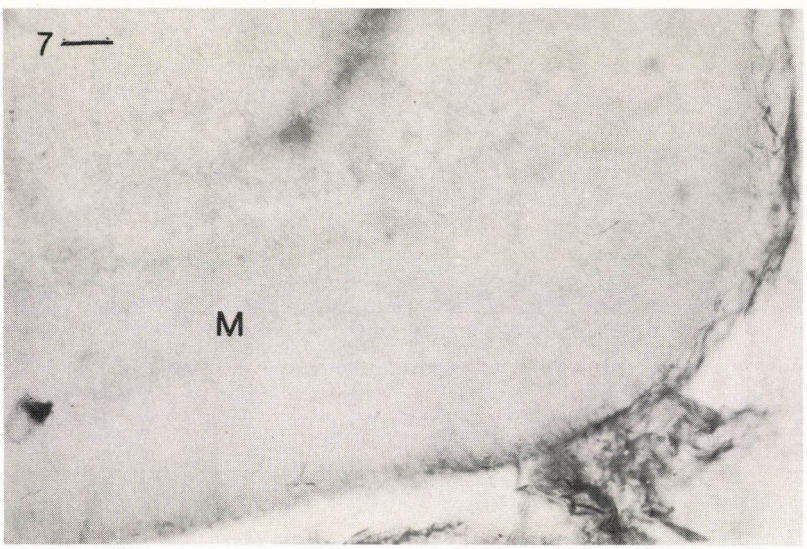

Fig. 7: Bergmann glia fibres are Vim 3B4 negative in the cerebellum of the adult rat. M=molecular layer. Scale bar: 50 μm

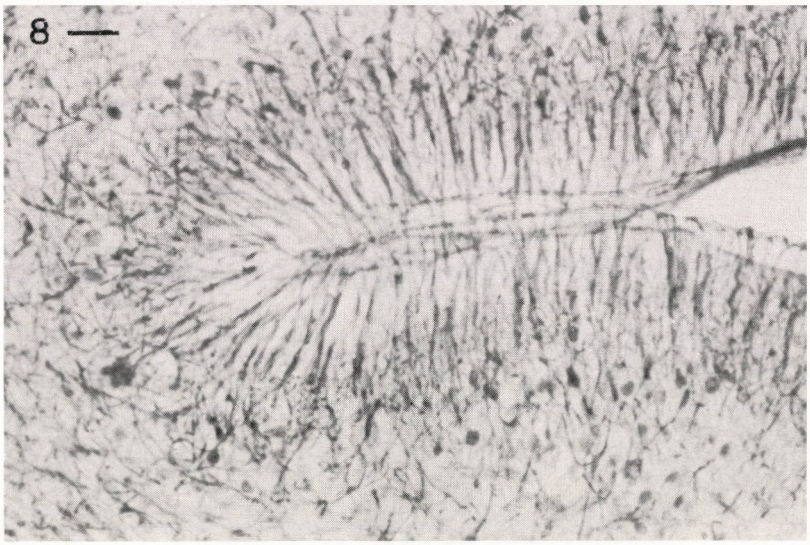

Fig. 8: V9 positive Bergmann glia fibres in the cerebellum of a 7-day-old rat. Scale bar: 50 μm

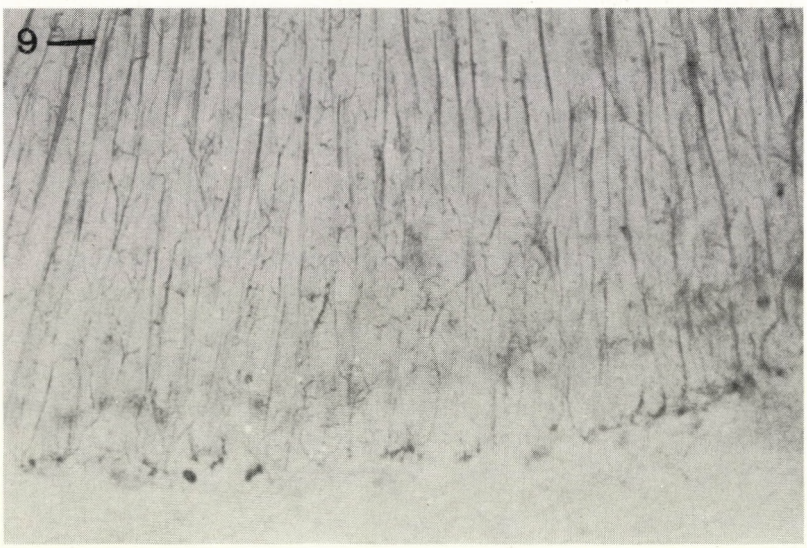

Fig. 9: V9 positive Bergmann glia fibres in the cerebellum of the adult rat. Scale bar: 50 μm

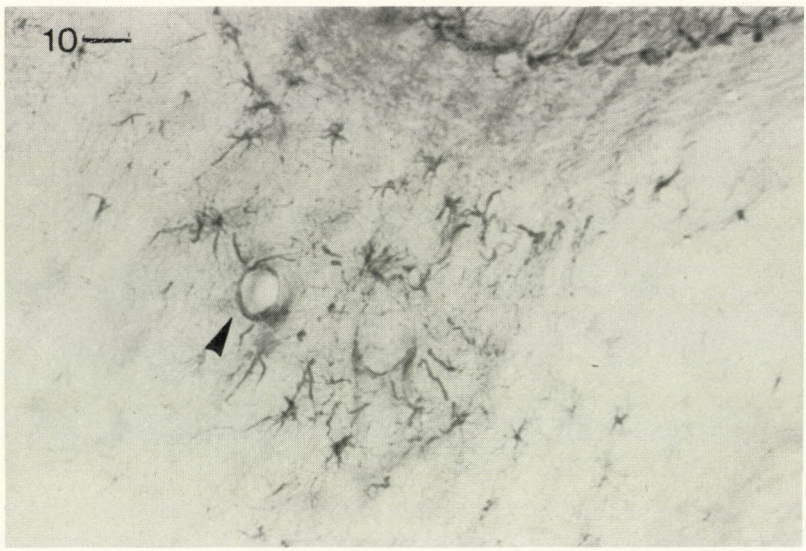

Fig. 10: V9 positive astrocytes and blood vessel (arrowhead) in the white matter of the cerebellum of an adult rat. Scale bar: 50 μm

DISCUSSION

Two monoclonal antibodies were recently described to show species-specific recognition pattern of vimentin when tested on different cell lines (Bohn et al., 1992). The authors localized the epitope recognized by Vim 3B4, applying sequence comparison and site-directed mutagenesis. A single amino acid replacement at residue 353 (coil 2 of the rod domain) - Val in the vimentin recognized, Leu in the non-recognized one - seems to control the antigen-antibody binding. Since the publication of the above article, amino acid sequence of vimentin from further species became to be accessible. The sequence of rat vimentin (Bussemakers et al., 1992), on one hand, lends further support to the suggested role of residue 353 in the antigen recognition by Vim 3B4. Furthermore, comparing the amino acid sequences with the species recognition pattern of V9 allows for the proposition that an Asn-Thr replacement at residue 417 might play an important role in the vimentin recognition by this antibody (Table 2). These data evidently do not exclude the possibily of the role of other factors (e.g. post-translational modifications).

Table 2

Correlation between the species recognition pattern of the two antibodies and vimentin amino acid sequence data.

Species		Vimentin recognition* with		
	residue 353	Vim 3B4	V9	residue 417
Human	Val	+	+	Asn
Rat	Leu	–	+	Asn
Mouse	Leu	–	–	Thr
Hamster	Leu	–	+	Asn
Chicken	Val	+	–	Thr

* Immunofluorescence recognition patterns from Bohn et al. (1992), cell lines studied: Human: HiKa, MRC-5, HeLa; Murine: 3T3, F9, MSF; Rat: NRK, C6, RAF; Hamster: BHK21, CHO; Chicken: primary fibroblasts.

For amino acid sequence data, see the following references: Human: (Perreau et al., 1988); Rat: (Bussemakers et al., 1992); Hamster: (Quax-Jeukenet al., 1983); Mouse: (Hennekes et al., 1990); Chicken: (Zehner et al., 1987)

We asked the question whether the species-specificity of these antibodies applied also to the vimentin expressed in glia cells. This question was based on several data showing multiple vimentin forms within one organism. The first indication of this type came from data obtained in *Xenopus laevis* (Hermann et al., 1989). Recently, different forms of vimentin have been detected in the goldfish central nervous system as well (Glasgow et al., 1994). Remarkable differences in the amino acid sequence of these forms, as well as in the tissue distribution of the corresponding mRNAs have been found. It is thus conceivable that distinct vimentin form(s) exist in the brain, where vimentin is expressed predominantly by early glial cells. Upon maturation, vimentin practically disappears from the mammalian brain (Dahl, 1981), persisting only in special cells, e.g. in Bergmann glia and some astrocytes (Pixley and de Vellis, 1984, Roeling and Feirabend, 1988).

Taking into account that Vim 3B4 recognized chicken, but not rat vimentin, while V9 showed an inverse species-specificity, we tested these antibodies on the brains of these two species, with respect to the ontogeny as well. In the non-glial cell lines, vimentin recognition has been tested by immunofluorescence (Bohn et al., 1992). We have done initial experiments to localize vimentin within the chicken brain with the same method, while detailed comparative studies were performed by the immunoperoxidase technique. To gain access of the developmental aspects of vimentin expression, we paid special attention to the cerebellum, since Bergmann glia are among the rare cells containing vimentin persisting into adulthood.

Our results show that in the glial cells of the chicken the epitope recognized by Vim 3B4 is clearly present both at the age of day 1 and in adults. This epitope could be detected in young rats, whereas it was not any more present in adults. On the contrary, the epitope recognized by V9, was detectable in both types of glial cells of the rat at both ages studied, but it was barely present in the chicken. In other words, the vimentin form bearing the epitope detected in the non-glial cell lines of the given species (Bohn et al., 1992) was present in both types of glia, at both ages studied. The second vimentin form - bearing the epitope virtually absent in the non-glial cell lines - seemed to be present mutually in the glial cells of both species, albeit expressed in a more restricted manner. Namely, the expression of the V9 epitope in the chicken is restricted to a distinct cell population (Bergmann glia at the top of each cerebellar folium) while the expression of the Vim 3B4 epitope in the rat is restricted in time, by being present only in young animals. Therefore, the expression of these two forms of vimentin has to be differently regulated.

In conclusion, our observations clearly speak for the coexistence of at least two vimentin forms in the rat and chicken brain. During ontogenesis, the expression of these forms seem to be distinctly and species-specifically regulated.

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REFERENCES

Bohn, W., Wiegers, W., Beuttenmüller, M. and Traub, P.(1992) Species-specific recognition patterns of monoclonal antibodies directed against vimentin. Exptl. Cell Res. 201, 1-7

Bussemakers, M.J.G., Verhaegh, G.W.C.T., Van Bokhoven, A., Debruyne, F.M.J. and Schalken, J.A. (1992) Differential expressions of vimentin in rat prostatic tumors. Biochem. Biophys. Res. Commun. 182, 1254-1259.

Dahl, D. (1981) The vimentin-GFA transition in rat neuroglia cytoskeleton occurs at the time of myelination. J. Neurosci. Res. 6, 741-748

Franke, W.W., Schmid, E., Osborn, M. and Weber, K. (1978) Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc. Natl. Acad. Sci. USA 75, 5034-5038

Glasgow, E., Druger, R.K., Fuchs, C., Levine, E.M., Giordano, S. and Schechter, N. (1994) Cloning of multiple forms of goldfish vimentin: Differential expression in CNS. J. Neurochem. 63, 470-481

Hennekes, H., Kuhn, S. and Traub, P. (1990) Coding sequence and flanking regions of the mouse vimentin gene. Mol. Gen. Genet. 221, 33-36

Herman, H., Fouquet, B. and Franke, W.W. (1989) Expression of intermediate filament proteins during development of Xenopus laevis. I. cDNA clones encoding different forms of vimentin. Development 105, 279-298

Perreau, J., Lilienbaum, A., Vasseur, M. and Paulin, D. (1988) Nucleotide sequence of the human vimentin gene and regulation of its transcription in tissues and cultured cells. Gene 62, 7-16.

Pixley, S.K.R. and de Vellis, J. (1984) Transition between immature radial glia and mature astrocytes studied with monoclonal antibody to vimentin. Developmental Brain Research 15, 201-209

Quax-Jeuken, Y.E., Quax, W.J. and Bloemendal, H. (1983) Primary and secondary structure of hamster vimentin predicted from the nucleotide sequence. Proc. Natl. Acad. Sci. U S A .80, 3548-3552

Roeling, T.A. and Feirabend, H.K.P. (1988) Glial fiber patterns in the developing chicken cerebellum: vimentin and glial fibrillary acidic protein (GFAP) immunostaining. Glia 1, 398-402

Zehner, Z.E., Li, Y., Roe, B.A., Paterson, B.M. and Sax, C.M. (1987) The chicken vimentin gene. Nucleotide sequence, regulatory elements, and comparison to the hamster gene. J. Biol. Chem. 262, 8112-8120

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SURVIVAL OF EMBRYONIC RAT MESENCEPHALIC TISSUE TRANSPLANTED INTO CEREBRAL CORTEX IN THE PRESENCE OF CO-GRAFTED EMBRYONIC STRIATUM

Kálmán, M. and Tuba, A.

1st Department of Anatomy, Semmelweis University of Medicine,
H-1450 Budapest, Tűzoltó u. 58, Hungary

Summary: The transplantation of embryonic dopaminergic tissue is an approach to the therapy of parkinsonism. In this study we compared the development of the rat embryonic ventral mesencephalon transplanted together or without striatal tissue into the cerebral cortex of adult rats. After one month, the survival ratio was 17 of 44 when striatal tissue was co-grafted, while only 4 of 45 transplants survived when the co-graft was omitted. In the mesencephalic graft, the appearance of tyrosine hydroxylase-containing cells was investigated by immunohistochemical method. Applying a fluorescent tracer dye, we demonstrated the growth of neural fibers from the mesencephalic tissue to the striatal co-graft. In situ, the embryonic striatal tissue is the natural target area for the developing nigrostriatal pathway. The possible role of trophic factors and postsynaptic target surfaces provided by the co-graft is discussed as basis of the adjuvant effect.

Key words: neurotransplantation, co-grafting, dopaminergic transplants, mesencephalon, striatum

INTRODUCTION

The transplantation of embryonic ventral mesencephalic tissue can ameliorate the symptoms of the experimental parkinsonism (Björklund and Stenevi, 1979, Fisher and Gage, 1993), the results however, are not always satisfactory enough for a confident clinical application (Björklund, 1991, Lindvall, 1991, Fisher and Gage, 1993). To increase the survival and functional effect of these transplants, we must take in consideration that the adult central nervous tissue of host animals is entirely adequate target for the embryonic tissue grafts. Developing neural cells need a proper set of neural growth factors and target area to form synapses (Oppenheim, 1981, Cunningham, 1982, Haun and Cunningham, 1984). We can suppose that co-grafted embryonic target tissue can provide these factors.

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The adjuvant effect of co-grafted target tissue have been demonstrated in case of several systems (muscle and spinal cord: Trok et al., 1994, cortical and subcortical tissues: Björklund et al., 1982, Haun and Cunningham, 1984). In the case of the dopaminergic cells of the substantia nigra, the target area is the striatum. Some previous data prove the adjuvant effect of the striatal cells on the development of the mesencephalic cells in cell cultures (Prochiantz et al., 1979, Di Porzio et al., 1980, Hemmendinger et al., 1981, Denis-Donini et al., 1983, Hoffmann et al., 1983) or in transplanted mixed cell suspension (Brundin et al., 1986, Yurek et al., 1990, Constantini et al., 1994). Development of interconnections has been demonstrated when solid mesencephalic and striatal tissues were co-grafted intra-ocularly (Olson et al., 1979) or intracerebrally (on the surface of the inferior collicle: Jaeger, 1986, in the striatum: De Beaurepaire and Freed, 1987, beside the host substantia nigra: Dunnett et al., 1989). In our experiments, the co-transplantation of rat embryonic striatum proved to be crucial for the survival of the rat embryonic ventral mesencephalon transplanted into the cerebral cortex of adult rats.

MATERIALS AND METHODS

To obtain dated embryos, vaginal smears were prepared from female albino rats mated with males overnight. The day of spermapositivity was considered as E0. On the 15th or16th day of gestation the embryos were removed in deep ketamine-xylazine narcosis (20 and 80 mg/kg body weight, respectively) from the mother, and the ventral part of the mesencephalon and the lateral part of the ganglionic eminence were dissected out. The use of the ventral mesencephalon instead of the substantia nigra is established, because the latter cannot be dissected out properly at this early age of development (Björklund and Stenevi, 1979, Fisher and Gage, 1993). The lateral part of the ganglionic eminence represents the primordium of the future caudate-putamen complex, the natural target of the nigrostriatal dopaminergic pathways. The adult recipient rats (150 to 250 body weight, of either sex) were also anaesthetised deeply by ketamine and xylazine (20 and 80 mg/kg body weight, respectively). At every recipient animal, an approximately 3X3 mm piece of the left parietal bone was removed, the dura was pierced and a pit was formed for the transplants in the cortex by a vacuum pump. The pit was above the host caudate-putamen complex but did not penetrate the corpus callosum. Only tissue of ventral mesencephalon was implanted into 45 animals while other 44 animals received ventral mesencephalon graft together with lateral ganglionic eminence. Then the bony piece removed formerly was repositioned, and the wound sutured accurately. Antibiotics were applied both locally and systematically.

After a one-month survival period the animals were overdosed by ether and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH7.4) and postfixed overnight in the same fixative. The transplants and the surrounding host tissue was dissected out, embedded into epoxy resin (Durcupan, Fluka) and cut into semithin sections.

The presence of the tyrosine hydroxylase, a marker of catecholaminergic neurons, was demonstrated by immunohistochemical procedure. In this case, the tissue

samples were embedded into agar and 70 μm thick sections were cut on a Vibratome. The floating sections were pre-treated with 3% H_2O_2 (5 min, room temperature) to suppress the endogenous peroxydase activity and 20% normal goat serum (1.5 hs, room temperature) to block the nonspecific antigen binding. The antiserum against tyrosine hydroxylase (polyclonal rabbit antiserum, produced by Eugene Tech Int., diluted 1:1000 in the presence of 0.5% Triton-X 100) was applied at 4°C, for 40 hs. Biotinylated anti-rabbit immunoglobulin and a complex of streptavidin and biotinylated horseradish peroxidase (both of them produced by Amersham, diluted 1:100) were then applied sequentially, each for 1.5 hs, at room temperature. All the immunoreagents were dissolved in 0.1 M phosphate buffer (pH 7.4). Between the incubations, the sections were washed in this phosphate buffer for 30 min. Finally, the immunocomplex was visualized by diaminobenzidine reaction in Tris-HCl buffer (0.05 M, pH 7.4). Sections of host substantia nigra were processed together with the transplant-containing sections during the immunohistochemical procedure. Parallel sections were incubated without anti-tyrosine hydroxylase antibodies. In this case no immunostained cells were observed.

In one series of experiments (6 animals), a DiI (1,1'-dioctadecyl-3,3,3',3'-teramethylindo-carbocyanine dye, produced by Molecular Probes) crystal was implanted into every piece of mesencephalic tissue before grafting. DiI is a fluorescent dye which diffuses along the axons and is a suitable neural pathway tracer (for refs., see Honig and Hume, 1989). Further processing followed as described above except that the Vibratome sections were mounted in glycerol, coverslipped and examined under fluorescent microscope. After the microphotographs were taken, we embedded the sections into epoxy resin (Durcupan, Fluka) and cut into serial semithin sections.

It should be noted that immunohistochemical and tracer studies were only performed on the co-grafts because the low survival ratio of the single mesencephalic grafts.

RESULTS

When co-grafted with embryonic striatum, 17 of the 44 rat embryonic mesencephalic transplants survived in the cerebral cortex of adult rats, while the survival ratio was only 4 of 45 when mesencephalic tissue was transplanted alone.

The mesencephalic grafts either the single or the co-grafted ones contained a rather dense population of large polygonal neurons (Fig. 1). They had well-developed Nissl substance and one or two satellite cells were attached to almost every neuron. The cells resembled substantia nigra (i.e. zona compacta) cells *in situ* but no melanin granules were observed. Only a few myelinated nerve fibers were seen (Fig. 2). The co-grafted striatum lay adjacent to the mesencephalic transplant (Fig. 3). The striatal neurons were smaller and their Nissl substance was not so conspicuous.

In the mesencephalic grafts some cells proved to be immunopositive against tyrosine hydroxylase (Fig. 4). They formed small groups but they were never so numerous than in the host substantia nigra incubated parallel with the sections of the transplants. Only immunopositive fibers but no cells were found in the adjacent areas corresponding to either the striatal co-graft or the host cerebral cortex.

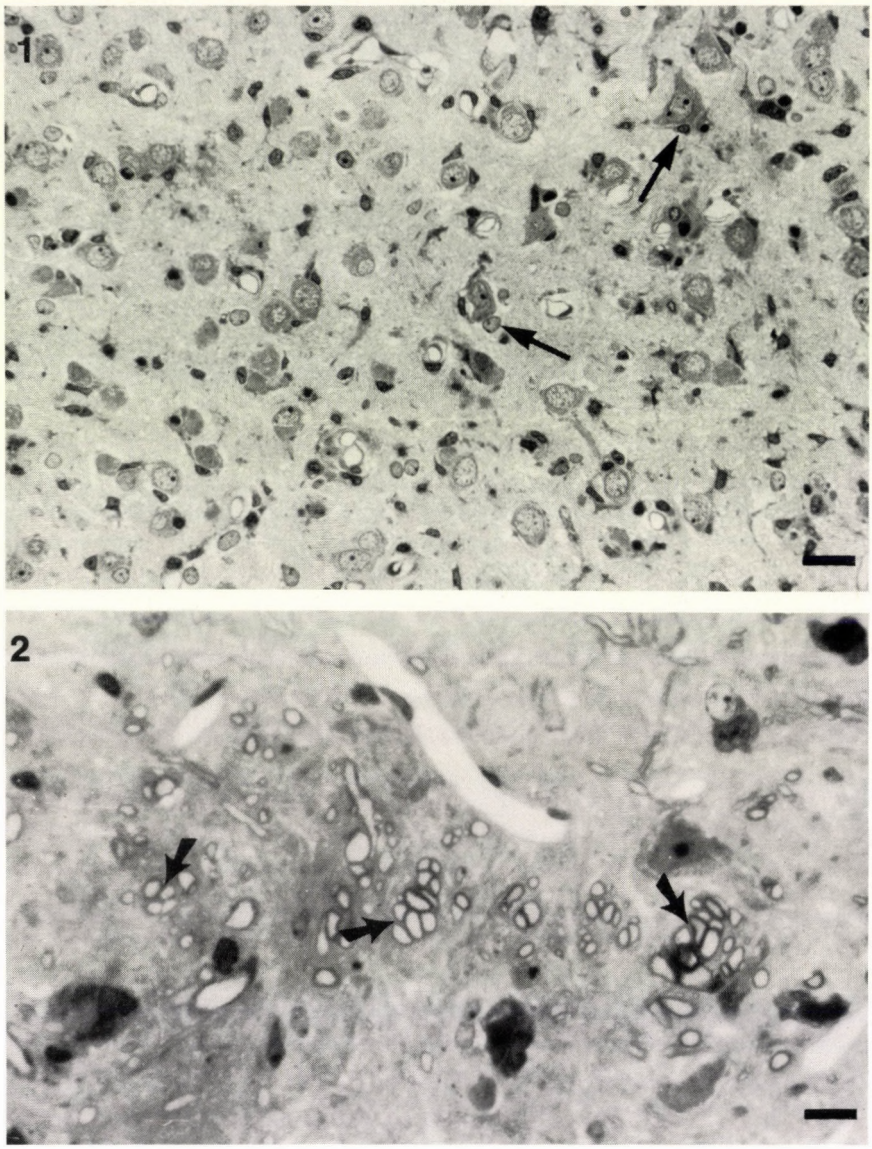
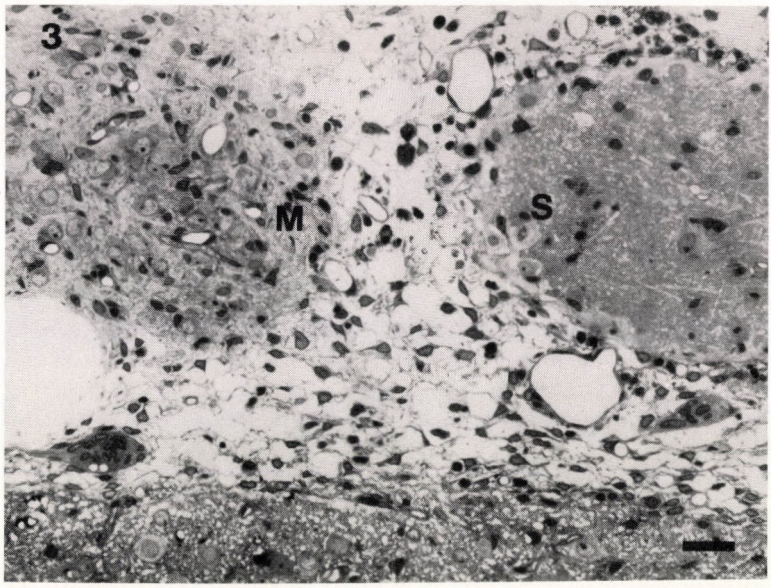


Fig. 1: A detail of a mesencephalic transplant. Note the large polygonal cells with the well-developed Nissl substance, and the satellite cells (arrow). Bar = 20 μm

Fig. 2: Myelinated nerve fibers in a mesencephalic transplant. Bar = 10 μm



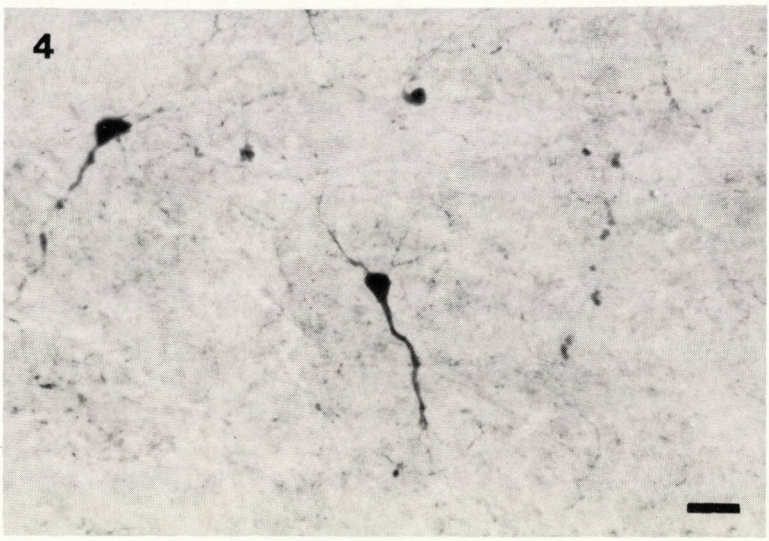


Fig. 3: Co-grafted striatal (S) and mesencephalic (M) tissues. In this case no neural connection can be seen in the section plane. Bar = 100 μm

Fig. 4: Tyrosine hydroxylase immunopositive cells in mesencephalic transplant. Bar = 25μm

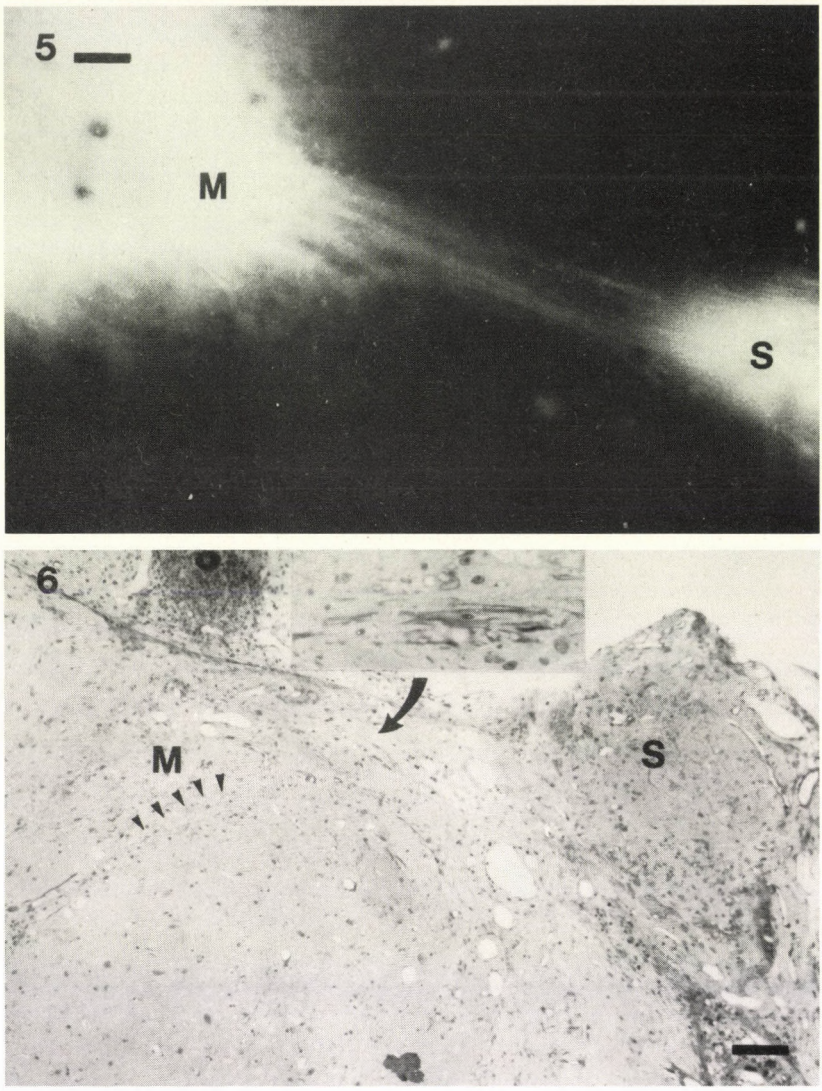


Fig. 5: Neural connection between the mesencephalic (M) and striatal (S) transplants, as demonstrated by the fluorescent tracer DiI. Bar = 200 μm

Fig. 6: Semithin section prepared from the specimen shown in Fig. 6. M-mesencephalic, S-striatal tissue, arrowheads - the border of the host tissue, inset - myelinated fibers between the transplants. Bar = 200 μm

When the mesencephalic graft was labelled with DiI, it showed strong fluorescence. From the bright mass of the transplant fainter straight strips emerged to approach an area of weaker fluorescence (Fig. 5). This latter area corresponded to the co-grafted striatal tissue as demonstrated in semithin section (Fig. 6) in which bundles of nerve fibers between the grafts were also observed (see inset). In any other direction, only short protrusions showed fluorescence (Fig. 5).

DISCUSSION

According to our results, the survival ratio of the mesencephalic transplants was significantly improved in the presence of embryonic striatal co-graft. The structure resembled to that described in previous papers on mesencephalic transplants (Jaeger, 1985). They displayed a characteristic feature of the substantia nigra cells, i.e. tyrosine hydroxylase immunopositivity. A relatively small number of tyrosine hydroxylase positive cells in comparison to that found *in situ*, and the formation of small groups of neurons have been described earlier (Jaeger, 1985). The paucity of myelinated fibers described also by Jaeger (1985), and the absence of melanin granules were indicative of a retarded maturation of the transplanted tissue. We could not rule out that the tyrosine hydroxylase immunopositive fibers seen around the transplants originate from other areas of the host brain. The results obtained by the tracer DiI, however, proved a direct growth of such fibers from transplant toward the striatal co-graft. No similar connections were formed with the host cortical tissue.

In previous experiments, when the mesencephalon was transplanted into different brain areas (on the surface of the inferior collicle: Jaeger, 1986, in the striatum: De Beaurepaire and Freed, 1987, beside the host substantia nigra: Dunnett et al., 1989) the adjuvant effect of the striatal co-graft were mainly functional. The amelioration of the biochemical and behavioral symptomes of the experimental parkinsonism was enhanced significantly when not only mesencephalon but also striatum was transplanted. Neural connections developed in these experiments between the co-grafts. Some authors described that the arborisation of dopaminergic cells was richer in the transplants of the mixed suspension of striatal and mesencephalic cells than in the graft of pure mesencephalic cell suspension (Brundin et al., 1986, Yurek et al., 1990). The significant effect on the survival ratio observed in the present study, can be explained by the data of Björklund et al., (1983), who reported that the cerebral cortex is not an optimal recipient area for mesencephalic grafts. In an adverse environment, the presence of an adjuvant co-graft is even more essential.

To explain the effect of co-graft on the survival ratio, we can implicate different factors. The importance of target cell location and synapse formation is well-known in

neurohistogenesis. The cells that fail to form proper contacts are "sentenced to death" (apoptosis) by their internal genetic program (Oppenheim, 1981, Cunningham, 1982), as it is described, for example, in the development of the sympathetic ganglia (Hendry and Campbell, 1976, Banks and Walter, 1977). Cell culture studies proved the benefits of cell contacts between co-cultured striatal and mesencephalic cells (Prochiantz et al., 1979, Di Porzio et al., 1980, Hemmendinger et al., 1981, Denis-Donini et al., 1983, Hoffmann et al., 1983). The importance of the target area was demonstrated well by the results that deprivation of the original nigrostriatal innervation promotes the development of the mesencephalic tissue transplanted into the striatum (for refs, see Beaurepaire and Freed, 1987, and Fisher and Gage, 1993) and even when the mesencephalon was transplanted into a deafferented striatum, the synapsing fibers of substantia nigra preferred the co-grafted embryonic cells to the deafferented adult host cells (De Beaurepaire and Freed, 1987).

Recent *in vitro* studies revealed that the development of dopaminergic cells is supported by special growth factors, BDNF (Brain Derived Growth Factor) and others, (for refs, see Zhou et al., 1994) and a similar effect could also be observed on transplanted cells (Sauer et al., 1983). Concerning the factors produced in the embryonic striatum, we could not find data in the literature. Only Tomozawa and Appel (1986) reported enhanced development of cultured striatal cells after that soluble extracts of striatal tissue were added to the medium.

On the basis of the data discussed here it seems to be clear that the striatal co-graft has an adjuvant effect on the grafted mesencephalic tissue itself, in experimental conditions. For the clinical application, however, one probleme has yet remained. The co-grafted tissue, as a competitive and preferred target can inhibit the innervation of the host tissue (De Beaurepaire and Freed, 1987). Considering that catecholamines have a rather diffuse effect around the site of release, we can suppose that the beneficial effect of the co-graft will not be counteracted, at least in the catecholaminergic transplants, by the "diversion" of the axones. Although some functional data cited above (Dunnett et al., 1989) seem to support this opinion, further examinations must forerun any clinical trials.

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REFERENCES

Banks, B.C. and Walter, S.J. (1977) The effects of postganglionic axotomy and nerve growth factor on the superior cervical ganglia of developing mice. J. Neurocytol. 6, 287-297.

Björklund, A. (1991) Neural transplantation - an experimental tool with clinical possibilities. Trends in Neurosci, 14, 319-322

Björklund, A. and Stenevi, U. (1979) Reconstruction of the nigrostriatal pathway by intracerebral nigral transplants. Brain Res. 177, 555-560

Björklund, A., Stenevi, U., Schmidt, R.H., Dunnett, S.B. and Gage, F.H. (1983) Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cell suspensions implanted in different brain sites. Acta Physiol. Scand. Suppl. 522, 9-18

Björklund, H., Seiger, A., Hoffer, B. and Olson, L. (1982) Trophic effects of brain areas on the developing cerebral cortex: I. Growth and histological organization of intraocular grafts. Dev. Brain Res. 6, 131-140

Brundin, P., Isacson, O., Gage, F.H. and Björklund, A. (1986) Intrastriatal grafting of dopamine-containing neuronal cell suspensions: Effects of mixing with target or non-target cells. Dev. Brain Res. 24, 77-84

Constantini, L.C., Vozza, B.M. and SnyderKeller, A.M. (1994) Enhanced efficacy of nigral-striatal cotransplants in bilaterally dopamine-depleted rats: an anatomical and behavioral analysis. Exp. Neurol. 127, 219-231

Cunningham, T.J. (1982) Naturally ocurring neuron death and its regulation by developing neural pathways. Int. Rev. Cytol. 74, 163-186

De Beaurepaire, R. and Freed,W. (1987) Embryonic substantia nigra grafts innervate embryonic striatal co-grafts in preference to mature host striatum. Exp. Neurol. 95, 448-454

Denis-Donini, S., Glowinski, J. and Prochiantz, A. (1983) Specific influence of striatal target neurons on the *in vitro* outgrowth of mesencephalic dopaminergic neurites: a morphological quantitative study. J. Neurosci 3, 2292-2299

DiPorzio U., Daguety, M., Glowinski, J. and Prochiantz, A. (1980) Effect of striatal cells on *in vitro* maturation of mesencephalic dopaminergic neurone grown in serum-free conditions. Nature, 288, 370-373

Dunnett, S., Rodgers, D. and Richards S (1989) Nigrostriatal reconstruction after 6-OHDA lesions in rats: combination of dopamine-rich nigral grafts and nigro-striatal "bridge" grafts. Exp. Brain Res. 75:525-535

Fisher, L.J. and Gage, F.H. (1993) Grafting in the mammalian central nervous system. Physiol. Rev. 73, 583-616

Haun, F. and Cunningham, T.J. (1984) Cortical transplants reveal CNS trophic interactions in situ. Dev. Brain Res. 15, 290-294

Hemmendinger, L.M., Garber, B.B., Hoffmann, P.C. and Heller, A. (1981) Target neuron-specific process formation by embryonic mesencephalic dopamine neurons *in vitro*. Proc. Natl. Acad. Sci. USA, 78, 1264-1268

Hendry, I.A. and Campbell, J. (1976) Morphometric analysis of rat superior ganglion after axotomy and NGF treatment. J. Neurocytol. 5, 351-360

Hoffmann, P.C., Hemmendinger, L.M., Kotake, C. and Heller, A. (1983) Enhanced dopamine cell survival in reaggregates containing telencephalic target cells. Brain Res. 274, 275-281

Honig, M.G. and Hume, R.I. (1989) DiI and DiO: versatile fluorescent dyes for neuronal labeling and pathway tracing. Trends in Neurosci. 12, 333-341

Jaeger, C.B. (1985) Cytoarchitectonics of substantia nigra grafts: a light and electron microscopic study of immunocytochemically identified dopaminergic neurons and fibrous astrocytes. J. Comp. Neurol. 231, 121-135

Jaeger, C.B. (1986) Axonal terminal clustering in nigrostriatal double grafts. Dev Brain Res. 24, 309-314

Lindvall, O. (1991) Prospects of transplantation in human neurodegenerative diseases. Trends in Neurosci, 8, 376-384

Olson, L., Seiger, A.,Hoffer, B. and Taylor, D. (1979) Isolated catecholaminergic projections from substantia nigra and locus coeruleus to caudate, hippocampus and cerebral cortex formed by intraocular sequential double brain grafts. Exp. Brain Res. 35, 47-67

Oppenheim, R.W. (1981) Neuronal cell death and some related regressive phenomena during neurogenesis: a selective historical review and progress report. In: Studies in Developmental Neurobiology, (ed. Cowan, W.M.), pp. 74-133, Oxford, New York

Prochiantz, A., Di Porzio, U., Kato, A., Berger, B. and Glowinski, J. (1979) *In vitro* maturation of mesencephalic dopaminergic neurons is enhanced in the presence of their striatal target cells. Proc. Natl. Acad. Sci. USA, 76, 5387-5391

Sauer, H., Fischer, W., Nikkhah, Wiegand, S.J., Brundin, P., Lindsay, R.M. and Björklund, A. (1993), Brain-derived neurotrophic factor enhances function rather than survival of intrastriatal dopamine cell-rich grafts. Brain Res. 626, 37-44

Tomozawa, Y. and Appel, S.T. (1986) Soluble striatal extracts enhance development of mesencephalic dopaminergic neurons *in vitro*. Brain Res. 399, 111-124

Trok, K., Freund, R.K., Palmer, M.R. and Olson, L. (1994) Spinal cord skeletal muscle cografts: trophic and functional interactions. Brain Res. 659, 138-146

Yurek, D., Collier, T. and Sladek, J. (1990) Embryonic mesencephalic and striatal co-grafts: Development of grafted dopamine neurons and functional recovery. Exp. Neurol. 109, 191-199

Zhou, J., Bradford, F.H. and Stern, G.M. (1994) The stimulatory effect of brain derived neurotrophic factor on dopaminergic phenotype expression of embryonic rat cortical neurons *in vitro*. Dev. Brain Res. 81, 318-324

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"NEUROTRANSMITTER SYSTEMS – MODERN TECHNICAL APPROACHES"

ABSTRACTS

from the International Symposium of the Drug Biochemistry Section
of the Hungarian Biochemical Society

Balatonőszöd, May 8 – 10, 1995

Neurobiology 3 (2), pp. 177–178, 1995

INTRODUCTION

Arányi, P.

President of the Drug Biochemistry Section of the Hungarian Biochemical Society

R & D Directorate of the Chinoin Pharmaceutical and Chemical Works Co. Ltd., Budapest H-1045, Hungary

The Drug Biochemistry Section of the Hungarian Biochemical Society had its yearly meeting in an unusually cold and wet spring (May 8–10, 1995, Balatonőszöd) at Lake Balaton.

We were however compensated by the active, sometimes heated discussions and by the lectures of high scientific standard.

The theme of the meeting "Neurotransmitter systems – modern technical approaches" was perhaps less focused than in previous years. Still it caught the interest of most of the participants (97 registered) and we felt no need for parallel sessions or small working groups. We had two distinguished guests from the USA, H. Sershen (Nathan S. Kline Institute) and U. V. Banakar (St. Louis College of Pharmacy).

The contributors had 30 – 45 minutes to introduce a technique and show applications from their own practice.

Topics included a whole series of talks on in vivo microdialysis focusing mostly on the dopaminergic system (F. Auth, Richter, J. Kiss, KOKI, O. Elekes, Richter), determination of intracellular ion concentrations and membrane potential by use of fluorescent dyes (B. Rosta, SOTE, L. Tretter, SOTE), microelectrophysiology (S. Erdő, Chinoin, S. Farkas, Richter) Positron Emission Tomography (L. Trón, DOTE) use of cell

culture systems in modelling neurodegeneration and neuroprotection (A. Kovács, EGIS, V. Lakics, Chinoin, E. Madarász, ELTE, F. Joó, SZBK) and molecular biology (P. Somogyi, GYKI, Z. Bősze, MBKM). This list meant to illustrate diversity rather than to cover all of the program.

Recent changes in the Hungarian economic and political environment that are a threat to research institutes and also precipitated serious changes in research and development strategies in the pharmaceutical industry were the topic of a round table discussion lasting late in the night. We have concluded that a more intense participation in each others' scientific and educational programs can be an important survival factor. As an immediate move in that direction we have decided that the theme of our next year's meeting should be proposed by universities and academic research centers from among the lines they feel can be of interest for the pharmaceutical industry and can eventually lead to the development of novel drugs.

The presidium of the Drug Biochemistry Section, where all major R & D based Hungarian pharmaceutical companies are represented, is waiting for the proposals for 1996.

Neurobiology 3 (2), p. 179, 1995

MICRODIALYSIS–SOME APPLICATIONS IN DRUG RESEARCH

Auth, F.

Pharmacological Research Centre, Chemical Works of G. Richter Ltd., Budapest, Hungary

Microdialysis is a powerful technique for studying extracellular concentrations of neurotransmitters and other substances in the brain and biological fluids. Following a brief overview of some critical aspects of the sampling and analytical requirements for this methodology its potential application in the process of drug development is discussed. In our laboratory microdialysis has been used among others to study the effects of the dopamine uptake inhibitor (DAUI) RGH-9623 on extracellular dopamine (DA) level. Comparison with other reference DAUIs following different routes of administration has been made. After local administration through the probe the enantiomers of RGH-9623 and the racemic compound itself have been compared. Results with RGH-2716, a compound with antiischemic and cognitive enhancing properties are also shown.

Since nigrostriatal and mesocortical dopaminergic pathways seem to be involved in the etiology and therapy of schizophrenia, it is of interest to look for neurochemical substrates of differences between classical and atypical antipsychotics. Comparing the effects of high and low doses of haloperidol we found that changes in extracellular levels of DA and its metabolites (DOPAC, HVA) follow very different dynamics, namely, the level of the neurotransmitter is elevated at low doses already, while levels of the metabolites increase only at higher doses. Thus, the effect of low dose of haloperidol resembles that of clozapine at higher dose suggesting that dopaminergic neurochemical changes following acute administration of haloperidol and clozapine may primarily reflect their different affinities for D2 receptors. In a dual-probe microdialysis experiment applying the neurochemically minimally effective dose of clozapine we were unable to find any preferential regional effect in the striatum and nucleus accumbens concerning the extracellular levels of DA, DOPAC and HVA. It seems therefore, that neurochemical data obtained following acute administration of haloperidol and clozapine neither predict nor reflect their different clinical side-effect profile observed during chronic administration.

Advantages and other configurations of dual-probe experiments is demonstrated with special reference to studying somatodendritic receptors. A model is presented revealing that the same experimental setting may theoretically result in different outcome in single vs. dual-probe experiments due to the different spatial distribution of neurons contributing to the delivery of infiltrated substances.

Akadémiai Kiadó, Budapest

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MEETING PHYSIOLOGICAL DEMANDS THROUGH SPECIALIZED DRUG DELIVERY

Banakar, U. V.

St. Louis College of Pharmacy, St. Louis, Missouri 63110 U. S. A.

The so-called ideal oral sustained-release, i. e., zero-order rate-controlled, drug delivery systems that are expected to maintain steady drug concentration levels in blood are based on the assumption that the drug's pharmacokinetic parameters are not influenced by circadian rhythms. It is now well established that nearly all body functions including heart rate, body temperature, blood pressure and also pharmacokinetic parameters display significant daily variations. Coupled with this observation, generally, the drug absorption is slow in stomach, rapid in the proximal portion of the intestine and once again slow in the distal portion of the intestine following oral drug delivery. Consequently, a drug delivery system releasing drug at zero order may not be able to provide a constant drug concentration level. On the contrary, a system which is in congruence with the changes in the absorption rate, if not compensating for them, will be able to provide more steady drug concentration levels.

A substantial body of theoretical and *in vivo* data are available in literature, especially, focusing on the improvement of sustained release dosage forms with respect to quick releasing systems to achieve constant plasma levels. However, there is a significant need for development of dosage form(s) that deliver drug complementary to the physiological/biological functional demands. Current developments in the modulated drug delivery, as opposed to strict rate controlled drug delivery will be presented. The presentation will discuss drug delivery performance data for products under development as well as of some recent market introductions.

Akadémiai Kiadó, Budapest

Neurobiology 3 (2), pp. 181 - 182, 1995

PRODUCTION OF PHARMACEUTICAL PROTEINS FROM TRANSGENIC RABBITS

Bősze, Zs., Aszódi, A., Baranyi, M. and Hiripi, L.

Institute for Animal Sciences, Agricultural Biotechnology Center, H-2100 Gödöllő, Hungary

Recombinant proteins – used as pharmaceuticals for human and animal therapy – produced in different systems. Bacteria are easily reprogrammed, usually they synthetize the foreign proteins very efficiently, but are unable to perform some of the post – translational modifications, more particularly glycosilation, which takes place in the Golgi apparatus of eukaryotic cells. Yeast and fungi offer interesting possibilities for relatively large – scale preparations of recombinant proteins, however the enzymatic machinery which glycosylates proteins is different in lower and higher eukaryotes, therefore the secreted recombinant molecules are frequently, inadequately glycosylated. Animal cells in culture offer all the possibilities to synthesize bona fide recombinant proteins, the limitation comes from the fact that animal cell culture is relatively costly and cannot be easily developed on a large scale.

Transgenic animals share most of the properties of animal cells in culture. They can properly carry out the post – translational modifications of recombinant protein. Transgenic animals are theoretically ideal fermentors – however many problems remain to be solved, before they can be used on a large scale. Predictive reports suggest, that 10% of recombinant proteins will be prepared from the milk of transgenic animals by the end of the century.

The predominant method used to transfer cloned genes into animals is direct microinjection into the pronuclei of fertilized eggs. The mechanism by which injected DNA integrates into a chromosome is unknown. The efficiency of gene integration into the genome tends to be lower for farm animals than for mice. The percentage of a gene – injected embryos that develop into transgenic animals varied from 0.1 to 4% for pigs, sheep and cattle. Transgenic yield in rabbits is comparable with mouse (10 – 15% of born animals). Rabbits represent a midpoint between laboratory and farm animals, transgenesis in rabbits offers considerable time saving over that required for sheep or cattle.

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Mammals vary in size, and several of them have been chosen to produce recombinant proteins in their milk. Ruminants, namely goat and sheep, appear the best candidates to produce proteins up to several tons per year. Pig is considered as a possible living frementor, although milk cannot be collected as easily as from ruminants. Rabbit produces up to 200 – 250 ml of milk per day. Its milk is particularly rich in protein (3 times higher than in cow's milk) and transgenic rabbits can be easily obtained at a relatively low cost. Theoretically 1 kg recombinant protein per year can be produced by transgenic rabbits. In addition, short pregnancy (30 days) and large litter size (up to 15) are additional advantages. Our group has been working on altering milk composition in transgenic rabbits (1, 2, 3). The same strategy can be adapted to produce valuable proteins of pharmaceutical interest.

References:

1. Bősze, Zs., Devinoy, E., Puissant, C., Fontaine, M. L. and Houdebine, L. M.: Characterization of rabbit κ–casein cDNA: control of κ–casein gene expression in vivo and in vitro. Journal of Molecular Endocrinology 11. 9 – 17. 1993.

2.Baranyi, M., Brignon, G., Anglage, P. and Ribadeau–Dumas, B.: New data on the proteins of rabbit (Oryctogalus cuniculus) milk. Comp. Biochem. Physiol. in press 1995.

3. Baranyi, M., Aszódi, A., Devinoy, E., Fontaine, M. L., Houdebine, L. M. and Bősze, Zs.: Cloning of rabbit κ–casein gene and in vivo expression in transgenic mice. (manuscript in preparation)

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IN VIVO MONITORING OF LACTATE, GLUCOSE AND URIC ACID USING ULTRAFILTRATION, MICRODIALYSIS AND ENZYME REACTORS

Elekes, O.[1], Muscone, D.[2], Venema, K.[3] and Korf, J.[3]

[1]Pharmacological Research Center, Chemical Works of Gedeon Richter Ltd., Hungary
[2]Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Italy
[3]Department of Biological Psychiatry, University of Groningen, The Netherlands

Continuous monitoring of metabolism in vivo is of interest in biomedical research and has the potential for clinical applications. This approach is currently possible only for few chemicals and practical solutions include the application of microdialysis. Recently an alternative technique for in vivo sampling has appeared, based on ultrafiltration. The devices thus fare developed for ultrafiltration give only a single point value and are not suitable for collection over a longer time period, e.g. 24 hours. Because of the little amount of samples such near non invasive sampling techniques need simple and sensitive analytical methods. Enzyme based amperometric flow injection analysis (FIA) systems for monitoring lactate, glucose and uric acid are described here. The oxidase and peroxidase enzymes are physically coimmobilised in a sandwich type reactor and ferrocene serves as a mediator. The assays are based on the measurement of a reduction current resulted from the enzymatic reactions, at a glassy carbon electrode held at a potential of 0.00 mV (vs Ag/AgCl).

High selectivity along with high sensitivity (the detection limit was of 30 nM for lactate, glucose and 60 nM for uric acid) and good stability (the enzymes remained active for more than 6 weeks at 30°C) was achieved. Only 6 µl of specimen is required and the precision and accuracy are very good. Moreover, due to constructional and operational simplicity, the present approach may be successfully completed with current microelectronic technology, and can also be miniaturised.

The lactate assay combined with microdialysis was applied in rats for cerebral monitoring. The usefulness of the assay in clinical chemistry is illustrated by the measurement of human serum uric acid and glucose concentration.

A new 24 hours sampling system, based on ultrafiltration, realised without the use of any mechanical device (peristaltic or syringe pump) will also be shown.

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SOME ELECTROPHYSIOLOGICAL METHODS FOR STUDYING FUNCTIONS RELATED TO EXCITATORY AMINO ACID RECEPTORS

Farkas, S., Horváth, Cs. and Kocsis, P.

Pharmacological Research Centre, Chemical Works of Gedeon Richter Ltd., H-1475 Budapest, POB. 27, Hungary

During the last fifteen years abundant evidence has accumulated proving that glutamate is the major excitatory transmitter in the central nervous system. Glutamate exerts its effect via ionotropic and metabotropic excitatory amino acid (EAA) receptors. Ionotropic receptors are further classified into NMDA, AMPA (or AMPA/kainate) and kainate receptors based on their agonist sensitivity. Although relatively well defined physiological functions have been attributed to AMPA and NMDA receptors, function of kainate receptors (or "high-affinity kainate binding sites") is a matter of debate. We will present our results obtained by some electrophysiological methods, suitable for testing functions of EAA receptors and using drugs acting on AMPA or NMDA receptors.

In the first part of our presentation we will discuss the basics, advantages and limitations of the "grease-gap" or "cortical wedge" method, which is a widely used method for pharmacological studies on brain slices (from rats):

Wedge shaped cortical slices are placed across the isolating wall of an electrically isolated two compartment perfusion chamber and perfused with artificial cerebrospinal fluid (ACSF). Thus, depolarisation of cells in the grey matter evoked by EAA agonists (glutamate, AMPA and NMDA) can be detected as a TTX insensitive shift in potential difference between the two compartment. Voltage shift responses evoked by glutamate are only partly attenuated by NMDA antagonists and are unaffected by AMPA antagonist drugs, suggesting that "electrogenic uptake" or an unidentified glutamate receptor takes part in the voltage shift caused by glutamate. On the other hand, responses induced by AMPA or by NMDA can easily be blocked by AMPA or NMDA antagonist drugs, respectively. The method is suitable for functional screening of EAA antagonist compounds and for quantitative pharmacological studies on agonist-antagonist interactions (dose-response curve shift, pA_2, IC_{50} etc.). The TTX and NMDA antagonist sensitive but AMPA antagonist insensitive spontaneous epileptiform activity (spike shaped depolarisations) which appears if the grey matter was perfused with Mg^{2+}-free ACSF is a characteristic phenomenon detectable on cortical slices.

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In the second part of the presentation studies on participation of AMPA and NMDA receptors in the spinal synaptic transmission will be discussed. In these investigations various components (monosynaptic reflex, disynaptic reflex, polysynaptic reflex - up to 10 ms poststimulus time - and the long lasting reflex discharge - up to 400 ms poststimulus time) of the dorsal root stimulation (single pulse or train - 500 Hz, 5 pulses) evoked ventral root reflex discharge were studied and "supramaximal" blocking doses of the AMPA antagonist GYKI 52466 (8-16 mg/kg, i.v.) and of the NMDA antagonist MK-801 (dizocilpine, 2 mg/kg, i.v.) were administered in C_1 spinal rats. The results indicate that all ventral root reflex activities were abolished by blockade of the AMPA receptors. On the other hand, blockade of NMDA receptors caused only partial inhibition in all reflex components. Our results conflict with the general view that AMPA and NMDA receptors mediate the monosynaptic- and the polysynaptic reflex, respectively. Instead, **AMPA receptors play a substantial role, in all excitatory synaptic transmission onto the spinal motoneurones while NMDA receptors give only a supplementary contribution. The relative importance of NMDA receptors in the mediation of short latency components is lower** (e.g. about 10 % in the monosynaptic reflex), while **in case of train stimulation and in later components, NMDA receptors gain relatively more significance** (about 45 % in the train stimulation evoked late reflex discharge). This is in agreement with predictions that may be drawn from differences in desensitisation kinetics of AMPA and NMDA receptors.

Desired selectivity of the applied pharmacological tools has been demonstrated testing electrical excitability of motoneurones and by microiontophoretic application of EAA agonists into the motoneurone pool.

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NEUROTRANSMISSION AND MICROSCOPY: RELIABILITY OF THE APPROACH

Halász, N.

Laboratory of Histology, R & D Directorate, Chinoin Pharmaceutical and Chemical Works Co. Ltd., Budapest, Hungary

A closer view at tissues and cells opened a new world for examiners of the nervous system when the silver impregnation technique, discovered by of Camillo Golgi and was applied for mapping the principal structure and main connections by Ramón y Cajal and many successors. The following century revealed, however, than never more than 5% (rather, 1%, in average) of neurons can be visualized by this technique, and we know nothing on the rest of neurons in the very same system of the actually impregnated tissue piece.

The second boom of extending broadly the contribution of neuromorphology to understand better the neurotransmission occurred when electron microscopy was capable of viewing even the closest junctions in between neuronal elements and constituents of synapses. Damage of the living cell through fixation, dehydration and embedding is, however, very substantial and so, relevance of the application of results to working systems can seriously be questioned.

Introduction and stepwise development of the main labelling techniques, mainly autoradiography and immunohistochemistry and more recently, the *in situ* hybridization enhanced enormously our understanding of transmitter-specific neuronal connections. Unfortunately, studies are focused to a very limited range of neurons when visualising one neurochemically distinct population of compartments only. In addition, "regular" artefacts should commonly be faced and calculated again.

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Refinement of fixation and visualisation techniques allowed us to ponder the significance of synaptic vesicles (as visualized by "conventional" techniques and also, by freeze-fracturing) and, as regards the events of the first milliseconds at chemical synapses, some intramembrane particles (Á. Párducz and co-workers). This technique can not take care simultaneously, however, of specific neurotransmitters at the pre- or postsynaptic site.

All these approaches allowed computer experts to build up models of interactions within more or less restricted neuronal networks of (mutually or exclusively) excitatory or inhibitory neurons (Lábos and collaborators) and, by this way, to give hints to morphologists which way to proceed for more reliable explorations.

Taking into consideration of the principles above, a discrete module of the central nervous system, the vertebrate olfactory bulb was introduced and illustrated as an assembly of neurons of various neurochemical features. Different components of this system were characterised by light- and electron microscopic morphology, double Golgi-electron microscopy, autioradiography and immunohistochemistry. Although results taken by a single -even advanced- technique, such a complex approach constitute a reliable basis of further neurochemical, developmental and physiological studies aimed to understand better the basic principles of neuronal transmission in this region of the vertebrate brain.

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IN VITRO RECONSTITUTION OF THE BLOOD-BRAIN BARRIER

Joó, F.

Laboratory of Molecular Neurobiology, Institute of Biophysics,
Biological Research Centre, H-6701 Szeged, Hungary

Unlike most organ systems, the central nervous system is separated from the blood by a protective cellular barrier, manifested by the continuous lining of capillary endothelial cells, which prevents the free passage of solutes and blood contituents from the circulation to the brain parenchyma. The capillary endothelial cells possess unique morphological, physiological and biochemical characteristics in the brain of essentially all vertebrates. After an era of studying with endogenous or exogenous tracers the unique permeability properties of cerebral endothelial cells in vivo, the next generation, i.e the freshly isolated cerebral microvessels model system was elaborated by us in 1973. Later, when conditions were provided for maintaining the cerebral endothelial cells in tissue culture systems, a further improvement of the model system was accomplished. Recent advances in our knowledge of the blood-brain barrier have in part been made by studying the properties and function of cerebral endothelial cells (CEC) with this in vitro approach. This review summarizes, in brief, the results obtained on isolated brain microvessels and on cultured cerebral endothelial cells and pinpoints different practical applications, in particular in drug research.

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SYNAPTIC AND NONSYNAPTIC NEUROTRANSMITTERS IN THE EXTRACELLULAR SPACE: THE SCOPE OF MICRODIALYSIS TECHNIQUE

Kiss, J.P. and Vizi, E.S.

Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, P.O.B. 67. Hungary

The extracellular space can be divided into synaptic and extrasynaptic areas. One-to-one communication of the neurons occurs in the synaptic cleft, however accumulating data show, that the synaptic neurotransmission is not the only one possibility for the information flow between neurons. Cells can send messages to many other neurons (one-to-many connection) through the extrasynaptic space. This communication is called nonsynaptic neurotransmission. The synaptic neurotransmission serves the information processing functions of the nervous system whereas the nonsynaptic interactions have a modulatory, fine-tuning role.

In vivo brain microdialysis is a suitable technique for acquiring information about the biochemical composition of the extracellular space in different regions of the central nervous system. Since the extracellular space is mainly of extrasynaptic nature, the data obtained from microdialysis experiments reflect primarily the nonsynaptic interactions.

In a recent microdialysis study Schneider et al. showed, that dopamine (DA) can diffuse over large distances (5-7 mm) in the striatum if the dopaminergic nerve terminals are destroyed by MPTP or the dopamine uptake carrier is blocked by nomifensine. The published results clearly demonstrate the significance of the nonsynaptic interactions of DA in the striatum and may explain the effectiveness of implanted dopaminergic cells in the treatment of Parkinson's disease.

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MEASUREMENT OF NEUROPROTECTION AGAINST QUISQUALATE EXCITOTOXICITY IN PRIMARY CULTURES FROM RAT EMBRYONIC TELENCEPHALON

Kovács, A. D.
Department of Biochemistry, EGIS Biological Laboratories, EGIS Pharmaceuticals Ltd., Budapest, Hungary

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.

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.

Neurons in tissue culture from rat embryonic telencephalon become very sensitive to glutamate receptor-mediated toxicity after two weeks in vitro, and medium change (MC) completely destroys them by activating the N-methyl-D-aspartate (NMDA) receptors. To avoid the neurotoxic effect of MC, cultures were exposed to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate (KA) receptor agonist quisqualate (10 μM) and to its antagonists, the 1-(aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466; 45 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 50 μM) for 21 h without medium change. In another approach treatments for 22 h with 10 μM quisqualate and 15 or 45 μM GYKI 52466 were carried out with MC but in the presence of 100 μM D-amino-5-phosphonopentanoic acid (D-AP5; an NMDA antagonist) to prevent neuronal cell destruction caused by fresh medium. Both approaches proved to be usable for measurement of neuroprotection against quisqualate excitotoxicity.

Based on LDH determination, a new neurotoxicity index (NI) was developed. Our new NI seems to be better parameter to evaluate neurotoxicity as compared to the two widely used NIs.

The primary telencephalic cultures - using our newly developed NI - are well suitable for screening large number of compounds for neuroprotective effect against AMPA/KA receptor-mediated excitotoxicity.

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CELL CULTURES IN MODELLING NEURODEGENERATION AND NEUROPROTECTION

Lakics, V., Molnár, P. and Erdő, S. L.

Chinoin Co., CNS Pharmacology, H-1045 Budapest, Tó u. 1-5, Hungary

The increasing incidence and significance of neurodegenerative disorders and acute brain injuries promote the research toward the possible mechanisms of these illnesses. The search for neuroprotective agents requires rapid, target-oriented screening of test compounds. Cell cultures have proved to fulfil these criteria. This study is an attempt to summarise the usefulness of neuronal cell cultures in the pharmacological research, focusing on the excitotoxic and Na^+ channel-mediated injury.

Among the advantages of cell cultures as experimental tools are the possibility to control the extracellular environment, the low cost and the lack of ethical, moral problems, compared to the animal experimentation. Variability, the small amount of biological material and the emphasised need for expertise should be taken into account as limitations of the method. Cell cultures can be classified into two major categories: primary cultures and continuous cell lines. Although, primary neuronal cultures are heterogeneous with respect to neuronal cell types, they contain postmitotic, well differentiated neurones compared to cell lines. In receptor mediated toxicity studies, such as the modelling of exitotoxicity, well differentiated and matured neurones can only be used. Unfortunately, for the time being, cell lines which bear glutamate receptors and are vulnerable to exitotoxic insult are not available.

Rat primary cortical cultures were chosen as a model system for studying exitotoxic cell death and Na^+ channel mediated injury. The extent of toxicity was

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detected using phase contrast microscopy and quantified via lactate-dehydrogenase leakage from the demaged cells.

Excitotoxins, N-methyl D-aspartate (NMDA) and kainate evoked marked, delayed type cell death in our cultures. The NMDA receptor antagonist, MK801 showed emphasised neuroprotective action against 1mM NMDA, in the nanomolar concentration range. Glycine site antagonists were also protective, but to a lesser extent. Increasing concentrations of glycine reversed this protection. AMPA receptor antagonists CNQX and DNQX ameliorated the 1 mM kainate-mediated cell death in the micromolar concentration range.

Veratridine, an opener of voltage-dependent sodium channels was 10 times more potent in inducing cell death in primary cultures than in SHSY5Y cell line. Blockers of sodium channels, phenytoin, carbamazepine and lidocaine as well as the neuroprotectant vinpocetine prevented veratridine-evoked toxicity in a concentration dependent fashion. The neuroprotective efficacies of these compounds did not correlate with their sodium channel blocking potencies revealed in patch clamp experiments. These example suggests that care should be taken with interpreting results obtained in neuroprotection studies with cell cultures.

In summary, using primary neuronal cultures, the possible neuroprotective action of compounds against excitotoxicity and veratridine-induced cell death can be detected, quantitated and compared with ease.

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IMMORTALIZED NEURAL PRECURSOR CELLS: THEIR USE IN PHARMACEUTICAL INVESTIGATIONS IN VITRO AND IN VIVO

Madarász, E.

Dept. of Comparative Physiology, Eötvös L. University, Budapest, Hungary

Immortalized cell lines of central nervous system origin might provide important tools in studies on the mechanisms of determination of neural tissue specific cellular phenotypes. Depending on their origin, such cell lines might represent different developmental potentials and might give rise to various neural phenotypes under different conditions. Immortalization of neural progenitor cells can be achieved by several routes : i)viral or cellular oncogenes can be transfected into cultivated brain cells[1-5]; ii) resting progenitor-populations of normal brain tissues can be activated, in vitro, by pharmacologycal doses of various growth factors[6,7]; iii) CNS tissues from transgenic animals with targeted activation / inactivation of appropriate regulatory genes can be dissociated and used for establishment of continuously proliferating neural precursor cell lines[8,9]. Immortalized neural progenitor cell lines were obtained from brains of double transgenic mice carrying genes coding the SV40 T-antigen and controlled by a LAP-responsive promoter of bacterial origin[8]. Neural characteristics were induced in such cells by reducing the expression of the T-antigen by IPTG and by growng the cells in aggregate cultures with bFGF. Different inducibility of neural phenotypes was found in cell lines obtained from brains of transgenic mouse embryos with targeted inactivation of p53 genes[9]. Cells of such lines developed neuronal characteristics upon induction by all-trans retinoic acid and are evaluable subjects of assays on neuronal fate-decision, in vitro. Inducible neural progenitor cell lines with different inducibility and developmental potential can be implantated into various regions of mouse brains of different ages, and are promising tools for studies on cell-replacement in damaged nervous tissue.

References:

1.Eves et al *Proc.Natl.Acad.Sci.* 89, 4373. (1992)
2.Eyrard et al *Proc.Natl.Acad.Sci.*87, 3062. (1990)
3.Ryder et al *J.Neurobiol.*23, 356. (1990)
4.Refranz et al *Cell* 66, 713. (1991)
5.Snyder et al *Cell* 68, 33. (1992)
6. Reynolds & Weiss *Science* 255, 1707 (1992)
7. Ray et al *Proc.Natl.Acad.Sci.*90,3602 (1993)
8. Tóth et al *J.Neurosci.Res.* 1995 in press
9. Madarász & Schlett *Eur.J.Neurosci. Suppl.*7, 2211 (1994)

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USE OF MOLECULAR MODELING METHODS FOR STRUCTURE AFFINITY ANALYSIS OF CENTRAL BENZODIAZEPINE RECEPTOR LIGANDS

Mátyus, P.[a], Barlin, G. B.[b], Balogh, T.[a] and Willis, A, C.[b]

[a] Institute for Drug Research, P.O.B. 82, Budapest H-1325, Hungary
[b] John Curtin School of Medical Research, The Australian National University, Canberra, GPOBox 334, A.C.T.2601, Australia

We have recently described that some 3-methoxy- and 3-acylaminomethyl-2-arylimidazo [1,2-*b*]pyridazines bind to the central benzodiazepine receptors (BZRs) with moderate to high affinities (IC_{50} ~ 100 - 1 nM).[1] Most of these compounds have been found to exert agonistic properties *in vivo* and *in vitro*.The structure-activity relationships for a subset of compounds (72) are now analysed by molecular modeling and CoMFA methods.

The pharmacophore and binding site models, proposed by superpositions, geometric and charge analyses, and semiempirical PM3 calculations on H-bonded complexes,[2] are consistent with known BZ receptor-ligand models. As minimum structural requirements, two hydrogen bonding sites (the nitrogen at position-1 and the oxygen in the 3-position) and a 2-substituted phenyl ring as lipopholic sites have been identified and characterized by their spatial arrangements.
For 6-benzylamino/benzyloxy/benzylthio/-3-methoxy/acetylamino and 6-halo-3-benzoylamino-methyl derivatives, the aromatic rings present in the 6- or 3-substituent, respectively, have also been proven to participate in the receptor binding.

The CoMFA analysis[2] (learning set: 30 compounds, $C(sp^3)$ probe atom with a +1 charge Sybyl standard options) gave satisfactory statistical parameters (r^2_{cv}=0.743; number of crossvalidation groups: 5; optimum number of components: 5; R^2=0.98). The predictability of this model was tested for a new set of compounds (19). Affinities were forecasted well for six moderately for seven and poorly for five compounds [the absolute value of $\log(1/IC_{50,pred.}) - \log(1/IC_{50,mean})$ is < 0.5; 0.5 - 1; 1 - 1.5, respectively].

References:

1. G.B. Barlin, L.P. Davies, R.A. Davis, P.W. Harrison: Aust. J. Chem. **1994**, *47*, 2001-2012, and references therein.
2. Semiempirical calculations were done by SPARTAN 3.1 (Wavefunction, CA), molecular mechanics, conformational analysis, superpositions and the CoMFA were carried out by the Sybyl 6.1 program (Tripos, MD).

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THE PATCH-CLAMP TECHNIQUE IN DRUG RESEARCH: EXAMPLES FOR APPLICATION

Molnár, P. and Erdő, S.L.

CNS Pharmacology Laboratory, Chinoin Co. Ltd., H-1045 Budapest, Hungary

During the past few years the patch-clamp technique has become a widely used microelectrophysiological method in drug research. The popularity of the method is due to a number of advantages over traditional in vitro methods, in terms of (a) direct information regarding agonist/antagonist actions at well-defined molecular targets, such as ion channels and ionotropic receptors, (b) relatively high capacity that may allow drug screening, (c) versatility, allowing the detection of various ion currents with minor modifications of the experimental conditions, (d) the possibility of studying indirect drug effects reflected by changes in transmembrane ion currents, and (e) of adjusting the composition of both intracellular and extracellular medium etc.

Nevertheless, the patch-clamp technique has it own limitations. In fact, only receptors forming ion channels of directly coupled to the channels can be examined. The artificial intracellular medium employed may not be taken as identical with the physiological intracellular fluid. Moreover, the patch-clamp instrumentation is relatively expensive.

The voltage-dependent sodium channel is considered to be an important pharmacological target for anticonvulsant, antiarrhytmic and local anaesthetic drugs. In additon, sodium channel blockers have been found to possess neuroprotective effects. Using the whole-cell patch-clamp technique, we have demonstrated that the clinically useful neuroprotectant, vinpocetine, is a specific blocker of the sodium channel (IC_{50} = 44 μM) in cultured cortical neurones.

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Prototype anticonvulsants, phenytoin and carbamazepine are considered to exert their effect via the blockade of sodium channels. Their IC_{50} values in whole-cell experiments on cortical neurones were about 50 and 200 μM, respectively. However, these concentrations of the drugs significantly inhibited high threshold calcium currents suggesting that the blockade of voltage-dependent calcium channels might also contribute to the pharmacological effects of phenytoin and carbamazepine.

Examples for GABA-A and NMDA receptor channel-currents were also obtained. Chloride currents evoked by the GABA-A agonist muscimol were antagonised by picrotoxinin; whereas the NMDA-evoked cation current was inhibited using antagonists of the co-agonist glycine site of the receptor complex.

Calcium-activated, large-conductance potassium channels may be considered as a unique type of ligand-gated ion channels. In whole-cell patch-clamp experiments on cultured tracheal smooth muscle cells the spasmolytic drug drotaverine blocked calcium-dependent potassium currents in a concentration-dependent fashion (IC_{50} = 48 μM at 40 mV). This effect of drotaverine was confirmed in single channel experiments indicating that the drug blocks potassium channels directly, rather than through changes in free calcium concentrations.

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THE DEVELOPMENT OF CHOLINERGIC NEURONS: RECEPTOR IMMUNOCYTOCHEMICAL STUDIES

Nyakas, Cs.[1,2], Gáspár, E.[1], Buwalda, B.[2], Felszeghy, K.[1], van der Zee, E.[2] and Luiten, P. G. M.[2]

[1]Central Research Department, Haynal University of Health Sciences, Budapest, Hungary and [2]Department of Animal Physiology, University of Groningen, The Netherlands

The structural and functional integrity between cholinergic axon terminals and cholinoceptive neurons in the neocortical and limbic brain areas play a major role in cognitive and other adaptive behavioral functions. The developmental patterns of cholinergic fiber ingrowth into these brain areas are strictly age-dependent and anatomically well organized.

The aim of present experiments was to study in rats the developmental profile of pre- and postsynaptic cholinergic markers 1) under normal condition, 2) after AF64A-induced marked loss of cholinergic fiber innervation of target cortical areas, 3) and after hypoxia-induced inhibition of cholinergic neurotransmitter function. The arborizing cholinergic fibers during development were visualized by acetylcholinesterase (AChE) histochemistry and choline acetyltransferase (ChAT) immunocytochemistry. The muscarinic acetylcholine receptors (mAChR) were immunostained with monoclonal antibody M35 raised against purified mAChR protein. To employ pre- and postsynaptic neurochemical cholinergic markers the activity ChAT and the number of ^{3}H-QNB binding sites were measured in hippocampal and neocortical brain areas.

The main findings show that: A) M35 immunoreactivity was present in all neuronal compartments of developing pyramidal cells in newborn rats well before the extrinsic cholinergic innervation reached the cholinoceptive neurons, B) a massive loss of cholinergic innervation of hippocampus, caused by icv AF64A injections at postnatal day 8, resulted in a severe retardation of mAChR development, C) perinatal hypoxia induced a transient delay of hippocampal and neocortical cholinergic innervation and a transient suppression of ChAT activity in the early postnatal period. This functional blockage of developing cholinergic neurons resulted in an up-regulation of postsynaptic receptor sites shown by ^{3}H-QNB binding measurement and mAChR autoradiography. Furthermore, the hypoxia-induced growth inhibition of cholinergic neurons was prevented by a calcium antagonist nimodipine.

In conclusion, during postnatal development remarkable plastic changes take place at both sides of cholinergic synaptic structures with might be subject of pathological influences. The developmental expression of mAChR antedate the extrinsic cholinergic afferentation in the cortical brain areas. The consequences of moderate functional blockage of afferentation up-regulates mAChR, while severe loss of afferentation permanently down-regulates the expression of mAChR.

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FLUORESCENT MEASUREMENT OF SYNAPTOSOMAL MEMBRANE POTENTIAL WITH ENHANCED ACCURACY

Roska, B., Csanády, L. and Adam-Vizi, V.

Department of Biochemistry II., Semmelweis University of Medicine, Budapest H-1444, Hungary

Synaptosomes are objects which cannot be penetrated by microelectrodes. Therefore fluorescent dyes were developed for membrane potential measurment. The major factor limiting the accuracy of these methods is the fact that the measured signal depends not only on membrane potential but on the total membrane surface of the sample as well.

In the case of synaptosomes the latter cannot be determined precisely, because the usually measured protein concentration of the synaptosome suspension doesn't specify the membrane area.

In order to overcome this difficulty we developed a procedure, and gained a highly accurate and reproducible method for membrane potential measurment.

Using 3,3'-diethylthiacarbocyanine iodide (C25), a slow response potential sensitive fluorescent probe we chose excitation and emission wavelengthes to be 566, 584 nm respectively in order to have the maximal sensitivity and the best linear relation between membrane potential and fluorescent signal. (This linearity has been observed in several previous studies).

Assuming that the fluorescent signal is influenced by the membrane potential and the membrane area independently, the following relation exists between the measured intensity ($I(c, V_m)$) and the membrane potential.

$$V_m = RT / F \cdot \left[I(c, V_m) / I(c, 0) - 1 \right] / a$$

where *a* is the slope of the linear intensity versus ln $[K^+]_{out}$ graph, *c* the total membrane area.

I (c,0) is the intensity at zero membrane potential which was achieved by adding 1 μM valinomycin, 3 μM gramicidin and 30 mM K^+. Since *a* was determined in the presence of valinomycin (which interferes with mitochondrial membrane potential), we used 1 μM rotenon, 5 mg/ml oligomycin to uncouple mitochondria during experiments. Applying this calibration method we were able to lower the relative error below 8 %. This was verified by measurments with depolarizing agents like veratridin, 4-AP and ouabain.

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SEROTONERGIC SYSTEM – THE TREASURE ISLAND OF DRUG RESEARCH

Schmidt, É. and Gacsályi, I.

Department of Biochemistry and Pharmacology, EGIS Pharmaceuticals Ltd., Budapest, Hungary

In the EGIS Pharmaceuticals the investigation of the serotonergic system and research of drugs affecting this system is one of the main research areas. In the 80's the serotonin project has been initiated . The aim of the serotonin project has been to synthesise and develop drugs effective in mental disorders and acting via 5-HT system. In this lecture we present deramciclane a putative antiserotonergic agent which is the prototype drug being developed within the framework of the serotonergic project. Deramciclane showed considerable affinity to 5-HT_{2C} (Ki = 8.7 nM) and 5-HT_{2A} (Ki = 35 nM) and moderate affinity to D_2 receptors. Results of studies on isolated organs also indicated the antiserotonin activity of deramciclane. Deramciclane proved to be active in test indicating anxiolytic activity, but showed no effect in tests predicting neuroleptic and antidepressant effects. Presently the compound is in the human Phase I. studies.

Simultaneously with the presentation of results of studies with deramciclane the possible role of serotonin in anxiety will also be discussed.

According to Briley and coworkers, 5,7-DHT evoked reduction of serotonin levels in the cortex and hippocampus, caused a significant anxiolytic effect in the elevated plus maze test (1). It was also revealed that administration of meta-chlorophenyl piperazine (mCPP, 5-HT_{2C} agonist) to patients with panic disorder and also to healthy volunteers provoked an anxiety like state (2). These findings suggest that compounds that decrease central serotonin transmission may reduce the level of anxiety, whereas those that increase serotonin transmission tend to enhance the level of anxiety.

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However, there are results which does not support the above mentioned hypothesis. File and coworkers collected microdialisate from dorsal hyppocampus after the administration of subconvulsant doses of pentylenetetrazole. Immediately after sampling the level of anxiety were measured in the elevated plus maze test, and the animals were separated into high-and low anxiety groups. The animals in the low -anxiety group had high levels of serotonin in their microdialisate, whereas the high-anxiety group showed low levels (3).

One possible explanation of this paradox is that anxiety is a state dependent reaction to the environmental situation and it is controlled by the serotonin mediated homeostatic mechanism (4).

These data in general support that there is a link between central serotonin system and anxiety states. Deramciclane a central antiserotonin agent is a possible candidate as an anxiolytic drug.

As far as the mechanism of action of deramciclane is considered an other possible indication is emerging - migraine prophylaxis. Recent preclinical and clinical findings provide evidences, that 5-HT_{2C} receptors involved the initiation of migraine. (5,6). Fozard and Kalkman hypothesised that nitric oxide (NO) is an important trigger for migraine and release of the endothelium derived NO may be modulated by 5-HT_{2C} active agent (7).

References:

1. Briley, M., Chopin, P. and Moret, C.: Psychopharmacology, 101, p.187 (1990).
2. Charney, D.S., Woods, S W., Goodman, W.K. and Heninger, G.R.: Psychpharmacology, 94, p.14 (1987).
3. File S.E., Curle, P.F, Baldwin, H.A. and Neal, M.J.: Neurosci. Lett., 83, p.318 (1987).
4. Briley, M., Chopin, P. and Moret, C: In: New concepts of anxiety, M. Briley and S.E. File (eds). Pierre Fabre Monograph Series, Macmillan Press, London, p.56 (1991).
5. Kahn R.S. and Wetzler S.: Biol. Psychiatr. 30. p.1139 (1991).
6. Fozard J.R.: In 5-Hydroxytryptamine mechanisms in primary headache, Olesen J. and Saxena P.R. (eds). Raven Press, New York, p. 200 (1992).
7. Fozard J.R. and Kalkman H.O.: Naun-Schmidedberg's Arch. Pharmacol., 350 p.225 (1994).

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MOLECULAR BIOLOGICAL METHODS IN THE RESEARCH OF DRUGS ACTING ON IONOTROPIC GLUTAMATE RECEPTORS

Somogyi P. and Tarnawa I.
Institute for Drug Research, H-1325 Budapest, P.O.Box 82,
Hungary

A new class of AMPA antagonist compounds has been developed recently at the Institute for Drug Research, Budapest (Tarnawa et al., 1993). To get a better understanding of the pharmacological actions of these compounds, new research techniques are going to be introduced, such as receptor cloning, and whole-cell voltage clamping on cultured mammalian cells expressing cloned glutamate receptors. The aim of this presentation is to render an account of these plans in the lights of the presently available pharmacological results.

Glutamate receptors play a central role in several central nervous system disorders, including epilepsy and acute- or chronic neurodegenerative diseases. Drugs that can attenuate glutamatergic excitation have a therapeutic potential for treating such diseases. On the basis of their pharmacological sensitivities, ionotropic glutamate receptors have been classified as NMDA, AMPA, or kainate receptors. Originally, NMDA receptors were suggested as a promising target for drug development, and the real apprehension of the involvement of AMPA receptors in pathological processes was hindered by the fact that no specific AMPA antagonists had been available until the end of the last decade. Shortly after the appearance of the competitive AMPA/kainate antagonist quinoxaline derivatives, the 2,3-benzodiazepine GYKI 52466 was reported to specifically inhibit AMPA/kainate receptor mediated responses. Although the in vitro potency of this compound (IC50 values around or above 10 μM) is inferior to the most effective quinoxaline-diones, its excellent bioavailablity makes it suitable as a pharmacological tool to study the physiological and pathological role of AMPA receptors.

Although the specific action of GYKI 52466 on the AMPA/kainate receptors has been confirmed by several investigators, the compound was found to be ineffective in displacing 3H-AMPA, or 3H-kainate from their binding sites. Further detailed analysis of its pharmacological effects revealed a non-competitive mode of action. Recently, a structure-activity relationship study with

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novel derivatives of GYKI 52466 was performed at the Institute for Drug Research, to find compounds with similar pharmacological profile but more potent actions. Among the 3-N acylated dihydro analogs, several such compounds were identified, one of them (GYKI 53773) is presently being tested in human volunteers. The most effective ones inhibited kainate induced whole-cell currents in acutely isolated cerebellar Purkinje cells at 5-12 times lower concentrations than GYKI 52466 did.

The importance of the single cell studies is that they make possible the measurement of specific drug effects. In addition to the quantitative characterization of the efficacies they are also suitable for analysing of the mode of action of the drugs. With the introduction of novel molecular cloning techniques it has become possible to perform these studies on identified glutamate receptors expressed in mammalian cells. Recent investigations on the sequence of different receptor subtypes confirmed the previous pharmacological classification of ionotropic glutamate receptors, and they revealed further receptor multiplicity. Thus, AMPA receptors are composed of four receptor subtypes such as GluR1, GluR2, GluR3, and GluR4. A combination of homomers or heteromers of these subunits can form a functional transmembrane channel.

The ionotropic glutamate receptors are of similar size, share 68-73% amino acid identity and show an overall structure similarity. They contain a very long N-terminal and a short C-terminal portion, and 5 hydrophobic regions, 4 of them are located in transmembrane domain. Both N-terminal and C-terminal domains are believed to be extracellular. Due to alternative splicing each of them have two splice variants (flip and flop). Ionotropic glutamate receptor - first GluR1 and GluR2 -specific DNA are planned to clone using reverse transcriptase to obtain cDNA from specific glutamate receptor messenger RNA extracted from rat brain. Applying polimerase chain reaction this cDNA will be to amplified and cloned into a special bacterial and mammalian vector, pCDNA3 (Invitrogene). Receptors expressed in mammalian cells (HEK-283, COS, or CHO) will be used for discovering the function of different AMPA antagonists, first of all GYKI 52466 and derivatives. The whole-cell voltage clamp technique will be applied for testing the expressed AMPA receptors functionally, and measuring drug effects.

Reference:

Tarnawa, I., Berzsenyi, P., Andrási, F., Botka, P., Hámori, T., Ling, I., and Körösi, J. (1993) Bioorg. Med. Chem. Lett. 3. 99-104.

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DETERMINATION OF THE RELEASE OF ENDOGENOUS NEUROTRANSMITTERS FROM THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM BY LUMINOMETRIC ASSAY

Sperlágh, B. and Vizi, E.S.

Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary

Due to the continuous exhaustion of natural resources the need to spread environment-friendly technologies and methods in the industry and scientific research became more and more important. Hence, on the field of neuropharmacology the radiolabelled neurotransmitter assays which has been used widespread during the past years tend to be followed by techniques, measuring the release of endogenous neurotransmitters. One of the most promising alternative of the radiolabelled neurotransmitter assays are the luminometric neurotransmitter assays which are capable to measure the level of acetylcholine and the purine neurotransmitters, ATP and ADP. Its major advantages are the high-sensitivity, simplicity and the lack of accumulation of radioactive waste material. Furthermore, since it is specific to the given neurotransmitter and does not detect the metabolites it can be regarded a more sophisticated method in order to montior the neural function. In our laboratory, a very sensitive luminometric method, the luciferin-luciferase assay has been utilized to measure the extracellular level of ATP in the peripheral and central nervous system. We investigated the source of endogenous ATP released in response to field stimulation in the guinea-pig vas deferens, where ATP functions as a cotransmitter with noradrenaline. Since the majority of the release of ATP could be inhibited by the α_1-adrennoceptor antagonist, prazosin (1 μM) and α_1- agonists, such as noradrenaline and methoxamine were able release ATP in both innervated and sypathectomized vas deferens, it could be concluded that a siginificant part of the release is is derived from postsynaptic sites, probably from the smooth muscle, in response to α_1-adrenoceptor activation by noradrenaline. Based on these results a new type of transmission, the cascade transmission is proposed, where a primary neurotransmitter of neural origin gines rise the release of secondary transmitter from the postsynaptic target cell, and the secondary transmitter may serve as an amplifier of the neurally released transmitters. In addition the stimulation - dependent release and extracellular breakdown of endogenous ATP from the central nervous sytem, from the medial habenula was also demonstrated.

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MEASUREMENT OF NEUROTRANSMITTER RECEPTORS, THEORETICAL AND PRACTICAL ASPECTS OF PARAMETER COMPUTATIONS

Szabó, G.

Department of Biochemistry, EGIS Pharmaceuticals LTD., Budapest, Hungary

Unveiling mechanism of action is an important step in drug research. Most neurotransmitters and drugs affecting neurotransmitter systems exert their effects through receptors. Receptors are generally characterized by their binding properties. Binding characteristics can be obtained by saturation or displacement measurements. Calculations of the receptor parameters (affinities and capacity) are based on the law of mass action. Computers made the calculations very easy and the number of software programs is increasing. All programs use the same equation (the law of mass action) but the way of computations may differ (for example fitting with least squares' method or iteration, treatment of NSB, weighing) resulting in different outcome. The differences are generally small, but sometimes can be significant and can further increase if more binding sites of the same ligand(s) are acting at the same time.

Examples are shown when confusing results can be obtained due to such complex systems.

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POSITRON EMISSION TOMOGRAPHY (PET) – ONE OF THE MOST ADVANCED IMAGING TECHNIQUES

Trón, L.[1], Balkay, L.[1], Boros, I.[1], Emri, M.[1], Márián, T.[1], Molnár, T.[1], Tóth, Gy.[1] and Gulyás, B[2].

[1]PET Centre, University Medical School, Debrecen, Hungary and [2]Laboratory for Brain Research and PET, Karolinska Institute, Stockholm, Sweden

PET is an in vivo imaging technique that measures body function. In contrast to the majority of biomedical tomographic methods PET presents a picture of physiology. This feature provides a remarkable importance for this method in a number of very basic application as functional imaging can assess changes in organ performance prior to structural changes.

To trace a bodily process a positron decaying element is put into a chemical known to take part in that process. This positron emitting compound is called a radiotracer, it can be either a normal body component, like water, or a drug. Positron emitting isotopes can be easily incorporated into metabolically important substrate, physiologically important compounds and therapeutic agents (e.g. FDG is a glucose analog labelled with F^{18}, a positron emitting fluorine representing a PET radiotracer used to measure metabolism).

PET scan provides three dimensional distribution of an administered radiopharmacon. The choice of the radiopharmacon is determined by the process to be investigated. Positron emitters most frequently used include common elements (oxygen, carbon, nitrogen fluorine, etc) that can easily be incorporated into compounds. Positron emitting isotopes have very short life time: O^{15} (2min), N^{15} (10 min), C^{11} (20 min), F^{18} (110 min) explaining why PET cameras are installed close to cyclotrons.

In general PET studies are of three types: (1) regional blood flow: (2) substrate metabolism: and (3) chemical recognition site, including hormone and neurotransmitter binding sites (receptors) investigations.

Perfusion imaging supports noninvasive diagnosis of coronary heart disease. In combination with metabolic tracers PET provides accurate detection of significant coronary artery disease and identifies viable but compromised myocardium in patients with advanced ischemic heart disease.

The investigation of glucose or amino acid metabolism provides a way of tumour diagnosis. Determination of the metabolic activity offers a possibility for tumour grading. Glucose metabolic rate measurements allow the distinction between persistence or recurrence of a tumour, from the one side and radiation necrosis, from the other.

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The receptors number often changes in certain tumours allowing for differential diagnosis. A tumour (e.g. breast cancer) containing oestrogen receptors is more likely to be treated with oestrogen receptor blocking drugs, than cancers that do not contain oestrogen receptors. Similarly, pituitary adenomas can be classified based on dopamine receptors. If the tumours contain such receptors they can be treated chemically rather then surgically by administering dopamine receptor agonists.

A single receptorligand labelled with a positron emitting isotope enables the investigation of the binding characteristics of any other ligand with the same specificity in simple competition experiments. Carefully designed measurements using labelled ligands can reveal receptor or receptor ligand turnover, identification of the binding site of certain drugs or biologically relevant molecules. The method is instrumental in studying the organ specific accumulation of labelled ligands and drugs. These investigations can reveal also the time course of these processes.

PET can examine biochemistry and pathologic processes in the brain and in other organs. It helps to decrease surgical morbidity and complications, improve selection of the type of the treatment, and provide an effective way to plan treatment. PET is used to assess the effectiveness of surgery, radiation therapy, and chemotherapy. It can document the extent of disease, progression or regression in response to different treatments. Such data permit early modification of inefficient treatment before the clinical response of patients. After treatment of a tumour measurement of metabolic activity helps to discriminate between persistence or recurrence of the tumour and the damage of normal tissue, such as that resulting from radiation necrosis.

Mapping human brain functions is another major focus of PET studies. These investigations yield information about brain's organization for perception, action, attention, emotion. These investigations can be combined with MRI scans and the fusion of anatomic MRI images with functional PET images result in a more comprehensive picture of the brain's organization.

A GE-4096^{PLUS} type PET camera has been installed in Debrecen in November 1993. It is the first equipment of this kind in Central-Eastern-Europe. Its 8 detector rings allow to study the distribution of the applied radiopharmacon in 15 slices simultaneously with a steric resolution of 6 mm. The equipment is capable to carry out whole body scans.

At the present time glucose metabolism investigations are in progress using Fluor^{18}-labelled dezoxy glucose. Radiochemical development programs have been started aiming the synthesis of C^{11}-labelled amino acids, N^{15} labelled ammonia (flow tracer). C^{11} labelled ligands specific for defined receptors (eg benzodiazepine receptors) will also be developed within a reasonably short time.

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STRUCTURE AND FUNCTION IN G-PROTEIN LINKED CLONED NEUROTRANSMITTER RECEPTORS

Wollemann, M.

Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521, Hungary

Cloned alpha- and beta-adrenergic, dopaminergic, muscarinergic, serotoninergic, histaminergic, metabotrop glutamate and their subtypes are discussed comparing primary amino-acid (AA) sequence structure, ligand binding and second messenger functions. All receptors of the above structure contain one N-terminal, seven transmembrane(TM), three extracellular, three intracellular and one C-terminal regions.

The N-terminal regions are glycosylated on the asparagine AA-s and they should be involved in the transport from processing microsomes to the membrane.

The transmembrane blocks consist of 20-25 mostly hydrophobic AA arranged in alpha-helixes forming a pocket after ligand binding between TM2-TM7. The TM2 and TM3 regions contain conservative AA-s as the aspartic acid in all biogenic amine receptors which bind to the amine group of the catechol (f.e.aspartic acid 113 in the beta2-adrenergic receptor.), whereas serine and phenylalanine are necessary for the catechol-hydroxyl group forming hydrogen bonds on TM5-7. Only aspartic acid bound ligands are capable of G-protein coupling, whereas antagonists do not cause conformation changes and G-protein activation.

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formation of the transmembrane regions. The cysteine at the C terminal region is palmitoylated and fixed to the membrane lipids.

The intracellular loops 2 and 3 and the C terminal part are all responsible for G-protein coupling and activation.Those G-protein linked receptors which are coupled to Gs-protein (activating adenylate cyclase) have a shorter intracellular loop 3 and a longer C-terminal region, (as the beta-adrenergic receptors), whereas those which have a longer intracellular loop 3 and shorter C terminal region couple to Gi-protein (inhibiting adenylate cyclase) as f.e. in the alpha2-adrenergic receptor. All these regions contain hydoxylated AA (serine and threonine) which are phosphorylated by different protein kinases (BARK, PKA and PKC etc.) and meantimely desensitized. The absence of the majority of these AA-s in these regions of the beta3-adrenergic receptor renders it insensitive against ligand desensitization. The construction of chimeric alpha2-beta2- adrenergic receptors proved also the importance of these regions in the specificity of coupling to the different G-proteins.

Mutation changes from 266-272 AA-s in the beta2-adrenergic and from 288-293 AAs in the alpha1B-adrenergic receptors leads to constitutive activation i.e. the receptor is active in the absence of ligand. The overexpression of the Gq protein can also induce constitutive activation in the muscarinic receptors M1,M3,M5.

Alternative splicing variants have been discovered in the beta1- and alpha1c-adrenergic, in D2-dopamine, 5HT5A- and 5HT5B-serotonin receptors.

Finally separate molecular mechanisms exist for rapid decoupling, for longterm sequestration and internalisation of the G-protein linked receptors. Decoupling is promoted by the various proteinkinases, whereas downregulation and endocytosis is promoted by the long C-terminal regions.

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BOOK REVIEW

NERVE—MUSCLE INTERACTION

Vrbová,G. (London, UK), Gordon, T. (Alberta, Canada)
and Jones, R. (Bristol, UK)

Chapman and Hall, London, Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras. 1995. Hardbound, 256 pages

In the preface of the first edition of this book (1978) the authors, at that time in the University College, London, paid tribute to Galvani's discoveries that paved the way for the up-to-date knowledge of an electrical nerve conduction and a chemical neuromuscular transmission, and to the memory of the late Ernst Gutmann, one of the leading scientists in this field of research. This second edition follows the outlines of the first one.

The first Chapter of the book, entitled "Early development of muscle" deals with the role of the "helix-loop-helix" proteins that are instrumental in the commitment of mesenchymal cells to myogenic lineage. Fusion of myoblasts and the development of several membrane characteristics, including first of all the acetylcholine receptor, and the emergence of phenotypic diversity, i. e. tonic and phasic muscle fibers and the distribution of myosin heavy chains, are also discussed in this first Chapter. – The second Chapter is devoted to the development of motoneurons, including their lineage, the expression of the cholinergic phenotype and the development of electrical excitability. When reading this chapter, the clinical neurologist will find confusing that there is no attempt to distinguish "upper" motoneurons from "lower" ones, on the other hand, the experimental neurobiologist will be puzzled by the fact that Prestige's "maintenance factor" is not mentioned at all. – Regulation of the acetylcholine

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receptor and that of (acetyl) cholinesterase as well as the structural and functional characteristics of early neuromuscular transmission can be found in the third Chapter, entitled "Encounter of motor nerves with muscle fibers". While the role of agrin is amply discussed, I did not find any reference to Fischbach's brainchild, the acetylcholine receptor inducing activity (ARIA). The role of calcitonin gene-related peptide in the regulation of the expression of the (nicotinic) acetylcholine receptor is briefly discussed. – Characteristics of tonic *versus* phasic muscle fibers of lower vertebrates are discussed in Chapter 4. – Chapter 5 is devoted to mammalian muscles and motor units. – The "emergence" of the motor unit, including the layout of its territory and the post-natal reorganization of synaptic contacts and muscle fiber properties, is summarized in Chapter 6. – Plasticity of muscles and motor units is discussed in Chapter 7. – The effect of axotomy on motoneurons, nerves and muscles (Chapter 8) and nerve regeneration in context to muscle reinnervation (Chapter 9) are the most important and well-written parts of the book, even though the reader will find several unrelated citations and misquotings also in these chapters. The last portion of the book (Chapter 10) deals with disturbances of nerve-muscle interaction and their consequences, like in Duchenne's muscular dystrophy, myasthenia gravis and the Lambert-Eaton syndrome. Here again, several important references, related to the structural aspects of calcium channels in muscle, especially in the neuromuscular junction, are missing. While the role of dystrophin is discussed, I did not find any mention of syntrophin and utrophin. – Each chapter has its own "Conclusion" and a separate List of References; in my humble opinion, a single and unified List of References would have been more useful; this way, many unnecessary repetitions could have been eliminated.

In this Reviewer's view, the first edition of this monography, summarizing the bulk of the essential knowledge related to the structural and functional implications of nerve-muscle interaction, was a standard work of reference in the late 70s, even though several important aspects of neuromuscular

transmission were omitted even at that time. In spite of this, the second edition of "Nerve – Muscle Interaction" contains a body of useful information which will be met with loyal understanding by scientists working in this expanding field of neurobiological research.

Bertalan Csillik

(contents continued)

Book review

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INSTRUCTIONS TO AUTHORS

Aims and Scope. *Neurobiology* is a multidisciplinary international journal, which publishes contributions from all traditionally important and newly emerging subdisciplines of the neurosciences including neuroanatomy, morphology, histochemistry, physiology, pharmacology, molecular neurobiology, developmental neuroscience, etc. Referees from a large international Editorial/Advisory Board will review submissions critically.

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Balázs, R. and Richter, D. (1973) Effects of hormones on the biochemical maturation of the brain. In: Biochemistry of the Developing Brain (ed. Himwich, W.) Vol. 1, pp. 254-304. M. Dekker, New York.

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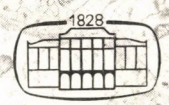

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NEUROBIOLOGY

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Akadémiai Kiadó, Budapest

PHYSIOLOGY OF THE HYPOTHALAMUS: FEEDING, DRINKING, LEARNING, AUTONOMIC AND IMMUNE REGULATION

Papers presented at a one day Symposium held on 17 May 1995 in Santiago de Compostela, Spain

Neurobiology 3 (3–4), p. 215, 1995

Special Address by the Symposium Organizers

Dr. Yukata Oomura and Kiyomi Koizumi are both 70 years old in 1995. Many colleagues and students who had been working with them wanted to organize a Memorial Symposium for the celebration of their great and long span contributions in the field of hypothalamic research. Thus, we, as the Organizing Committee, proposed the organization of a Satellite Symposium on "Physiology of the Hypothalamus: Feeding, Drinking, Learning, Autonomic and Immune Regulation" in the frame of with the Fourth Conference of the International Behavioral Neuroscience Society (IBNS) at Santiago de Compostela. We thank very much the Program Committee, Council Meeting, and also the President of the IBNS for accepting our Symposium as an official Satellite. We would also like to thank all the participants and local committee for their efficient collaboration in holding the Symposium. This special issue of Neurobiology is devoted to the proceedings of the Meeting.

Hitoo Nishino
Hiroshi Yamashita
Toshile Sakata
László Lénárd

Neurobiology 3 (3-4), pp. 216-217, 1995

Brief curriculum of the two distinguished scientists honoured by presentation of papers at this Symposium

Dr. Yukata Oomura

Dr. Oomura started his research carrier in 1947 as a graduate student in Kyushu University School of Medicine. His first interest was to record an action potential from Ranvier's node in a single nerve fiber. He joined the research group of Tokyo University School of Medicine and got basic training in electrophysiology by Drs. M. Sato, I. Tasaki, I. Hagiwara and Y. Katsuki. At that time he studied the relationship between the threshold for action potential, membrane potential (capacity transformation) and action of chemicals such as ATP, creatine phosphate, inhibitors and so on.

In 1953, he became a postdoc of the Brain Institute, University Illinois and worked with Drs. R. W. Gerard and N. G. Ling on the excitability of mitral and granular cells in the frog olfactory bulb using intracellular recording. Then, he moved to Johns Hopkins University, Department of Biophysics and worked with Dr. M. G. Larrabee as a research associate. He worked on the effect of glucose-deprivation on the excitability of the pre- and postsynaptic membranes in the sympathetic ganglia.

After returning from the U. S. A. in 1956 he started to investigate the excitability of the membrane in the neuromuscular junction, Ranvier's node, and also Onchidium using voltage clamping in Na+ free medium or by the application of phospholipase A and C and botulinus toxin. Thus, Dr. Oomura`s early research carrier was mostly focused on the ionic mechanisms of excitable or synaptic membrane.

Meanwhile he planned to study the synaptic mechanism of CNS neurons in association with behavioral output. One day he read an article written by Cross, B. A. and Green, J. D., "Activity of single neurons in the hypothalamus: effect of osmotic and other stimuli" (J. Physiol., 1959). He read that paper with much interest, and based on it performed single neuronal analysis of feeding and satiety centres in the hypothalamus in relation to behavior. He recorded single neuronal activities from the LHA and VMH and investigated their reciprocal excitability using time-series analysis. Using multibarreled microelectrodes he recognized the glucose-sensitive neurons in the LHA and glucoreceptor neurons in the VMH which work reciprocally in the regulation of feeding. By microelectrophoretic application of glucose, the glucose-sensitive neurons became

hyperpolarized with no change in membrane resistance due to the activation of the Na+-K+ pump. Glucoreceptor neurons were depolarised by glucose with the suppression in K+ channel.

In the research for hunger and satiety substances, he found that an endogenous sugar acid works as a hunger substance and FFA, 2-buten-4-olide, and aFGF work as satiety substances in the regulation of hunger.

He was also involved in other hypothalamic research fields: autonomic nervous system, circadian rhythm, sexual behavior, endocrine control as well as sleep and temperature regulation.

More than 300 scientific papers derive from his department and more than 50 physiologists joined work in his department from all over the world.

More recently he moved to NIPPON ZOKI Pharmaceutical Co., Ltd. as the president of the Institute of Bio-Active Science and now he is serving as the highest adviser.

He is still very active and highly motivated in studying the action of a FGF in relation with feeding and memory functions.

Dr. Oomura was awarded a number of highly esteemed prices, honoured by the membership of national and international scientific bodies and academies. He is also a Board Member of 8 neuroscience journals.

Hitoo Nishino

Neurobiology 3 (3-4), pp. 218-219, 1995

Kiyomi Koizumi

It was my good fortune to meet Kiyomi Koizumi in 1960 at Kobe where she was a visiting lecturer of the Department of Physiology in Kobe Medical College: she has just arrived from New York with the late Chandler McC Brooks and I was a medical student studying physiology. At that time I never imagined that our friendship would last for 35 years. Now she is already 70 years old but is still working hard and energetically at the Interim Chair and professor of the Department of Physiology, State University of New York. She greatly contributed to the researches on the spinal cord, hypothalamus and the autonomic nervous system. She published about 150 papers and was invited to participate in many international conferences. She also took part in the activity of the Society as Associate Editor of the Journal of Autonomic Nervous System and member of the study section of NIH. She was liked by everyone who worked with her because of her personality. She is so kind, modest, warm hearted and attractive lady. It is my privilege to introduce this tribute to her on behalf of scientists who feel their appreciation to her.

Professor Koizumi started her research carrier working at the State University of New York, Downstate Medical Center, in Brooklyn, with Prof. Chandler McC Brooks (who was her life-span collaborator). She studied the mechanisms of spinal reflexes and their affective factors, temperature, reticular formation and chemical agents. One of the best known papers published in this time was on iontophoretic work carried out with D. R. Curt in 1961.

In 1960 she spent one year in Kobe at Professor Brooks' desire to work on supraoptic neurons of the hypothalamus. This project was performed in collaboration with Professor Isamu Suda in Kobe Medical College who had outstanding ideas and skill. They recorded single unit activities of supraoptic neurons using floating electrodes and hemispherectomized preparations. After returning from Japan, she worked with Junji Ushiyama, Tomoe Ishikawa and Guilermo Zaballos on the hypothalamus.

In 1967 Richard Dyball, a Bristol scientist joined her and they succeeded to record from antidromically identified supraoptic neurons in rats. This had great impact on neuroendocrinology and led to new discoveries. Soon after it Kiyomi Koizumi and I started to make intracellular recordings from magnocellular neurons and after two years laborious work we finally succeeded and published our results in 1971. In 1973 Hitoo Nishino arrived to her laboratory. He worked on suprachiasmatic neurons and the transmitters, while Tomoe Ishikawa studied the pineal gland.

Meanwhile, Akio Sato came to New York for studying the autonomic nervous system that was her third step. This line of work was performed with Horst Seller, Naohito Terui and Mark Kollai who was her co-worker for many years. Her interest was to clarify the hypothalamic control of the autonomic nervous system. Hiroshi Kannan worked on an interdisciplinary study involving the magnocellular nucleus and the autonomic nervous system. This work was continued until 1986. Then she started to work again on the hypothalamus in collaboration with Toshimasa Osaka, Toshihiko Katafuchi, Yukio Hattori and Itsugi Nagatomo, until 1989. Since around 1989 she has studied with interest polydipsic inbred mice. Most recently, cooperative projects for studying mice were organized by many researchers involving genetic, molecular, cellular and behavioral aspects, in which she participated with enthusiasm.

During her past and extremely successful decades as a neuroscientist, Dr. Koizumi became a member of a number of important scientific bodies, national and international Societies and achieved highly appreciated honours.

Hiroshi Yamashita

Full Papers

based on lectures, presented at this Symposium to honour the scientific achievements of Drs. Yukata Oomura and Kiyomi Koizumi

Neurobiology 3 (3–4), pp. 223–239, 1995

ROLE OF FOREBRAIN GLUCOSE-MONITORING NEURONS IN THE CENTRAL CONTROL OF FEEDING: I. BEHAVIORAL PROPERTIES AND NEUROTRANSMITTER SENSITIVITIES

Lénárd, L.[1,2], Karádi, Z.[1], Faludi, B.[1,2] and Hernádi, I.[2]

[1]Neurophysiology Research Group of the Hungarian Academy of Sciences, Institute of Physiology, Pécs University Medical School, H-7643 Pécs, Szigeti út 12, Hungary and [2]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University, H-7601 Pécs, Ifjúság út 6, Hungary

Summary. Extracellular single neuron recording experiments were performed in the lateral hypothalamic area (LHA), amygdaloid body (AMY) and globus pallidus (GP) of anesthetized rats and anesthetized or alert rhesus monkeys during microelectrophoretic administration of different neurochemicals including glucose. Neuron activity in the behaving primate was also investigated during a conditioned bar press alimentary task, as well as during presentation of food and non-food objects. In the LHA, AMY and GP specific glucose-sensitive (GS) neurons were found, as their activity were suppressed by glucose. The proportion of GS neurons was approximately 29%, 11% and 14%, respectively. The GS neurons in the monkey were especially likely to respond to phases of the conditioned alimentary task, and these same neurons appeared to be particularly influenced by sensorimotor and motivational factors. LHA, AMY and GP GS neurons displayed distinct sensitivities to various neurotransmitters applied microelectrophoretically. The present results, along with previous data, indicate that a hirerarchically organized network of the brainstem and forebrain glucose-monitoring neurons exist and this system is invovled in the regulation of feeding.

Key words: Feeding Behavior - Unit Activity - Glucose-Sensitive Neurons - Neurotransmitter Sensitivity - Lateral Hypothalamic Area - Amygdala - Globus Pallidus - Monkey and Rat

Correspondence should be addressed to: László Lénárd, MD., Ph.D., D.Sc.
Institute of Physiology, Pécs University, Medical School,
H-7643 Pécs, Szigeti út 12; Hungary

INTRODUCTION

The lateral hypothalamic area (LHA), amygdaloid body (AMY) and globus pallidus (GP) have already been known to play essential roles in various processes of the central control of feeding behavior [Anand and Brobeck, 1951; Berridge and Cromwell, 1990; Box and Mogenson, 1975; Fonberg, 1974; Hahn et al., 1988; Hajnal et al., 1992; Lénárd, 1977, Lénárd and Hahn, 1982; Lénárd et al., 1975, 1982, 1988, 1989, 1991, Morgane and Jacobs, 1969; Oomura, 1980; Sándor et al., 1992; Teitelbaum and Epstein, 1962; Ungerstedt, 1971]. Although physiological characteristics of neurons of these structures have also been intensively studied [Oomura et al., 1970, 1975], little was known of their specific, feeding associated functions. Our knowledge was especially poor concerning neurochemical characteristics of these cells until specific glucose-sensitive (GS) neurons have been discovered in the LH of both rats and monkeys [Aou et al., 1984, Oomura et al., 1969, 1974]. It has been found that activity of these GS cells are influenced by endogenous, hunger- and satiety-related signals and suppressed by local microelectrophoretical application of glucose [Oomura, 1976, 1980, 1981; Oomura and Yoshimatsu, 1984; Shimizu et al., 1983, 1984]. A long series of studies were then designed to determine the characteristics of LHA GS neurons [Karádi et al., 1988, 1989, 1990, 1992; Nishino et al., 1988;] and to identify similar GS cells in feeding-related forebrain regions, i. e., in the AMY and GP [Karádi et al., 1988, 1994, 1995a, Lénárd et al., 1994, 1995; Nakano et al., 1986;], in structures well known for their mutual functional interconnections with the LHA [Nauta and Mehler, 1966; Oomura et al., 1970, 1975; Parent et al., 1984; Price, 1981]. In order to provide complex and comparative characterization of neuronal activity of these feeding-related forebrain regions, in the present experiments, extracellular single neuron recordings were performed in the LHA, AMY and GP of anesthetized rats and anesthetized or alert rhesus monkeys during microelectrophoretic administration of various neurotransmitters and glucose. Feeding-associated behavioral attributes of the cells in the behaving primate were investigated during performing a conditioned bar press alimentary task, and during presentation of food- and non-food objects.

MATERIALS AND METHODS

Animals

Thirty-five male CFY rats (LATI, Gödöllő, Hungary, weighing 275-360 g) and 4 rhesus monkeys (Macaca mulatta, weighing 4-8.5 kg) of both sexes were used. Animals were cared for in accordance with the NIH Guidelines. Experimental arrangements and details of recording and data analysis have been described previously [Karádi et al.,1992, 1995; Lénárd et al., 1994, 1995; Niedetzky et al., 1993]. A brief outline of the methods will be presented here.

Conditioned Alimentary Task

Before surgery, monkeys were trained to perform a fixed ratio bar-press feeding task. The monkeys, mildly food deprived (for 12 h) were seated in primate chairs facing a panel equipped with discriminative cue lamps, a bar and a feeder box. After learning to press the bar for food on a continuous reinforcement schedule, monkeys were trained to a high fixed-ratio bar press schedule (FR 20-30) consisting of four phases: 1) Presentation of a cue lamp to signal the start of bar pressing (CL); 2) 20-30 bar presses for a single food reinforcement (BP); 3) presentation of a short cue tone (CT) followed by a food reward with a 0.5-3 s delay; and 4) grasping and eating the food (reward period, RW). A small (5-6 mm in diameter) ball of bread served as standard reward. More palatable foods (apple, potatoe, raisin) were also used in specific cases. Intertrial intervals were changed randomly between 30 and 120 s. During a daily 4-7 h experimental session, 40-80 trials were performed. Water was offered to the monkey once per hour. Task related activity changes of forebrain neurons were evaluated in 6-20 consecutive trials with the same reward.

Surgery

After the monkeys had been well trained in the alimentary task, they were operated on under pentobarbital anesthesia (Nembutal, 40 mg/kg IP) and aseptic conditions to fix a plastic plate stereotaxically to their skulls. The plate restrained the monkeys in the stereotaxic apparatus during the daily experimental sessions and, thus, allowed insertions of multibarreled microelectrodes through a preceedingly drilled small hole (3 mm in diameter) in the skull and the incised dura mater. In monkeys the stereotaxic coordinates for electrode placements into the LHA, AMY or GP were chosen according to the atlas of Snider and Lee [Snider and Lee, 1961]: A: 11-15; L: 1.5-3.8; V: 3-0.5, A: 11.5-16; L: 4-10; V: 2.5-(-)3, A: 11-16; L: 3.5-8.5; V: 3-8, respectively. After 2 weeks recovery from surgery, the recording experiments started under awake conditions. Forebrain single neuron activity was recorded during: a) bar-press feeding behavior, b) presentation of food and nonfood objects (familiar foods with different reward qualities [e.g. piece of bread, potatoe, apple, or banana] and nonfood objects [familiar or unfamiliar, e.g. syringe, brush, pen, etc.] were presented in the monkey's central visual field), c) presentation of taste and odor stimuli and d) microelectrophoretic application of neurochemicals. In specific cases feeding-task related activity changes were also studied during extinction trials and satiation. Results of chemosensory stimulations are presented in the accompanying paper [Karádi et al., 1995b]. In the awake condition, electrophoretic applications of neurochemicals were performed between blocks of behavioral trials with random intervals. Chemicals were also electrophoretized in lightly anesthetized primates (ketamine, 10 mg/kg/hr; IM). Monkeys were used for 6-9 months.

Rats were operated on stereotaxically under urethane anesthesia (3.5 ml/kg, IP, of 25% fresh solution) to drill a small, 2.5 mm diameter hole in the skull for inserting the electrodes. Single neuron activity recordings were made from the areas of the LHA, AMY or GP corresponding to the stereotaxic coordinates of Pellegrino, Pellegrino and Cushman [Pellegrino et al., 1979]: A: 4.2-6.6; L: 1.5-3; V: -2.5-(-)4, A: 4.2-6.6; L: 3-6; V: -1.5-(-)4.5, A: 6-7; L: 2.5-4; V:1.5-(-)1.5, respectively. Recordings usually proceeded for 3-7 h daily. The same rat was used several times in these acute experiments, however, only three microelectrode insertions were allowed at one hole. Drill sites were covered by antibiotic ointment, and the severed edges of the hairy skin on the skull were attached to each other by

surgical clips after each session. Experiments with the same animal were separated in time by at least 2 days.

Recording, Microelectrophoresis and Data Analysis

In both rats and monkeys, extracellular recording and microelectrophoretic application of neurochemicals were accomplished by means of nine-barreled glass microelectrodes. Single neuron activity was recorded via the central barrel containing a carbon fiber (7 μm in diameter, impedance 1-6 MΩ at 50 Hz). Neurochemicals were applied through the micropipettes surrounding the central recording electrode. Each barrel was filled with one of the following solutions: 0.5 M D-glucose (Gluc, in 0.15 M NaCl), 0.5 M monosodium L-glutamate (Gt, pH 8.0), 0.5 M GABA (pH 7.0), 0.5 M dopamine HCl (DA, in 2% ascorbic acid, pH 4.0), 0.5 M noradrenaline HCl (NA, in 2% ascorbic acid, pH 4.0) and 0.5 M acetylcholine HCl (Ach, pH 4.5). Two barrels were filled with physiological saline: one of them was used as a current balancing channel and the other one as part of a driven shield to improve signal-to-noise ratio [Yamamoto et al., 1985]. Neurochemicals were released by constant currents of appropriate polarity from a constant current source (NeuroPhore BH-2). Electrodes were advanced under microscopic control by means of a hydraulic microdrive (Narishige, MO-10). Extracellular action potentials were passed into a preamplifier, a high gain amplifier with low- and high-cut filters, and to a window discriminator to form standard pulses. In a parallel processing, signals were also led to a computerized A/D converter device (CED 1401 plus). Neuronal spikes and formed pulses were continuously monitored on oscilloscopes (HAMEG HM-2035 and HM-2037). Pulses were fed into an IBM PC/AT microcomputer to construct frequency histograms and perievent histograms. All raw data, the original action potentials, formed pulses, and marker signals were stored on magnetic tapes and floppy disks for off-line analyses.

The extracellularly recorded action potentials of well-isolated, spontaneously active neurons were only studied. Cells that showed nonspecific current effects (response to Na^+ or Cl^-) were excluded from the analysis. Neurons were considered to be responsive to glucose and neurochemicals if their firing rates changed at least 30% from the baseline level, and if the activity changes to drugs proved to be dose dependent (by using different current intensities) and replicable.

Localization of Recording Sites

In order to reconstruct the recording sites different methods were applied. As the microelectrode was advanced by means of the hydraulic microdrive, the depth of each neuron from the brain surface was measured and registered in μm. In monkeys, at the end of each penetration, X-ray photographs of the head were taken to examine the position of a tungsten wire obligately inserted into the central recording barrel. After finishing all experiments, in both rats and monkeys, an elgiloy electrode was inserted in various locations within the LH, AMY and GP at coordinates identical to those of the recording sites, and electrolytic microlesions were made by passing negative 0.1 mA DC current for 15 s through the tip of the electrode. The positions of microlesions were later determined histologically. After completion of the marking procedure, animals were deeply anesthetized with an overdose of

Nembutal and the brains were perfused with physiological saline followed by fomaldehyde solution. Brains were removed and cut in 40 μm serial sections and stained with cresyl violet.

RESULTS

Activity of 989 neurons (672 in the anesthetized or alert monkey and 317 in the anesthetized rat) was studied. Of these, 233 neurons were recorded in the monkey LHA, 243 in the AMY and 196 in the GP. The number of recorded neurons in the rat LHA, AMY and GP was 73, 97 and 147, respectively. The mean spontaneous firing rates in the LHA, AMY and GP were 9.6 ± 5.9, 13.7 ± 7.7 and 11.8 ± 7.5 spikes/s, respectively. Although the spontaneous activity of neurons in the subdivisions of recorded regions were somewhat different, no significant difference in the mean spontaneous discharge rates was found between the two species (for more details: see the accompanying paper [Karádi et al., 1995b]).

Responses to Microelectrophoretically Applied Glucose

In anesthetized or alert monkeys the sensitivity to microelectrophoretically administered glucose was examined in 616 neurons. In anesthetized rats, glucose-sensitivity was tested in 313 neurons. Intensity of application current varied from 10 to 90 nA. Results are demonstarted in Table 1. In the monkey, 66 LHA cells (30%) of 212 proved to be GS, i.e. were suppressed by administration of glucose. These inhibitory responses, in a dose-dependent manner, occurred within 8-30 s after beginning of glucose ejection, and neurons recovered to their initial discharge rates within 30 s to 3 min after termination of glucose application. The remaining 156 cells (70%) did not show activity changes to glucose and were classified as glucose-insensitive (GIS) neurons. In the monkey AMY 24 neurons (11%) of 216 showed definite inhibitory responses to the electrophoretically applied glucose, and in the monkey GP 26 GS neurons were found (15%, 26/178 cells, see Table 1.). It is remarcable that in the LHA, AMY and GP of the rat the proportion of GS neurons were very similar to those observed in the monkey (27 %, 20/73, 10%, 10/97 and 13%, 19/124 neurons, respectively, see Table 1.).

Neurotransmitter Sensitivity

In anesthetized or alert monkeys, neurotransmitters and glucose were tested by microelectrophoretic application in 152 lateral hypothalamic, 101 amygdaloid and 105 pallidal

neurons. In anesthetized rats, respectively, 69, 92 and 104 cells were examined. Within the recorded forebrain regions the percentual distribution of characteristic responses to neurotransmitters did not differ significantly between the primate and the rodent.

TABLE 1

Proportion of glucose-sensitive (GS) and glucose-insensitive (GIS) neurons in the monkey and rat. Single neuron activity was recorded from the LHA, AMY and GP

MONKEY

	LHA	AMY	GP	Total
GS	66 (30%)	24 (11%)	26 (15%)	116
GIS	156 (70%)	192 (89%)	152 (85%)	500
Sum	222 (100%)	216 (100%)	178 (100%)	616

RAT

	LHA	AMY	GP	Total
GS	20 (27%)	10 (10%)	19 (13%)	49
GIS	53 (73%)	87 (90%)	124 (87%)	264
Sum	73 (100%)	97 (100%)	143 (100%)	313

Microelectrophoretic application of Gt in the LHA, AMY or GP was followed by short latency sharp facilitatory responses with the exception that in both monkey and rat pallida a minority of neurons (10 of 105 cells, 10% in the monkey and 8 of 104 cells, 8% in the rat) exhibited definite, dose-dependent and replicable activity decreases. No significant difference was found in Gt sensitivity between GS and GIS cells. Application of GABA resulted in sharp inhibition with a short latency in both GS and GIS neurons. No facilitatory response was observed when GABA was used. Ach exhibited facilitatory or inhibitory responses with no characteristic distribution between the GS or GIS neurons. DA and NA microelectrophoresis resulted either in activity increase, or decrease in the LHA, AMY and GP. GS neurons of both monkey and rat displayed distinct sensitivities to catecholamines, i.e. a majority of them could be influenced by DA (LHA: 69%, AMY: 67%, GP: 75% of neurons tested in both

primate and rat) or NA (LHA: 65%, AMY: 71% and GP: 62%, respectively). As shown in Table 2., the predominant response of GS neurons in the monkey LHA, AMY and GP was inhibition to DA, and this typical response of GS cells in the LHA was significant (GS vs. GIS neurons, $p < 0.05$, χ^2 test). On the other hand, GIS neurons in the LHA and AMY exhibited characteristic excitatory responses to DA (GIS vs. GS neurons, $p < 0.01$, $p < 0.05$, respectively).

TABLE 2

Characteristic catecholamine sensitivities of GS neurons in the monkey LHA, AMY and GP

		Activity changes DA				Activity changes NA			
		↑	↓	∅	Σ	↑	↓	∅	Σ
LHA									
	GS	5	31*	15	51	2	36*	20	58
	GIS	33**	22	31	86	7	28	52	87
AMY									
	GS	3	10	6	19	2	13*	6	21
	GIS	40*	12	13	65	5	16	49	69
GP									
	GS	7	10	5	22	3	10	8	21
	GIS	12	30	28	70	1	18	31	50

↑ and ↓, characteristic exciatory and inhibitory responses, respectively. ∅, no response. Σ, number of GS or GIS neurons tested. * $p < 0.05$, ** $p < 0.01$, χ^2 test.

In the AMY, 3 GS neurons were excited and 10 GS cells were inhibited by DA. The inhibitory effect of DA in an AMY GS neuron is demonstrated in Fig. 1.A. In the monkey GP the distribution of excitatory and inhibitory responses of GS neurons was somewhat different in that 7 GP cells were excited and 10 neurons were inhibited by DA. An example of the excitatory response to DA in a GP GS neuron is shown in Fig. 1.B. A majority of both GS and GIS neurons examined in the monkey LHA, AMY or GP displayed activity decreases to

NA (see Table 2.). These characteristic inhibitory responses to NA, however, were more frequently seen in GS cells of the LHA and AMY (see Fig. 1.A.) than observed in those of GIS neurons ($p < 0.5$). In the GP 3 GS cells of 21 were excited and 10 neurons were inhibited by NA, while excitatory response to NA was seen only in 1 GIS cell. In the rat the catecholamine sensitivity of GS neurons was essentially similar to those seen in the primate, i. e. the predominant response to DA or NA was activity decrease or inhibition.

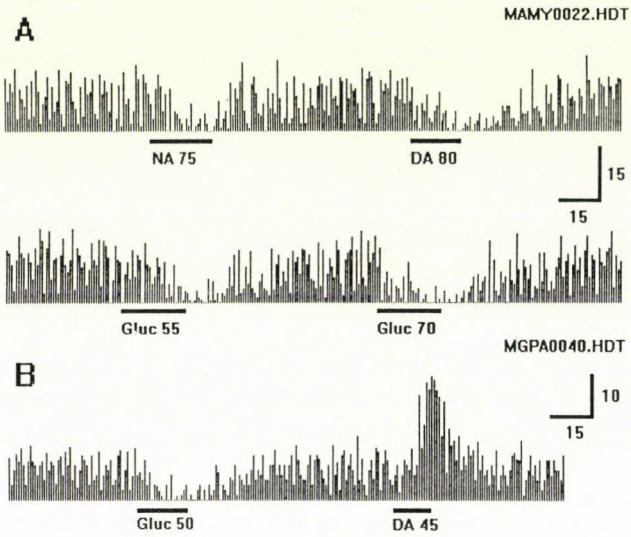

Fig. 1. Responses of glucose-sensitive (GS) neurons to catecholamines. A: Activity changes of a monkey GS cell recorded from the central amygdaloid nucleus. Activity decrease to electrophoretically applied noradrenaline (NA) and dopamine (DA). Dose dependent inhibition to glucose (Gluc). B: DA sensitivity of a GS neuron recorded from the monkey globus pallidus. Inhibition to Gluc, excitatory response to DA. Horizontal lines, duration of drug applications. Numbers below histograms, electrophoretic current intensities in nA. Calibration: time (s), impulses/s, respectively

Feeding-task Related Activity Patterns and Food-oriented Responses

In the monkey LHA, AMY and GP althogether 285 neurons were studied during conditioned discriminative bar-press feeding task. Characteristic activity patterns with firing changes in at least one of the four phases of the task were frequently seen in these neurons (LHA: 80/120 cells, 67%; AMY: 59/99 neurons, 60%; GP: 31/66 cells, 47%, respectively). Activity patterns exhibited remarkable changes during presentation of food with different

reward qualities (bread, raisin, banana), and during extinction (i.e. when successful completion of the task was not followed by presentation of food reward). Neurons that responded to the cue light did not respond to other light signals that were irrelevant to the task. Cue-tone sensitive cells did not show activity changes to acoustic tones presented at random, independent of the presentation of food.

Both glucose-sensitivity and task related activity were examined in 99 LHA, 79 AMY and 51 GP neurons. The occurrance of task related activity changes in the three brain regions tested were more frequent in GS than in GIS neurons (LHA: 36 of 41 GS neurons, 88%, GS vs. GIS cells, $p < 0.05$; AMY: 18/20 cells, 90 %, $p < 0.05$; GP: 14/17 cells, 82%, $p < 0.05$, respectively). In good agreement with previous results (Aou et al., 1984; Nishino et al., 1988; Karádi et al., 1990, 1992) the LHA GS neurons were usually depressed during BP and the predominant response of GIS cells were facilitation at CL. Both GS and GIS neurons showed characteristic activity changes during RW and the activity of GS cells were easily modified by extinction, satiation or by different food rewards. In the AMY GS cells were depressed or facilitated during BP or RW and cue-related phasic responses were mainly observed in GIS cells. Similarly to those observed in the LHA, AMY GS neurons showed distinct responsiveness to more palatable foods (raisin, banana), and the original discharge rates were modified or diminished after satiation. During the task, the GP GS neurons were not only more responsive than GIS cells (9/34 neurons, 26%), but showed more complex task-related responses. Tonic activity changes during BP with sharp facilitation during RW and phasic responses (facilitation or inhibition) at CT were frequently observed. In GP GIS neurons the predominant response was facilitation at CL and BP with activity increase or decrease during RW. Examples of these characteristic feeding task-related activity changes are shown in Fig. 2.B. In the GP, however, movement-related neuronal responses were also observed. Eleven GIS neurons, recorded from the caudodorsal part of the GP, changed in activity in contiguity with arm, head, eye or chewing movements. Four GIS neurons, recorded also from the dorsal pallidum, appeared to be driven by hand movements during bar pressing. In GS neurons no such motor-action related firing changes were observed. Instead, GS neurons exhibited complex task-related patterns with responses in two or more phases of the task, and displayed definite discharge-rate changes to different food rewards.

During presentation of food vs. nonfood objects only a minority of neurons displayed discriminative firing rate changes (see Fig. 2.A.). Activity decrease or facilitation at the sight of foods with no response to nonfood objects were observed in 6 LHA, 7 AMY and 6 GP neurons (6/48 cells, 12%; 7/49, 14% and 6/58 units, 10%, respectively). Among these 4 LHA neurons and 3 AMY cells proved to be GS, i.e. were suppressed during administration of

glucose. In the GP, however, only GS cells (6 units) exhibited these specific, discriminative firing rate changes to presentation of food (Fig. 2 A).

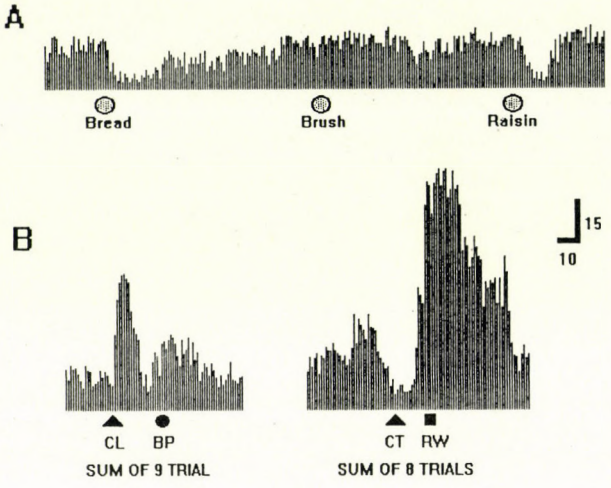

Fig. 2. Feeding-related activity changes of monkey GP neurons. A: Responses of a GS neuron during presentation of food and non-food. Short latency inhibitory response to presentation of bread and inhibition to raisin. No response to presentation of brush. B: Feeding-task related activity patterns in GP GIS and GS neurons. Left: GIS neuron. Sharp, phasic facilitation to cue light (CL) and excitatory response during bar press (BP). Right: GS neuron. Inhibition at cue tone (CT) and facilitation to the food reward (RW). Calibration: time (s), impulses/s

Location of Recording Site

Reconstruction of the recording sites showed that GS cells exhibited specific topographical locations in both rodent and primate. In the LHA GS cells were located in the ventral-medial subdivision, while GIS cells were found without such topography, i.e. they were distributed in the whole extent of the LHA. In both rat and monkey AMY, GS neurons were found to be located within the central nucleus. Some GS cells, however, appeared in the adjacent dorsal region of the AMY. In rats, GS neurons were exclusively located in the ventral-medial quarter of the GP, adjacent to the entopeduncular region. In monkey experiments, GS neurons were found at the ventromedial apex of the internal segment of the GP, while 5 GS cells were located somewhat more rostrally in the ventromedial subdivision.

In contrast to the location of GS cells, GIS neurons and movement related units were localized dorsally and caudally in the monkey GP.

DISCUSSION

The experiments presented above were designed to examine in a single study the specific characteristics of GS neurons in the LHA, AMY and GP, and to compare the feeding-associated attributes of the GS cells in these structures. GS neurons with similar characteristics were found in the LHA, AMY and GP of two different species under different conditions, i.e. in anesthetized rats and anesthetized or awake monkeys. The electrophoretically (electro-osmotically) applied D-glucose resulted in activity decrease or inhibition in these specific cells. Latency, time course and intensity of these effects were very similar in the GS neurons recorded from rats or monkeys. On the basis of theoretical considerations and experimental measurements [Aou et al., 1984; Oomura, 1983; Oomura et al., 1969] the threshold concentration for the effect of glucose applied to a neuron was estimated at 2 mM. This quantity is the same order of magnitude as normal blood glucose level (3.6-5.5 mM), and it seems to be in the physiological range.

Neurotransmitter Sensitivities

The electrophoresis experiments showed complex neurochemical sensitivities of neurons examined in the three brain regions. Effects of Gt, GABA and Ach were similar in both GS and GIS neurons, however, GS cells exhibited distinct sensitivities to catecholamines. In the LHA and AMY, DA and NA inhibited the GS cells, while GIS neurons showed facilitatory responses to DA. Similar observations have been made in our earlier experiments when single neuron activity was recorded from the monkey [Karádi et al., 1988, 1992, Nishino et al., 1988]. In the LHA, neurons (not examined for glucose-sensitivity) showing facilitatory responses to both electrophoretic application of DA and conditioned light stimuli [Lénárd et al., 1986; Nishino et al., 1987] exhibited monosynaptic responses to electric stimulation of the ventral tegmental area, the region of origin of the mesolimbic DA system [Fallon et al., 1978; Nishino et al., 1987]. Both the AMY and GP are also innervated by the ascending catecholaminergic fibers [Fallon et al., 1978; Ungerstedt, 1971] and the appearance of characteristic responses to catecholamines in these structures were not unexpected, therefore. On the basis of the present and previous results [Karádi et al., 1988, 1994; Lénárd et al., 1994, 1995], one may suppose that different responses of either GS, or GIS neurons to

catecholamines in the AMY and GP depend on the specific arrangements of catecholamine terminals and other elements of the local neuronal networks. For the better understanding the neurochemical characteristics of GS cells and their postsynaptic receptor mechanisms, however, further experiments with specific receptor blockers in combination with electrophysiological stimulation and recording methods are required.

Behavioral Results

As the present results showed, the LHA, AMY and GP GS neurons exhibited significant activity changes during the conditioned alimentary task. The appearance of task-related activity patterns were more frequent in GS than in GIS neurons. GS neurons were highly sensitive to the motivational state of the monkey as discharge patterns changed during extinction or satiation and the GS cells exhibited distinct responsiveness to foods with different reward qualities. The very same neurons showed definite taste and odor responsiveness (see the accompanying paper [Karádi et al., 1995b]). These results clearly show that GS neurons of the three brain regions in question are, indeed, involved in the neural mechanisms of feeding behavior. Previously it was found that both LHA and AMY GS neurons are depressed during BP and GIS cells exhibit characteristic responses to CL [Nakano et al., 1986; Nishino et al., 1988; Karádi et al., 1988, 1992, 1994]. In fact, it was suggested that GIS neurons are responsive to the external sensory cues, while GS cells are coupled with the effective bar-presses to obtain food reward. The present results support these data, though our observations, in this respect, were not statistically significant. In the GP, however, phasic responses at CT were frequently observed in GS cells. One of the common characteristics of HLA, AMY and GP GS neurons were that they displayed definite discharge-rate changes during RW.

General Considerations

In both species GS neurons exhibited a distinct topographical arrangement in that GS units were exclusively recorded from the ventral-medial region of the LHA, the central nucleus of the AMY and the ventral-medial part of the GP. A large amount of experimental data suggest that lesions or neurochemical manipulations of these distinct regions can modify the basic metabolic rate, body weight and hunger motivation [Anand and Brobeck, 1951; Box and Mogenson, 1975; Fonberg, 1974; Lénárd, 1977; Lénárd et al., 1975, 1982, 1988, 1991; Morgane and Jacobs, 1969; Oomura, 1980; Teitelbaum and Epstein, 1962; Ungerstedt, 1971]. Embryological studies show [Kuhlenbeck and Haymaker, 1949] that the ventral-medial part of the pallida and the LHA have common origin and the existence of two-way

interconnections are indicated between these two structures [Oomura et al., 1975]. In rats the lesion of the GP results in serious feeding disturbances, metabolic deficits and elevation of blood glucose level [Hahn et al., 1988; Lénárd, 1977; Lénárd et al., 1975; Morgane and Jacobs, 1969]. The central-medial part of the AMY was postulated as a "hunger-center", and in early experiments in rats it has been shown that unilateral lesion of the AMY combined with contralateral LHA destruction can elicit the well known lateral hypothalamic syndrome [Fonberg, 1974; Teitelbaum and Epstein 1962]. On the basis of these results it is obvious that the LHA and AMY work in concert, as far as the basic regulation of feeding concerns, and that the ventral-medial part of the pallida belongs to this neural network. Food-associated neuronal responses were described in the ventral-medial areas of the primate GP [Nishino et al., 1985], and GS cells, in the present experiments, were found exactly in this region. Furthermore, in the GP only these GS neurons exhibited discriminative activity changes to food vs. nonfood objects.

On the other hand, electrical stimulation of the dorsal pallida in rats elicits consummatory motor acts of eating, and the threshold of these motor responses decreases on food deprivation. [Szabó et al., 1978]. Previous findings demonstrated motor-related discharges in the intermediate and posterior-dorsal but not the anterior regions of the monkey GP [Iansek and Porter, 1980] which is in good agreement with the present results, i. e. GIS neurons recorded from these regions displayed motor characteristics. These observations support our view on differential roles of GS and GIS pallidal neurons in food recognition and motivationally determined food acquisition processes and are in concordance with previous and present data on distinct functions of LHA and AMY GS and GIS neurons in feeding-associated motor initiation and their involvement in reward-reinforcement mechanisms [Karádi et al., 1988, 1989, 1990, 1992; Lénárd et al., 1986, 1989; Nakano et al., 1986, 1987; Nishino et al., 1988].

Besides LHA, AMY and GP, GS neurons were described previously in the area postrema (AP) and the nucleus of the solitary tract (NTS) [Adachi and Kobashi, 1986; Oomura and Yoshimatsu, 1984]. In fact, the existence of a hierarchically organized glucose-monitoring system, located along the central neuraxis, has been postulated [Oomura and Yoshimatsu, 1984]. It was shown that hepatoportal glucose-sensitive afferent signals [Niijima, 1982] converge on the NTS and LHA GS cells and GS neurons of the AP were also found to respond to the local changes of glucose level [Adachi et al., 1984; Funahashi and Adachi, 1993; Shimizu et al., 1983]. Activity of the GS cells of the glucose-monitoring network is modified by systemic or local, electrophoretic application of glucose, free fatty acids, and other metabolites and hormones [Ishibashi et al., 1989; Oomura, 1980; Oomura and Yoshimatsu, 1984; Shimizu et al., 1983], the basic humoral signals of the internal milieu involved in the

regulation of hunger and satiety. On the basis of previous and present results, it is reasonable to suppose that GS neurons of the AMY and GP also belong to the glucose-monitoring neuronal network essential in the regulation of feeding. The possible effects of hormones and non-glucose metabolites on AMY and GP GS neurons, however, are yet to be determined.

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References

Anand, B.K., Brobeck, J.R. (1951) Localization of a "feeding center" in the hypothalamus of the rat. Proc. Soc. Exp. Biol. Med. 77, 323-324.

Adachi, A. and Kobashi, M. (1986) Consequence of hepatic gluco-and osmosensitive inputs on chemosensitive units in the medulla oblongata of rat. In: Emotions: Neuronal and Cemical Control (ed. Oomura, Y.) pp. 103-113. Jap. Sci. Soc. Press, Karger AG, Tokyo/Basel

Adachi,A., Shimizu, N., Oomura, Y. and Kobashi, M. (1984) Convergence of hepatoportal glucose-sensitive afferent signals of glucose-sensitive units within the nucleus of the solitary tract. Neurosci. Lett. 46, 215-218.

Aou,S., Oomura,Y., Lénárd,L., Nishino,H., Inokuchi,A., Minami,T. and Misaki,H.(1984) Behavioral significance of monkey hypothalamic glucose-sensitive neurons. Brain Res. 302, 69-74.

Berridge, K.C., Cromwell, H.C. (1990) Motivational-sensorimotor interaction controls aphagia and exaggerated treating after striatopallidal lesions. Behav. Neurosci 104, 778-795.

Box, B.M. and Mogenson, G.J. (1975) Alteration in ingestive hebavior after bilateral lesions of amygdala in rat. Physiol. Behav. 15, 679-688.

Fallon, J.H., Koziell, D.A. and Moore, R.Y. (1978) Catecholamine innervation of the basal forebrain. J. Comp. Neurol. 18, 509-532.

Fonberg, E. (1974) Amygdala functions within the alimentary system. Acta Neurobiol.Exp. 34, 435-466.

Funahashi, M. and Adachi, A. (1993) Glucose-responsive neurons exist within the area postrema of the rat: In vitro study on the isolated slice preparation. Brain Res. Bull. 32, 531-535.

Hahn,Z., Karádi, Z. and Lénárd, L. (1988) Sex-dependent increase of blood glucose concentration after bilateral pallidal lesion in the rat. Acta Physiol. Hung. 72, 99-101.

Hajnal, A., Sándor, P., Jandó, G., Vida, I., Czurkó, A., Karádi, Z. and Lénárd, L. (1992) Feeding disturbances and EEG activity changes after amygdaloid kainate lesions in the rat. Brain Res. Bull. 29, 909-916.

Iansek,R. and Porter, R. (1980) The monkey globus pallidus. Neuronal discharge properties in relation to movement. J. Physiol. 301, 439-455.

Ishibashi, S., Oomura, Y. and Okayama, T. (1989) Facilitatory and inhibitory effects of TRH on lateral hypothalamic and ventromadial neurons. Physiol. Behav. 22, 785-787.

Karádi, Z., Faludi, B., Czurkó, A., Niedetzky Cs. and Lénárd, L. (1994) Gustatory and olfactory responses of chemosensitive pallidal neurons. In: Olfaction and Taste XI. (ed. Kurihara, K. et al.) pp. 537-538, Springer, Tokyo.

Karádi, Z., Faludi, B., Lénárd, L., Czurkó, A., Niedetzky, Cs., Vida, I. and Nishino, H. (1995a) Glucose-sensitive neurons of the globus pallidus: II. Complex functional attributes. Brain Res. Bull. 37, 157-162.

Karádi, Z., Faludi, B., Hernádi, I. and Lénárd, L. (1995b) Role of forebrain glucose-monitoring neurons in the central control of feeding: II. Complex functional attributes. Neurobiology, in press.

Karádi, Z., Oomura, Y., Nishino, H. and Aou, S. (1989) Olfactory coding in the monkey lateral hypothalamus: Behavioral and neurochemical properties of odor-responding neurons. Physiol. Behav. 45, 1249-1257.

Karádi, Z.,Oomura, Y., Nishino, H., Scott, T.R., Lénárd,L. and Aou, S. (1988) Lateral hypothalamic and amygdaloid neuronal responses to chemical stimuli in the rhesus monkey. In: Proc.of the 22nd Japanese Symposium on Taste and Smell, JASTS (ed. Morita, H. et al.) pp.121-124, Asahi University Press, Gifu.

Karádi, Z., Oomura, Y., Nishino, H., Scott, T.R., Lénárd, L. and Aou,S.(1990) Complex attributes of lateral hypothalamic neurons in the regulation of feeding of aleart rhesus monkeys. Brain Ress. Bull. 25, 933-939.

Karádi, Z., Oomura, Y., Nishino, H., Scott, T.R., Lénárd, L. and Aou, S. (1992) Responses of lateral hypothalamic glucose-sensitive and glucose insensitive neurons to chemical stimuli in behaving rhesus monkeys. J. Neurophysiol. 67, 389-400.

Kuhlenbeck, H. and Haymaker, W. (1949) The derivates of the hypothalamus in the human brain: Their relation to the extrapyramidal and autonomic system. Milit. Surg. 105, 26-52.

Lénárd, L.(1977) Sex-dependent body weight loss after 6-hydroxydopamine injection into the globus pallidus. Brain Res. 128, 559-568.

Lénárd, L., Jandó, G., Karádi, Z., Hajnal, A. and Sándor,P. (1988) Lateral hypothalamic feeding mechanisms: Iontophoretic effects of kainic acid, ibotenic acid and 6-hydroxydopamine. Brain Res. Bull. 20, 847-856.

Lénárd, L. and Hahn, Z. (1982) Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of the hunger and thirst motivated behavior. Brain Res. 233, 115-132.

Lénárd, L., Karádi, Z., Faludi, B., Czurkó, A., Niedetzky, Cs., Vida, I and Nishino, H.(1995) Glucose-sensitive neurons of the globus pallidus: I. Neurochemical characteristics. Brain Res. Bull. 37, 149-155.

Lénárd, L., Karádi, Z., Szabó, I. and Hahn, Z. (1982) Pallidal mechanisms in the organization of feeding and sensorimotor integration. In: Recent Developments in Neurobiology in Hungary. IX. (ed. Lissák, K.) pp. 79-113. Akadémia Kiadó, Budapest.

Lénárd, L., Oomura, Y., Nakano, Y., Aou, S. and Nishino, H. (1989) Influence of acetylcholine on neuronal activity of monkey amygdala during bar press feeding behavior. Brain Res. 500, 359-368.

Lénárd, L., Oomura, Y., Nishino, H., Aou, S., Nakano, Y. and Yamamoto, T. (1986) Activity in monkey lateral hypothalamus during operant feeding: Modulation by catecholamines and opiate. In: Emotions: Neuronal and Chemical Control *ed. Oomura, Y.) pp. 45-53. Japan Sci. Soc. Press, Tokyo/Basel.

Lénárd, L., Sarkisjan, J. and Szabó, I. (1975) Sex-dependent survival of rats after bilateral pallidal lesions. Physiol. Behav. 15, 389-397.

Lénárd, L., Sándor, P., Hajnal, A., Jandó, G., Karádi, Z. and Kai, Y. (1991) Sex-dependent body weight changes after iontophoretic applications of kainic acid into the LH or VMH. Brain Res. Bull. 26, 141-148.

Lénárd, L., Faludi, B., Karádi, Z., Czurkó, A., Vida, I. and Niedetzky, Cs. (1994) Responses of pallidal neurons to microelectrophoretically applied glucose and neurochemicals. In: The Basal Ganglia IV: New Ideas and Date on Structure and Function (ed. Percheron, G. et al.) pp. 239-244, Plenum Press, New York.

Morgane, P.J. and Jacobs, H.L. (1969) Hunger and satiety. World Rev. Nutr. Diet. 10, 100-213.

Nakano, Y., Lénárd, L., Oomura, Y., Nishino, H., Aou, S. and Yamamoto, T. (1987) Functional involvement of catecholamines in reward related neuronal activity of the monkey amygdala. J. Neurophysiol. 57, 72-91.

Nakano, Y., Oomura, Y., Lénárd, L., Nishino, H., Aou, S., Yamamoto, T. and Aoyagi, K. (1986) Feeding-related activity of glucose- and morphine-sensitive neurons in the monkey amygdala. Brain Res. 399, 167-172.

Nauta, H.W.J. and Mehler, W.R. (1966) Projections of the lentiform nucleus in the monkey. Brain Res. 1, 3-42.

Niedetzky, Cs., Karádi, Z., Czurkó, A., Faludi B., Vida,I., Jandó,G. and Lénárd.L. (1993) Computer analysis of single neurons activity during conditioned feeding task. Neurobiology. 1, 147-155.

Niijima, A. (1982) Glucose-sensitive afferent nerve fibers in the hepatic branch of the vagus nerve in the guinea pig. J. Physiol. 332, 315-323.

Nishino, H., Ono, T., Muramoto, K.I., Fukuda, M. and Sasaki, K. (1985) Movement and nonmovement related pallidal unit activity during bar press feeding behavior in the monkey. Behav. Brain Res. 15, 27-42.

Nishino, H., Oomura, Y., Aou, S. and Lénárd, L. (1987) Catecholaminergic mechanisms of feeding-related lateral hypothalamic activity in the monkey. Brain Res. 405, 56-67.

Nishino, H., Oomura, Y., Karádi, Z., Aou, S., Lénárd, L., Kai, Y., Fukuda, A., Ito, C., Min, B.I. and Plata-Salaman, C.R. (1988) Internal and external information processing by lateral hypothalamic glucose-sensitive and insensitive neurons during bar press feeding in the monkey. Brain Res. Bull. 20, 839-845.

Oomura, Y.(1976) Significance of glucose, insulin, and free fatty acid on the hyporthalamic feeding and satiety neurons. In: Hunger, Basic Mechanisms and Clinical Implications (eds. Novin, D. et al.) pp. 145-157. Raven Press, New York.

Oomura.Y. (1980) Input-output organization in the hypothalamus relating to food intake behavior. In: Handbook of the Hypothalamus (eds. Morgane, P. and, Panksepp, J) Vol.2, pp. 557-620. Marcel Dekker, New York.

Oomura, Y. (1981) Chemical sensations and their perception. In: Sensory Functions. Adv. Physiol. Sci. (eds. Grastyán, E. et al.) pp. 343-348, Akadámiai Kiadó, Budapest.

Oomura, Y. (1983) Recent techniques and investigations on the influence of noradrenaline on feeding behavior. In: Methods in Biogenic Amine Research (eds. Parvez, S. et al.) pp. 959-979, Elsevier, Amsterdam.

Oomura, Y., Nakamura, T., Machanda, S.K. (1975) Excitatory and inhibitory effects of globus pallidus and substantia nigra on the lateral hypothalamic activity in the rat. Pharmacol. Biochem. Behav. SI,23-36.

Oomura, Y., Ono, T. and Ooyama, H. (1970) Inhibitory action of the amygdala on the lateral hypothalamic area in rats. Nature Lond. 228, 1108-1110.

Oomura, Y., Ono, T., Ooyama, H. and Wayner, M. (1969) Glucose and osmosensitive neurons of the rat hypothalamus. Nature Lond. 222, 282-284.

Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T. and Yamada, Y. (1974) Glucose inhibition of the glucose-sensitive neurons in the lateral hypothalamus. Nature Lond. 247, 284-286.

Oomura, Y., Yoshimatsu, H. (1984) Neural network of the glucose monitoring system. J. Autonom Nerv. Syst. 10, 359-372.

Parent, A., Smith, Y., Bellefeuille, L.D. (1984) The output organization of the pallidum and substantia nigra in primate as revealed by a retrograde double-labeling method. In: The basal ganglia (eds. McKenzie, J.S. et al.) pp. 147-160, Plenum Press, New York.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J. (1979) A Stereotaxic Atlas of the Rat Brain. New York, Plenum Press.

Price, J.L. (1981) The efferent projections of the amygdaloid complex in the rat, cat and monkey. In: The Amygdaloid Complex, INSERM Symposium (ed. Ben-Ari, Y.) No. 20, pp. 121-132, Elsevier, Amsterdam.

Sándor, P., Hajnal, A., Jandó, G., Karádi, Z. and Lénárd, L. (1992): Microelectrophoretic applications of kainic acid into the globus pallidus: Disturbances in feeding behavior. Brain Res. Bull. 28, 751-765.

Shimizu, N., Oomura, Y., Novin, D., Grijalva, C.V. and Cooper, P.H. (1983) Functional correlations between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rat. Brain Res. 265, 49-54.

Shimizu, N., Oomura, Y. and Sakata,T. (1984) Modulation of feeding by endogenous sugar acids acting as hunger or satiety factors. Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) 15, R542-550.

Snider, S. and Lee, J.C. (1961) A Stereotaxic Atlas of the Monkey Brain (Macaca mulatta). Chicago, IL: Univ. of Chicago Press.

Szabó, I., Sarkisian, J., Lénárd, L. and Németh, L. (1977) Pallidal stimulation in rats: Facilitation of stimulus-bound chewing by food deprivation. Physiol. Behav. 18, 361-368.

Teitelbaum, P. and Epstein, A.N. (1962) The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. Psychol. Rew. 69, 74-90.

Ungerstedt, U. (1971) Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol. Scand. Suppl. 367, 95-122.

Yamamoto, T., and Oomura, Y., Nishino, H., Aou, S. and Nakano, Y. (1985) Driven shield for multibarrel electrode. Brain Res. Bull. 14, 103-104.

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ROLE OF FOREBRAIN GLUCOSE-MONITORING NEURONS IN THE CENTRAL CONTROL OF FEEDING: II. COMPLEX FUNCTIONAL ATTRIBUTES

Karádi, Z.[1], Faludi, B.[1,2], Hernádi, I.[2] and Lénárd, L.[1,2]

[1]Neurophysiology Research Group of the Hungarian Academy of Sciences and Institute of Physiology, Pécs University, Medical School, H-7643 Pécs, Szigeti út 12, Hungary and
[2]Comparative Physiology Group, Department of Zoology, Faculty of Natural Sciences, Janus Pannonius University, H-7601 Pécs, Ifjúság út 6, Hungary

Our parallel investigations in the lateral hypothalamic area (LHA), amygdaloid body (AMY) and globus pallidus (GP) provided evidence for the existence of glucose-sensitive (GS) neurons in these forebrain regions. To examine exogenous chemosensory responsiveness of these cells, extracellular single neuron activity was recorded in anesthetized or alert rhesus monkeys and in anesthetized rats during 1) microelectrophoretic administration of chemicals and 2) gustatory and 3) olfactory stimulations. The GS cells in all three forebrain structures were more likely than the glucose-insensitive (GIS) neurons to change in firing rate in response to tastes and smells. The gustatory (and olfactory) GS neurons, compared to the non-gustatory GS or both types of GIS cells, displayed significantly higher sensitivities to catecholamines. Neurons with both "endogenous" and "exogenous" chemosensitivity were found to be topographically organized in the LHA, AMY and GP as well. While receiving further evidence for the substantial morphological and functional overlapping of the brain's glucose-monitoring neural network and the central gustatory representations, on the basis of the present and previous findings, it is suggested that constituents of this complex system accomplish a simultaneous monitoring, integration and control of a broad variety of feeding-associated signals of the internal and external milieux for the biological welfare of the organism.

Key words: Glucose-Sensitive Neurons - Gustatory and Olfactory Cells - Lateral Hypothalamic Area - Amygdaloid Body - Globus Pallidus - Feeding Regulation - Rhesus Monkey and Rat

Correspondence should be addressed to : Zoltán Karádi, M.D., Ph.D.
Institute of Physiology, Pécs University, Medical School,
H-7643 PÉCS, Szigeti u. 12, Hungary
Phone: 36-72-324-122/1760 ext., Fax: 36-72-312-488, E-mail: karadi@physiol.pote.hu

INTRODUCTION

A biologically adaptive regulation of feeding is achieved through detection of endogenous chemical signals and their continuous integration with exogenous chemosensory information and other, emotional-motivational determinants of the animals' behavior. The lateral hypothalamic area (LHA), amygdaloid body (AMY) and globus pallidus (GP) have already been considered to play important roles in several mechanisms (maintenance of body weight, metabolic control, sensory-motor integration, perceptual, motivational and memory processes, learning, etc.) of these feeding-related regulatory functions [Anand and Brobeck, 1951; Berridge and Cromwell, 1990; Cromwell and Berridge, 1993; Czech, 1973; Fonberg, 1974; Hahn et al., 1979,1988; Levine and Schwartzbaum, 1973; Lénárd, 1977; Lénárd and Hahn, 1982; Lénárd et al., 1982a,b,1988; Morgane and Jacobs, 1969; cf.. Oomura, 1980; Sándor et al., 1992; Yoshimatsu et al., 1988].

In a series of behavioral-neurochemical-electrophysiological investigations, evidence has been obtained for the existence of so-called "glucose-sensitive" (GS) neurons in the LHA, AMY and GP, in both rats and rhesus monkeys [Aou et al., 1984; Karádi et al., 1988,1989,1990b,1992,1994,1995; Lénárd et al., 1994,1995a; Nakano et al., 1986; cf. Oomura, 1980; Oomura et al., 1969,1974]. These neurons, as their uniform characteristic, were specifically suppressed in activity by glucose administered microelectrophoretically. Gustatory and olfactory information have also been demonstrated to be processed in these same ventral forebrain regions [Burton et al., 1975; Karádi et al., 1988,1989,1990b,1992,1994,1995; Nishino et al., 1988; Norgren, 1974,1976; Schwartzbaum, 1988; Soltysik et al., 1975; Takagi, 1986; Yamamoto et al., 1989]. To date, however, no complex characterization has been provided - in a single study - concerning feeding-associated (especially chemosensory) properties of local lateral hypothalamic, amygdaloid and pallidal cells.

Since related knowledge of these above functions in the primate - presumably closely corresponding to those in humans - is especially poor, the present study was designed to be performed with alert rhesus monkeys. In each of the three structures, single neurons were first classified according to their responsiveness to electrophoretically administered glucose, then action potentials were recorded extracellularly during: 1) performing a conditioned alimentary task (related data presented in the accompanying paper) [Lénárd et al., 1995b], 2) microiontophoretic application of chemicals, and 3) gustatory and 4) olfactory stimulations. With regard to possible species-specificities as well as to differences between the awake and anesthetized conditions, similar experiments were also conducted in anesthetized rats and monkeys.

MATERIALS AND METHODS

Animals and Basic Experimental Design

Thirty-seven male CFY rats (LATI, Godollo, Hungary; weighing 275-365 g) and 4 rhesus monkeys (*Macaca mulatta*; weighing 4-8.5 kg) of both sexes were used. Both rats and primates were caged individually in temperature and humidity controlled rooms with 12-12 hrs light/dark cycle. All animals were cared for in accordance with the NIH Guidelines. Single neuron recordings in anesthetized rats and monkeys were performed during microelectrophoretic administration of chemicals as well as during gustatory and olfactory stimulations. In the alert primate, single neuron activity was also recorded during the performance of a high fixed-ratio schedule conditioned bar press feeding task as well as during the presentation of food or non-food objects (technical description and results reported in the accompanying paper) [Lénárd et al., 1995b]. Each experimental session (with both species) lasted from 3.5 to 7 hrs. Investigations with the same animal were separated in time by at least 2 days. Details of the experimental procedures and techniques have already been described previously [Karádi et al., 1992,1995; Lénárd et al., 1995a; Niedetzky et al., 1993], so that only a brief outline of the methods will be given here.

Surgery

After the primates had been well trained (more than 85% proficiency level), they were aseptically operated on (pentobarbital anesthesia, i.p. 40 mg/kg; introduced by i.m. ketamine, 35 mg/kg) to stereotaxically fix (with dental acrylic) a U-shaped plastic plate to their skulls. Short re-training sessions (when the heads of the monkeys were restrained in a stereotaxic frame with the aid of the plastic plate) followed an approx. two-week recovery period. The animals were subsequently anesthetized briefly to drill a small hole (3 mm in diam) in the skull for insertion of microelectrodes. At the beginning of each recording session, to permit electrode penetration, the dura was locally anesthetized (Lidocaine) and incised with a sterile hypodermic needle. Recording sites were chosen according to the atlas of Snider and Lee [Snider and Lee, 1961].

Rats were used in acute experiments. The stereotaxic operations were carried out under urethane anesthesia (3.5 ml/kg, i.p., of 25% fresh solution) when a small hole (2.5 mm in diam) was drilled in the skull for insertion of microelectrodes. The penetration was allowed by incising the dura by means of a sterile hypodermic needle (under local Lidocaine anesthesia) at the start of each recording session. Stereotaxic coordinates for electrode placements were chosen according to Pellegrino, Pellegrino and Cushman [Pellegrino et al., 1979].

Recording and Microelectrophoresis

Extracellular single neuron recordings and microelectrophoretic administration of chemicals in both species were made by means of 9-barreled carbon fiber multibarreled glass microelectrodes (1-6 MΩ impedance of the central, recording pipette at 50 Hz). Chemicals were applied electrophoretically through micropipettes surrounding the central barrel. They were respectively filled with 0.5 M D-glucose (Gluc, in 0.15 M NaCl), 0.5 M monosodium L-

glutamate (Gt, pH 8.0), 0.5 M GABA (pH 7), 0.5 M dopamine HCl (DA, in 2% ascorbic acid, pH 4.0), 0.5 M noradrenaline HCl (NA, in 2% ascorbic acid, pH 4.0) and 0.5 M acetylcholine-HCl (Ach, pH 4.5). Constant currents of appropriate polarity were used to release the drugs from their respective barrels (NeuroPhore BH-2). The electrodes were advanced to the target areas under microscopic control by means of a hydraulic microdrive (Narishige MO-10). Action potentials were passed into a preamplifier, then to a high gain amplifier with low and high cut filters and to a window discriminator to form standard pulses. In an additional, parallel, processing, signals were also led to a computerized A/D converter device (CED 1401*plus*). Both the spike discharges and pulses were continuously monitored on oscilloscopes (HAMEG HM-2035 and -2037). All the pulses and trigger marker signals were fed into an IBM PC / AT compatible microcomputer for real-time and off-line analyses. Raw data, formed pulses, marker signals and voice commentaries were stored on magnetic tapes and floppy disks for subsequent analyses.

Gustatory and Olfactory Stimulations

For well-quantified, replicable application of tastants in the primate [Karádi et al., 1990a], a soft polyethylene or silicone tube was intraorally implanted in the monkeys []. One opening of the tubing was sutured at the buccal surface just posterior to the last molars and the other end was drawn up subcutaneously and fixed to the dental acrylic headpiece. In the rat, a similar (but smaller in diam) polyethylene tubing was oriented towards the mouth of the animal, and was fixed in a position that enabled us to stimulate large areas of the tongue and palate. In addition to concentrations of the four "primary" taste qualities (*salty*: NaCl; *sour*: HCl and citric acid; *bitter*: quinine hydrochloride; *sweet*: glucose and sucrose), the so-called *umami* taste (monosodium L-glutamate) and *orange juice* (as complex sapid solution) were also employed as stimuli. Applications of tastants were followed by two injections of deionized water and air.

For olfactory stimulations, eleven synthetic volatile substances (orange, banana, isoamyl acetate, potato, skatole, borneol, pine-needle, leather, musk, 2,4,6-trymethylpiridine and trimethylamine) in appropriate concentrations were employed. In the monkey, the originally described delivery technique was used [Karádi et al., 1989], whereas in the rat experiments, fine polyethylene tubes were led to the vicinity of the nostrils and the various odorants (each through different pairs of tubings) were simultaneously applied at both sides. Each stimulation was followed by two air cleansings. All studies were carried out in a temperature- and humidity-controlled, well-ventillated room.

Data Analysis

The action potentials of well-isolated, spontaneously active neurons were recorded extracellularly. Those neurons that did not respond to electrophoretically applied glutamate or GABA, and that showed activity changes to either Na^+ or Cl^- (nonspecific current effect), were excluded from the analysis.

If during the respective stimulations a neuron's discharge rate changed by at least 30% from the baseline level and if the results were replicable, the cell was considered to respond to gustatory and olfactory signals. In the microelectrophoresis studies, an additional criterion of

dose-dependence (increasing ejection current intensities with enhanced effects on firing rates) was also introduced.

For statistical analysis of data, the Student's t and χ^2 tests were employed.

Localization of Recording Sites

Coordinates of the electrode placements were determined stereotaxically, and the depth of each neuron from the brain's surface was measured and registered in micrometers. In addition to the above procedure, bilateral X-ray photographs of the head were taken in the monkey after each penetration. The reconstruction of recording sites was also aided by MRI pictures taken before the main stereotaxic surgery. Final localization, both in the rat and monkey, was based on histological examination of microlesions made at coordinates identical to those of recording sites after finishing all experiments.

RESULTS

The activity of altogether 971 neurons (324 in the anesthetized rat and 647 in the alert or anesthetized monkey) was recorded in these series of experiments. The mean spontaneous discharge rates in the LHA, AMY and GP were 9.8 ± 6.4, 14.2 ± 8.9 and 11.5 ± 7.9 spikes/s, respectively. Neuronal firing did not vary significantly between the two types (anesthetized or awake) of preparations, nor between the two species. There were, however, essential differences among regional spontaneous firing rates in the GP (with much lower activity ventrally), and smaller differences in the LHA (ventromedial vs dorsolateral areas, the latter displaying higher rates) and the AMY (central-dorsal vs ventrobasal-lateral divisions, cells of the former firing subtly slower).

Sensitivity to glucose applied microelectrophoretically was examined in 929 neurons. Of these, 295 cells were recorded in the LHA, 313 in the AMY as well as 321 in the GP. The proportion of GS cells (i.e., whose activity was suppressed by glucose) was found to be 29% (86 units), 11% (34 neurons) and 14% (45 cells), respectively, in the 3 forebrain structures. The remaining neurons (209 in the LHA, 279 in the AMY and 276 in the GP) did not change in activity in response to glucose. Thus, they were assigned as glucose-insensitive (GIS) units. These differential proportions of GS vs GIS cells in the ventral forebrain were remarkably similar in the two species as well as in the awake or anesthetized preparations.

Results of the feeding task related experiments as well as of those investigating motivational aspects of the alimentary behavior - referring to a highly overlapping population of lateral hypothalamic, amygdaloid and pallidal *chemoneurons* -, are presented and discussed in the accompanying paper of this same issue [Lénárd et al., 1995b].

Responses to Exogenous Chemosensory Stimuli

The effect of taste stimuli was tested in 786 neurons (485 in the monkey and 301 in the rat) forebrain. Characteristic proportions of gustatory cells (these and the general firing attributes did not differ significantly in the two species and in anesthetized or awake preparations) are

TABLE 1

Proportion of taste-responsive neurons in ventral forebrain structures (%)

	GS		GIS		
	Taste (+/-)	Taste (∅)	Taste (+/-)	Taste (∅)	Total
LHA	60 (68) *	10 (32)	29 (17) *	142 (83)	241
AMY	23 (77) #	7 (23)	8 (3) #	230 (97)	268
GP	34 (87) ^	5 (13)	10 (4) ^	228 (96)	277

Abbreviations: Taste +/-, excitatory and/or inhibitory gustatory responses; Taste ∅, no taste response. *, #, ^, $p < 0.01$, χ^2 test. (Recordings performed in both rats and rhesus monkeys.)

summarized and demonstrated in Table 1. As seen in Table 1, GS neurons change in activity in response to gustatory stimulation, in all three forebrain regions. In addition to their higher responsiveness to sapid stimuli, these chemosensitive cells were also found to be tuned broadly across qualities of the stimulus array, i.e., they responded predominantly to two or more tastants. Figure 1 demonstrates typical GS neurons in the primate AMY (A) and GP (B) with multiple gustatory sensitivities. Amygdaloid gustatory neurons, as distinct from lateral hypothalamic and pallidal taste units, often displayed (as it is also shown in Figure 1A) 'complementary' firing rate changes to tastants with opposite hedonic values (QHCl vs sucrose). The GIS cells, in contrast to the broad tuning of GS neurons, exhibited more "discriminative" gustatory responses: they were usually facilitated or inhibited by only one taste quality in all 3 forebrain areas.

Responsiveness to olfactory stimulation was examined in 108 lateral hypothalamic, 81 amygadaloid and 89 pallidal neurons (total 278). More than two-thirds of recordings (in 196 cells) were performed in the primate forebrain, and the response and neuronal firing

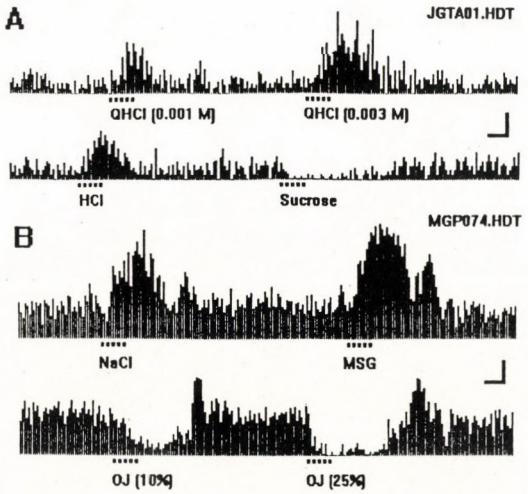

Fig.1. Multiple taste responses of an amygdaloid (A) and pallidal (B) chemosensitive neuron in the monkey. A: Concentration /dose/-dependent facilitation to quinine hydrochloride (QHCl), excitation to hydrochloric acid (HCl) and prolonged decrease in firing to sucrose. B: High excitation to Na-salts, and a dose-dependent complex response pattern to orange juice (OJ). Dotted lines, onset and duration of gustatory stimulations. Calibration, time in sec, and neuronal discharge rate in spikes/sec, respectively

characteristics did not differ significantly between the two species and alert or anesthetized preparations. Of the three regions in question, neurons of the LHA were the most sensitive to smells, with more than 50% (55 cells) of all cells tested responding to one or more odorants. Amygdaloid and pallidal units displayed a similarly lower responsiveness to olfactory stimulation: The proportion of smell-sensitive neurons in these structures was found to be 15% (12 cells) and 12% (11 units), respectively. In all three areas, the chemosensitive (GS) neurons were the most likely to respond to odor stimuli: Their respective proportions in the LHA, AMY and GP were 89%, 77% and 91%. The majority of these GS cells, in sharp contrast to the GIS neurons, were not only broadly tuned, i.e., responded to two or more smells, but exhibited gustatory sensitivity as well. The chemosensitive units, in addition, proved to be 'selectively' driven (or inhibited) by familar vs non familiar (LHA and AMY) and food-related vs non-food related smells (LHA and GP).

TABLE 2

Characteristic catecholamine sensitivities of gustatory neurons in the ventral forebrain

	LHA		AMY		GP	
	Taste +/-	Taste ∅	Taste +/-	Taste ∅	Taste +/-	Taste ∅
NA	----	∅	----	∅	---/+	∅
DA	--/+	∅	--/+	∅	--/++	∅
ΣN=	217		188		205	

Abbreviations, symbols are identical to those in Table 1. + and - , characteristically excitatory and inhibitory responses, respectively; ∅, no response. (Results obtained from experiments with both rats and monkeys.)

Neurotransmitter Sensitivity of the Chemosensory Cells

The effect of microelectrophoretically administered chemicals has been examined in the present series of experiments in 272 lateral hypothalamic, 241 amygdaloid and 250 pallidal cells. Neurons in the primate and rodent forebrain (both in the anesthetized and alert preparations) exhibited variable and non-characteristic sensitivities to acetylcholine and glutamate, and exclusively inhibitory responses to GABA. These very same units, however, depending on their gustatory (and olfactory) responsiveness, displayed differential sensitivity to microionto-phoretically applied catecholamines (CAs), dopamine (DA) and noradrenaline (NA). As shown in Table 2, predominantly the taste-responsive cells were those (in all three forebrain structures) that were usually inhibited by NA, and either inhibited or excited by DA.

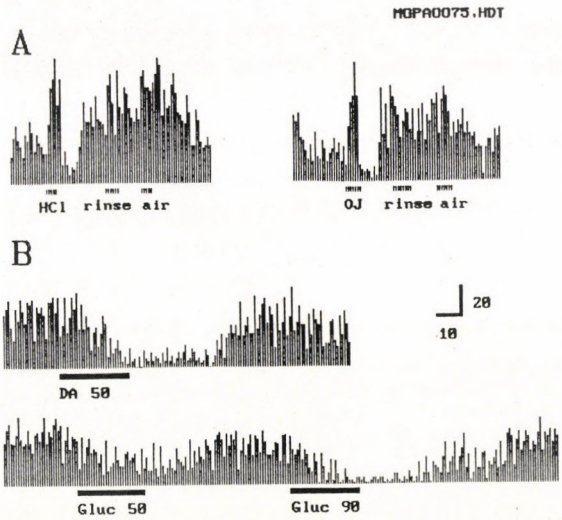

Fig. 2. Gustatory responsiveness and catecholamine-sensitivity of a typical GS cell in the monkey GP. A: Complex (excitation-inhibition-excitation) response pattern to HCl and OJ. Dotted lines, onset and duration of taste stimulation as well as those of rinsing with deionized water and air. B: Lasting decrease in activity to DA applied microiontophoretically. Dose-dependent inhibition to glucose microelectrophoresis. Horizontal lines, numbers, onset and duration of microelectrophoretic applications, and ejection current intensities, respectively. Calibration is identical with that in Fig.1

Figure 2 also demonstrates CA-sensitivity of a characteristic GS neuron recorded in the ventromedial region of the monkey GP.

Localization of Chemosensory Neurons

In good agreement with our previously reported findings [Karádi et al., 1988,1989,1990b,1992,1995], careful analysis of the recording sites revealed specific topographical organization of chemosensory neurons in all three forebrain areas tested. These GS, taste- (and smell-) responsive cells (with a characteristic likelihood to change in firing rate during motivated feeding actions) were found to be located in the ventral-medial divisions of the monkey and rat LHA. The GIS units had no such specific topography: they were intermingled with other types of neurons throughout the whole extent of the area. The same kind of amygdaloid chemoneurons, in contrast to the non-specific topography of GIS cells, were concentrated in the central nucleus of the AMY and its surroundings in the dorsal region

of this complex structure in both species. Feeding-associated gustatory (and olfactory) GS neurons were found in rostral and ventro-medial divisions of the GP (internal segment of the dorsal GP in the primate and the corresponding area of the lentiform nucleus in the rodent), clearly distinguishing their topographical organization from the GIS (and non-feeding related GS) cells that were localized more caudally and dorsolaterally in the pallidum.

DISCUSSION

To reveal complex, feeding-associated attributes of neurons of reportedly interrelated forebrain areas, i.e., the LHA, AMY and GP [Aggleton et al., 1980; Nauta and Mehler, 1966; Oomura et al., 1970,1975; Parent et al., 1984; Price, 1981], a complex, behavioral-neurochemical-electrophysiological methodology was employed in the present study. Experiements have been performed in both primates and rodents, data were, however, mainly obtained from awake rhesus monkeys whose feeding-related regulatory functions are supposed to greatly correspond to those in humans.

Feeding-Associated and Motivational Attributes of Chemosensory Neurons

In agreement with findings of previous investigations [Aou et al., 1984; Karádi et al., 1988,1989,1990b,1992,1995; Nakano et al., 1986], the GS cells were those in all three forebrain regions that were the most likely to change in activity during phases of the conditioned alimentary task (data in details reported in the accompanying paper in this same issue) [Lénárd et al., 1995b]. These neurons, at the same time, were also responsive to a variety of gustatory (and olfactory) stimuli [Karádi et al., 1988,1989,1990b,1992,1994,1995; Oomura, 1987]. The chemosensory cells (with both "endogenous" and "exogenous"chemosensitivity), as their general characteristic, proved to be greatly influenced by shifts in the motivational state of the animals. In the LHA, AMY and GP, these neurons showed differential responsiveness to pleasant vs aversive tastes [Karádi et al., 1988,1992], or to familar vs non-familiar [Karádi et al., 1989] as well as to food-related vs non-food related odors [Karádi et al., 1995]. Evidence has also been obtained for hunger or (sensory-specific) satiety [Rolls et al., 1986] modulation of lateral hypothalamic chemoneurons [Karádi et al., 1990b], and similar tendencies were found in amygdaloid and pallidal recordings as well. It is, therefore, reasonable to suppose that chemosensitive neurons of the LHA, AMY and GP, along with the GIS cells that possess more discriminative response properties, may constitute the neural basis of a perceptual-motivational regulation of feeding-associated central taste (and smell) information processing.

This above hypothesis is also supported by our findings on distinct but characteristic CA-sensitivities of GS and GIS gustatory neurons. The ascending dopaminergic and noradrenergic pathways that project through and partly terminate in the three forebrain structures examined, have already been broadly implicated in sensory-motor integration, perceptual, emotional and reinforcement-reward processes as well as, more specifically, in feeding-related motivational control mechanisms [Aou et al., 1983; Lénárd, 1977; Lénárd and Hahn, 1982; Lénárd et al., 1982a,b; Lénárd et al., 1986; Marshall et al., 1974; Nakano et al., 1987; Nishino et al., 1987; Oltmans and Harvey, 1976; Oomura, 1980,1987; Palfai et al., 1984; Ungerstedt, 1971].

General Considerations

Data previously available in the literature [Karádi et al., 1992,1995; Norgren, 1974; Scott and Giza, 1987; Travers et al., 1987] demonstrated essential functional and morphological differences between the central taste (and smell) representations of lower mammals (especially rodents) and primates (macaque monkeys). While in the rat it is characteristic that the hindbrain relay stations (nucleus of the solitari tract, parabrachial nucleus) have direct and reciprocal interconnections with forebrain gustatory 'centers' (thalamus, LHA, AMY, insular cortex), such "taste loops" have not been reported yet in the monkey. By contrast, it is now evident that a more 'serial' gustatory information processing exist in the primate brain [Karádi et al., 1992,1995; Mesulam and Mufson, 1982]. Nevertheless, it seems to be uniformly characteristic for both species that 'exogenously' chemosensory neurons also possess 'endogenous' chemosensitivity (or *vice versa*). These chemosensitive (GS) gustatory (and olfactory) cells appear to have a specific topographic organization in the ventral forebrain: In all three areas examined in the present study, they were localized only in well circumscribed subdivisions of these structures.

Considering the above findings and the intimate relationships among all these areas in the LHA, AMY and GP, furthermore, their well known connections with associative orbitofrontal cortical regions [Heimer et al., 1982; Karádi et al., 1990b; Kievit and Kuypers, 1977; Oomura, 1980,1987; Parent et al., 1984; Price, 1981; Scott and Giza, 1987], it is reasonable to suppose that these chemosensory neurons possess more complex functional attributes than processing "pure" taste and smell signals. Indeed, this particular sub-set of neurons was likely to change in activity in response to not only chemical cues but also to various other, feeding-related sensory (visual, acoustic) stimuli as well as during motivated motor actions or relevant motivational manipulations. While organized hierarchically, as

constituents of a central glucose-monitoring neural network [Karádi et al., 1992,1995; Lénárd et al., 1994,1995b; Oomura and Yoshimatsu, 1984], these *chemoneurons* - along with the GIS units - appear to perfectuate a complex integration of feeding-associated information from the internal and external milieux. Their multiple functional attributes may be indispensable in a biologically adaptive feeding control when the animals' endogenous homeostatic needs and the exogenous environmental signals have to be evaluated and incorporated - moment-to-moment - in the ongoing behavioral actions.

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REFERENCES

Aggleton, J.P., Burton, M.J. and Passingham, R.E. (1980) Cortical and subcortical afferents to the amygdala of the rhesus monkey (Macaca mulatta). Brain Res. 190, 347-368.

Amaral, D.G., Veazey, R.B. and Cowan, W.M. (1982) Some observations on hypothalamo-amygdaloid connections in the monkey. Brain Res. 252, 13-27.

Anand, B.K., Brobeck, J.R. (1951) Localization of a "feeding center" in the hypothalamus of the rat. Proc. Soc. Exp. Biol. Med. 77, 323-324.

Aou, S., Oomura, Y., Lénárd, L., Nishino, H., Inokuchi, A., Minami, T. and Misaki, H. (1984) Behavioral significance of monkey hypothalamic glucose-sensitive neurons. Brain Res. 302, 69-74.

Aou, S., Oomura, Y., Nishino, H., Inokuchi, A. and Mizuno, Y. (1983) Influence of catecholamines on reward related neuronal activity in monkey orbitofrontal cortex. Brain Res. 267, 165-170.

Berridge, K.C., Cromwell, H.C. (1990) Motivational-sensorimotor interaction controls aphagia and exaggerated treating after striatopallidal lesions. Behav. Neurosci. 104, 778-795.

Burton, M.J., Mora, F., Rolls, E.T. (1975) Visual and taste neurones in the lateral hypothalamus and substantia innominata: Modulation of responsiveness by hunger. J. Physiol. 252, 50-51.

Cromwell, H.C., Berridge, K.C. (1993) Where does damage lead to enhanced food aversion. The central pallidum/substantia innominata or lateral hypothalamus? Brain Res. 624, 1-10.

Czech, D.A. (1973) Effects of amygdalar lesions on eating and drinking and saline preference in the rat. Physiol. Behav. 10, 821-823.

Fonberg, E. (1974) Amygdala functions within the alimentary system. Acta Neurobiol.Exp. 34, 435-466.

Hahn, Z., Karádi, Z., Lénárd, L. (1988) Sex-dependent increase of blood glucose concentration after bilateral pallidal lesion in the rat. Acta Physiol. Hung. 72, 99-101.

Hahn, Z., Lénárd, L., Ruppert, F. (1979) The connection of plasma triiodothyronine levels with the sex-dependent body weight loss after bilateral pallidal lesion in rats. Acta Physiol. Hung. 53, 17- 21.

Heimer, L., Switzer, R.D., Van Hoesen, G.W. (1982) Ventral striatum and ventral pallidum. Components of the motor system? Trends Neurosci. 5, 83-87.

Karádi, Z., Faludi, B., Czurkó, A., Niedetzky, Cs. and Lénárd, L. (1994) Gustatory and olfactory responses of chemosesnitive pallidal neurons. In: Olfaction and Taste XI. (eds. Kurihara,K. et al.) pp. 537-538. Springer Verlag, Tokyo.

Karádi, Z., Faludi, B., Lénárd, L., Czurkó, A., Niedetzky, Cs., Vida, I., Nishino, H. (1995) Glucose-sensitive neurons of the globus pallidus: II. Complex functional attributes. Brain Res. Bull. 37(2), 157-162.

Karádi, Z., Nishino, H., Oomura, Y., Scott, T.R. and Aou, S. (1990a) A method for gustatory stimulus delivery in awake rhesus monkeys. Brain Res. Bull. 25, 665-668.

Karádi, Z., Oomura, Y., Nishino, H. and Aou, S. (1989) Olfactory coding in the monkey lateral hypothalamus: Behavioral and neurochemical properties of odor-responding neurons. Physiol. Behav. 45, 1249-1257.

Karádi, Z.,Oomura, Y., Nishino, H., Scott, T.R., Lénárd, L., Aou, S. (1988) Lateral hypothalamic and amygdaloid neuronal responses to chemical stimuli in the rhesus monkey. In: Proc. 22nd JASTS (ed. Morita, H.) pp. 121-124. Asahi University Press, Gifu.

Karádi, Z., Oomura, Y., Nishino, H., Scott, T.R., Lénárd, L. and Aou, S. (1990b) Complex attributes of lateral hypothalamic neurons in the regulation of feeding of alert rhesus monkeys. Brain Res. Bull. 25, 933-939.

Karádi, Z., Oomura, Y., Nishino, H., Scott, T.R., Lénárd, L. and Aou, S. (1992) Responses of lateral hypothalamic glucose-sensitive and glucose insensitive neurons to chemical stimuli in behaving rhesus monkeys. J. Neurophysiol. 67, 389-400.

Kievit, J., Kuypers, H.G.J.M. (1977) Organization of the thalamo-cortical connections to the frontal lobe in the rhesus monkey. Exp. Brain. Res. 29, 299-322.

Levine, M.S., Schwartzbaum, J.S. (1973) Sensorimotor functions of the striatopallidal system and lateral hypothalamus. J. Comp. Physiol. Phychol. 85, 615-635.

Lénárd, L. (1977) Sex-dependent body weight loss after 6-hydroxydopamine injection into the globus pallidus. Brain Res. 128, 559-568.

Lénárd, L., Faludi, B., Karádi, Z., Czurkó, A., Vida, I. and Niedetzky, Cs. (1994) Responses of pallidal neurons to microelectrophoretically applied glucose and neurochemicals. In: The Basal Ganglia IV. (eds. Percheron,G. et al.) pp. 239-244. Plenum Press, New York.

Lénárd, L., Hahn, Z. (1982) Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of hunger and thirst-motivated behavior. Brain Res. 233, 115-132.

Lénárd, L., Hahn, Z. and Karádi, Z. (1982a) Body weight changes after neurochemmical manipulations of lateral amygdala: Noradrenergic and dopaminergic mechanisms. Brain Res. 249, 95-101.

Lénárd, L., Jandó, G., Karádi, Z., Hajnal, A., Sándor, P. (1988) Lateral hypothalamic feeding mechanisms: Iontophoretic effects of kainic acid, ibotenic acid and 6-hydroxydopamine. Brain Res. Bull. 20, 847-856.

Lénárd, L., Karádi, Z., Faludi, B., Czurkó, A., Niedetzky, Cs., Vida, I., Nishino, H. (1995a) Glucose-sensitive neurons of the globus pallidus: I. Neurochemical characteristics. Brain Res. Bull. 37, 149-155.

Lénárd, L., Karádi, Z., Faludi, B. and Hernádi, I. (1995b) Role of forebrain glucose-monitoring neurons in the central control of feeding: I. Behavioral properties and neurotransmitter sensitivities. (in press) Neurobiology

Lénárd, L., Karádi, Z., Szabó, I., Hahn, Z. (1982b) Pallidal mechanisms in the organization of feeding and sensorimotor integration. In: Recent developments in neurobiology in Hungary. IX. (ed. Lissák, K.) pp. 79-113. Akadémia Kiadó, Budapest.

Lénárd, L., Oomura, Y., Nishino, H., Aou, S., Nakano, Y., Yamamoto, T. (1986) Activity in monkey lateral hypothalamus during operant feeding: Modulation by catecholamines and opiate. In: Emotions: Neuronal and chemical control. (ed. Oomura, Y.) pp. 45-53. Japan Sci. Soc. Press, Tokyo.

Marshall, J.F., Richardson, J.S., Teitelbaum, P. (1974) Nigrostriatal bundle damage and the lateral hypothalamic syndrome. J. Comp. Physiol. Phychol. 87, 808-830.

Mesulam, M.M. and Mufson, E.J. (1982) Insula of the old world monkey. III. Efferent cortical output and comments on function. J. Comp. Neurol. 212, 38-52.

Morgane, P.J. and Jacobs, H.L. (1969) Hunger and satiety. World Rev. Nutr. Diet. 10, 100-213.

Nakano, Y., Lenard, L., Oomura, Y., Nishino, H., Aou, S. and Yamamoto, T. (1987) Functional involvement of catecholamines in reward related neuronal activity of the monkey amygdala. J. Neurophysiol. 57, 72-91.

Nakano, Y., Oomura, Y., Lenard, L., Nishino, H., Aou, S., Yamamoto, T. and Aoyagi, K. (1986) Feeding-related activity of glucose- and morphine-sensitive neurons in the monkey amygdala. Brain Res. 399, 167-172.

Nauta, H.W.J., Mehler, W.R. (1966) Projections of the lentiform nucleus in the monkey. Brain Res. 1, 3-42.

Niedetzky, Cs., Karadi, Z., Czurkó, A., Faludi, B., Vida, I., Jandó, G., Lenard, L. (1993) Computer analysis of single neuron activity during conditioned feeding task. Neurobiology 1, 147-155.

Nishino, H., Oomura, Y., Aou, S. and Lénárd. L. (1987) Catecholaminergic mechanisms of feeding-related lateral hypothalamic activity in the monkey. Brain Res. 405, 56-67.

Nishino, H., Oomura, Y., Karádi, Z., Aou, S., Lénárd, L., Kai, Y., Fukuda, A., Ito, C., Min, B.I. and Plata-Salaman, C.R. (1988) Internal and external information processing by lateral hypothalamic glucose-sensitive and insensitive neurons during bar press feeding in the monkey. Brain Res. Bull. 20, 839-845.

Norgren, R. (1974) Gustatory afferents to the ventral forebrain. Brain Res. 81, 285-295.

Norgren, R. (1976) Taste pathways to hypothalamus and amygdala. J. Comp. Neurol. 166, 17-30.

Oltmans, G.A., Harvey, J.A. (1976) Lateral hypothalamic syndrome in rats: A comparison of the behavioral and neurochemical effects of lesions placed in the lateral hypothalamus and nigrostriatal bundle. J. Comp. Physiol. Psychol. 90, 1051-1062.

Oomura. Y. (1980) Input-output organization in the hypothalamus relating to food intake behavior. In: Handbook of the hypothalamus (eds. Morgane, P., Panksepp, J.) Vol. 2. pp. 557-620. Marcel Dekker. New York.

Oomura, Y. (1987) Modulation of prefrontal and hypothalamic activity by chemical senses in the chronic monkey. In: Umami: A basic taste. (eds. Kawamura, Y., Kare, M.R.) pp. 481-509. Marcel Dekker, New York.

Oomura, Y., Nakamura, T., Manchanda, S.K. (1975) Excitatory and inhibitory effects of globus pallidus and substantia nigra on the lateral hypothalamic activity in the rat. Pharmacol. Biochem. Behav. S1, 23-36.

Oomura, Y., Ono, T. and Ooyama, H. (1970) Inhibitory action of the amygdala on the lateral hypothalamic area in rats. Nature Lond. 228, 1108-1110.

Oomura, Y., Ono, T., Ooyama, H. and Wayner, M.J. (1969) Glucose and osmosensitive neurons of the rat hypothalamus. Nature Lond. 222, 282-284.

Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T. and Yamada, Y. (1974) Glucose inhibition of the glucose-sensitive neurons in the lateral hypothalamus. Nature Lond. 247, 284-286.

Oomura, Y., Yoshimatsu, H. (1984) Neural network of the glucose monitoring system. J. Autonom Nerv. Syst. 10, 359-372.

Palfai, T., Armstrong, D., Courtney, C.L. (1984) Effect of L-DOPA or bromocriptine on feeding and motor behavior of rats with lesions in the globus pallidus. Physiol. Behav. 33, 283-289.

Parent, A., Smith, Y., Bellefeuille, L.D. (1984) The output organization of the pallidum and substantia nigra in primate as revealed by a retrograde double-labeling method. In: The Basal Ganglia II. (eds. McKenzie, J.S. et. al.) pp. 147-160. Plenum Press, New York.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J. (1979) A stereotaxic atlas of the rat brain. Plenum Press, New York.

Price, J.L. (1981) The efferent projections of the amygdaloid complex in the rat, cat and monkey. In: The Amygdaloid Complex, INSERM Symposium No.20. (ed. Ben-Ari, Y.) pp. 121-132. Elsevier, Amsterdam.

Rolls, E.T., Murzi, E., Yaxley, S., Thorpe, S.J., Simpson, S.J. (1986) Sensory-specific satiety: Food specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. Brain Res. 368, 79-86.

Sándor, P., Hajnal, A., Jandó, G., Karádi, Z., Lénárd, L. (1992) Microelectrophoretic applications of kainic acid into the globus pallidus: Disturbances in feeding behavior. Brain Res. Bull. 28, 751-765.

Schwartzbaum, J.S. (1988) Electrophysiology of taste, feeding and reward in lateral hypothalamus of rabbit. Physiol Behav. 44, 507-526.

Scott, J.W. and Pfaffmann, C. (1967) Olfactory input to the hypothalamus: Electrophysiological evidence. Science Wash. DC 158, 1592-1594.

Scott, T.R. and Giza, B.K. (1987) The effect of physiological condition on taste in rats and primates. In: Umami: A Basic Taste. (eds. Kawamura Y., Kare, M.R.) pp. 409-437. Marcel Dekker, New York.

Snider, S. and Lee, J.C. (1961) A Stereotaxic Atlas of the Monkey Brain (Macaca mulatta). Univ. of Chicago Press, Chicago, IL.

Soltysik, S., Hull, C.D., Buchwald, N.A., Fekete, T. (1975) Single unit activity in basal ganglia of monkeys during performance of a delayed response task. Electroencephalogr. Clin. Neurophysiol. 39, 65-78.

Takagi, S.F. (1986) Studies on the olfactory nervous system of the old world monkey. Prog. Neurobiol. 27, 195-250.

Travers, J.B., Travers, S.P. and Norgren, R. (1987) Gustatory neural processing in the hindbrain. Annu. Rev. Neurosci. 10, 595-632.

Turner, B.H. (1973) A sensorymotor syndrome produced by lesions of the amygdala and lateral hypothalamus. J. comp. physiol. Psychol. 82, 37-47.

Ungerstedt, U. (1971) Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol. Scand. Suppl. 367, 95-122.

Yamamoto, T., Matsuo, R., Kiomitsu, Y. and Kitamura, R. (1989) Response properties of lateral hypothalamic neurons during ingestive behavior with special reference to licking of various taste solutions. Brain Res. 481, 286-297.

Yoshimatsu, H., Niijima, A., Katafuchi, T. and Oomura, Y. (1988) Lateral and ventromedial hypothalamic influences on hepatic autonomic nerve activity in the rat. Brain Res. Bull. 21, 239-244.

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HYPOTHALAMIC NEURONS ARE RESISTANT TO THE INTOXICATION WITH 3-NITROPROPIONIC ACID THAT INDUCES LESIONS IN THE STRIATUM AND HIPPOCAMPUS VIA THE DAMAGE IN THE BLOOD-BRAIN BARRIER

Nishino, H., Shimano, Y., Kumazaki, M., Sakurai, T., Hida, H., Fujimoto, I. and Fukuda, A.

Department of Physiology, Nagoya City University Medical School, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan

Summary: Modulation of the function of the blood-brain barrier (BBB) in the hypothalamus was investigated after the intoxication with 3-nitropropionic acid (3-NPA) that inhibits the succinate dehydrogenase. 3-NPA was administered to rats for three days. Following transcardial perfusion, brain sections were studied by immunohistochemistry. On the 2nd or 3rd day after 3-NPA, strong immunoreactions for blood-borne macromolecules, IgG, appeared in the striatum and hippocampus. Glial fibrillary acidic protein (GFAP) positive astroglias distributed heterogeneously, and induced nitric oxide synthase (iNOS) positive cells appeared around the vessels. A week later, bilateral lesions were detected in these areas. In the hypothalamus, there appeared a moderate immunoreaction for IgG, but no expression of iNOS. GFAP positive astroglias were rich especially around the vessels, and no loss in microtubule-associated protein 2 (MAP2) immunoreaction was detected, suggesting an intact BBB structure and no neuronal loss following 3-NPA intoxication. Data indicate that hypothalamic neurons are resistant to 3-NPA that induces specific lesions in the striatum and hippocampus via the damage in the BBB.

Key Words: 3-nitropropionic acid; Neural death; Blood-brain barrier; Rat; Hypothalamus; Striatum

INTRODUCTION

The hypothalamus is involved in various vital functions. This is due to its strategic position in the brain : firstly it locates in the basal diencephalon, thus has intimate connections with both forebrain and mid-/hind- brains [Haymaker et al., 1969], secondly it is a higher center regulating the autonomic as well as hormonal systems [Reichlin et al., 1978] and thirdly there are chemosensitive neurons [Grossman, 1962; Oomura, 1973,1980] that respond to blood-borne and/or cerebrospinal fluid substances, thus monitoring chemical information concerned with feeding, drinking, sexual behavior and so on [Koizumi et al., Oomura, 1980; Wauquier et al., 1976]. The last nature could be attained by the characteristic that the BBB function in the hypothalamus is not so tight [Hashimoto et al., 1967; Sluga et al., 1967] and the blood-borne chemical information is rather well transmitted to the hypothalamus. 3-NPA, an irreversible inhibitor of the succinate dehydrogenase, disturbs the oxidative phosphorylation in the mitochondrial respiratory chain and induces energy (ATP) deficiency, depolarization and excitatory neurotoxicity especially in the striatum [Beal et al., 1993; Hamilton et al., 1987; Ludolph et al., 1991]. In the present study, we administered 3-NPA systemically to rats and investigated how the BBB function and neuronal as well as glial viability in the hypothalamus were disturbed. We report here that the 3-NPA intoxication hits the striatum severely and sometimes hippocampus by damaging the BBB function, but it induces no significant damage in the hypothalamus.

MATERIALS AND METHODS

Animals

Male Wistar/St rats (body weight 230-260 g) were used. They were kept in a temperature and light-dark cycle controlled animal room. Lab chow and water were given *ad libitum*. Animal cares were according to the guidelines of Institution for Animal Experiment, Nagoya City University Medical School.

3-NPA administration

3-NPA (Aldrich Chemical Co.) dissolved in saline and pH adjusted to 7.4 was administered subcutaneously (20 mg/kg) every day for two or three days. Control animals were given saline in the same way.

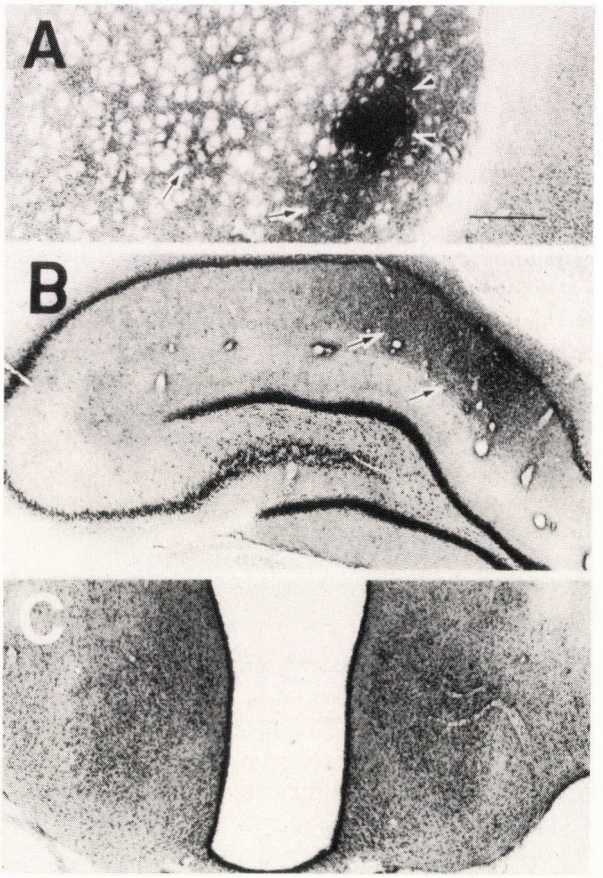

Fig. 1: Histological damages in the striatum (A) and hippocampal CA1 (B) but not in the hypothalamus (C) following the acute intoxication of 3-NPA. Neuronal loss, lymphocytes invasions (arrows) and sometimes bleeding (arrow heads) appeared in the lateral striatum and CA1. The hypothalamus remained quite intact. Scale, 500 μm

Behavioral observation

LD_{50} of 3-NPA has been calculated to be about 24 mg/kg in Wistar rats. Since 20 mg/kg of 3-NPA (the present dose) is a rather high dose, we observed carefully the general behavior of the animals in an openfield after each injection of 3-NPA.

Immunocytochemistry and histology

On the 2nd, 3rd and 7th day, under deep pentobarbital anesthesia, animals were perfusion-fixed through the aorta with saline followed by a fixative with 4 % paraformaldehyde in phosphate buffer. Brains were removed and 50 μm thick coronal sections were made. For immunocytochemistry [Czurko and Nishino, 1994], antibodies against cow GFAP (Dakopatts, rabbit anti-cow GFAP, x 100), rat IgG (Vector Lab., purified biotinylated rabbit anti-rat IgG, x 100), NOS (Upstate Biotech. Inc., rabbit anti-iNOS, x 200) antibodies and anti-MAP2 (Chemicon Int'l Inc., anti-MAP2, x 100) monoclonal antibody were used. After the reaction with biotinylated IgG, avidin-biotin complex was made and diaminobenzidine was used as a chromogen. Sections were also stained by hematoxylin-eosin for histological observation.

RESULTS

Behavioral abnormality

On the first day after 3-NPA administration, animals became a little motionless but no abnormal behavior was observed. However, on the 2nd or 3rd day, half of the intoxicated animals showed paddling, rolling, abnormal gait, and opisthotonoid posture for several hours after 3-NPA injection. Later on they became recumbent, somnolent and suffered from dyspnea.

Lymphocyte emigration

Under hematoxylin-eosin staining, a strong emigration of small cells with dense nucleus (majority, lymphocytes) was observed in the striatum on the 2nd or 3rd day. There were often intra-striatal edema and hemorrhage. A similar emigration was sometimes detected in the CA1 but not in other part of the hippocampus. In the hypothalamus there were no such emigrations in any nucleus (Fig. 1).

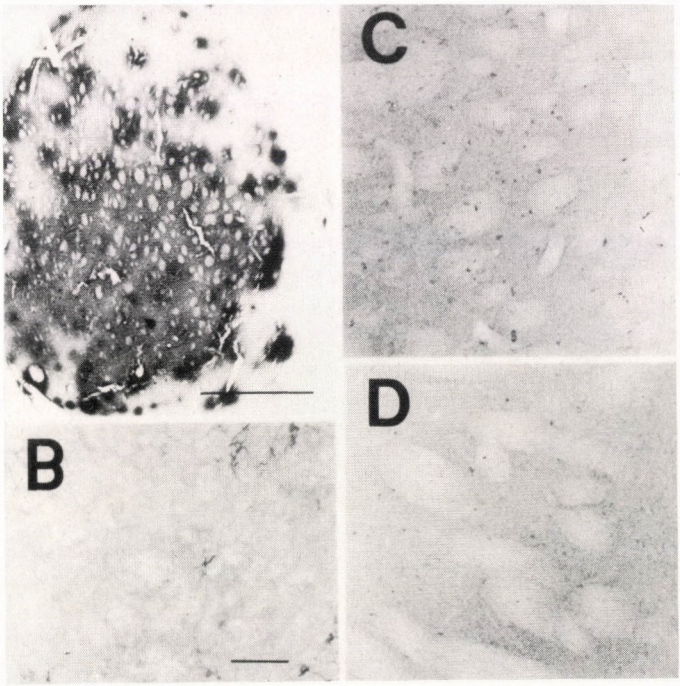

Fig. 2: Characteristics of the striatal lesion by 3-NPA intoxication. In intoxicated animals there were IgG extravasation (A), loss of GFAP glias (B) and expression of iNOS (C) in the striatum. No such iNOS positive cells in intact striatum (D). Scale in A, 1 mm; Scale in B 100 μm applied to C and D

Striatal damage

Among the whole CNS structure, the striatum was damaged most severely (Fig. 2). On the 2nd or 3rd day there was a strong extravasation of IgG in the centrolateral striatum. In this area GFAP positive astroglias were almost gone. iNOS immunopositive small cells (mostly glias) were detected around the vessels in the centrolateral striatum. A week later there were specific bilateral lesions in the striatum with the loss of MAP2- and GFAP- immunoreactions. Above striatal damages were detected in more than a half of 3-NPA intoxicated rats. Control animals showed no such abnormalities.

Hippocampal damage

On the 2nd or 3rd day, the extravasation of IgG and the emigration of lymphocytes were found in CA1 area in one third of intoxicated animals. MAP2

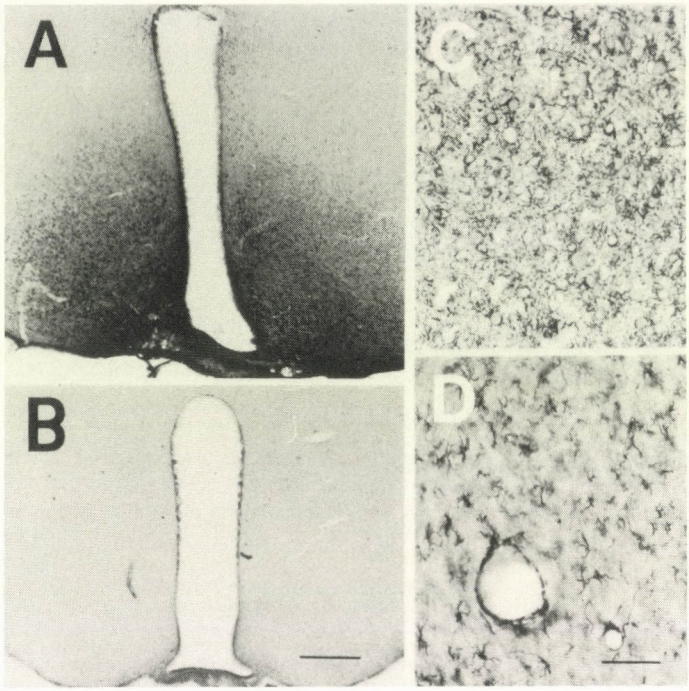

Fig. 3: The hypothalamus remained intact after intoxication of 3-NPA. Although there was a moderate extravasation of IgG in the ventral part (A), no iNOS expression was observed (B). MAP2 immunoreaction (C) and GFAP positive glias (D) remained intact. Scale in B, 500 μm applied to A. Scale in D, 100 μm applied to C

immunoreaction on somas and apical dendrites of CA1 pyramidal cells disappeared on the 7th day and GFAP immunoreaction was not detected in CA1 area.

Histology in the hypothalamus

There was a moderate immunoreaction for IgG in the ventral part of the hypothalamus (arcuate, ventromedial nuclei) on the 2nd and 3rd day after 3-NPA administration. However, no iNOS positive cells were detected in any part of the hypothalamus. The pattern of the distribution of MAP2 positive cells in the hypothalamus is quite similar in 3-NPA treated and control animals. GFAP positive astroglias are rich especially around vessels in the hypothalamus in any stage of the intoxication. Thus, no histological damages were found in the hypothalamus following 3-NPA intoxication (Fig. 3).

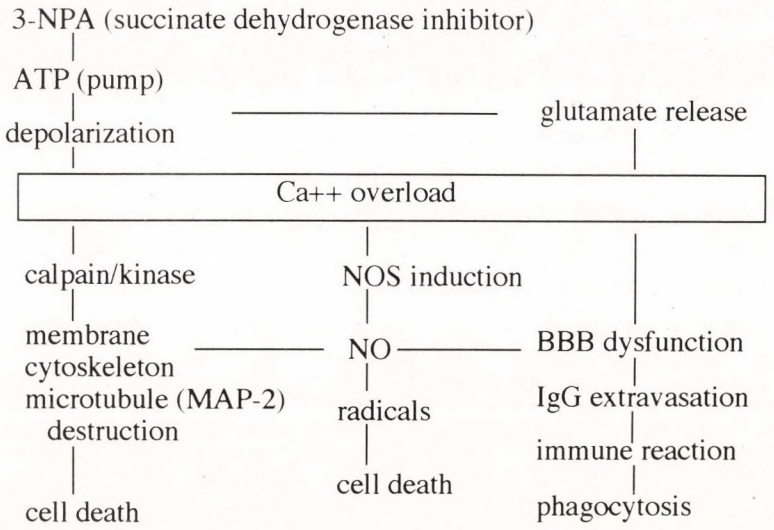

Fig. 4: Putative events involved in neuronal cell death following 3-NPA intoxication

DISCUSSION

3-NPA is a mycotoxin [He et al., 1990; Hu et al., 1986] that inhibits irreversibly the succinate dehydrogenase, a key enzyme in mitochondrial oxidative phosphorylation. Because of the suppression in ATP production [Hamilton et al., 1987], 3-NPA induces the disturbance in energy-coupled pump function, depolarization and so on. The neurotoxicity with 3-NPA is proposed to be mainly via the excitatory neurotoxicity [Beal et al., 1993; Simpson., 1993]. We have proposed that the BBB dysfunction that leads to the extravasation of serum proteins and the enhancement of immune and phogocytotic activities would be deeply involved in systemically administered 3-NPA intoxication [Nishino et al., 1995; Shimano et al., 1995].

The hypothalamus is a unique structure where the BBB function is not so tight [Hashimoto et al. 1967; Sluga et al., 1967], thus hypothalamic neurons evolved a high chemosensitivity [Oomura, 1973,1980] for circulating bioactive substances to gain their vital functions. The purpose of the present study is to investigate the effect of 3-NPA intoxication on hypothalamic BBB and neurons as well as glias in comparison with those in other structures.

Following the administration of 3-NPA, there were a strong extravasation of IgG, an emigration of lymphocytes and the loss of GFAP reactive astroglias in the striatum and hypocampal CA1. These results indicate the dysfunction of the BBB [Nishino et al., 1995; Shimano et al., 1995] since, in the normal brain, the astroglial feet lap vessels to make the BBB and protect the extravasation of blood-borne macromolecules as well as the emigration of blood cells. In well accordance with the above disturbances, a stout expression of iNOS was often observed in glia-like small cells that located around the vessels in the striatum [Nishino et al., 1996a] and less frequently in the CA1. In the hypothalamus, there was a moderate immunoreaction for IgG especially in the ventral part but no disturbance in GFAP reactive astroglias nor any expression of iNOS, and the MAP2 immunoreaction in neurons remained intact.

Based on these data, we hypothesize that 3-NPA at first induces the depolarization that leads to the overload of intracellular Ca^{++} ($[Ca^{++}]_i$) . Ca^{++} overload then induces the activation of protease/kinase that leads to the destruction of microtubules (MAP2) [Nishino et al., 1996b], the expression of iNOS [Gilbert et al., 1993], the production of NO/radicals [Moncada et al., 1991; Priitz et al., 1986], the dysfuction of the BBB, and finally the cell death (Fig. 4).

However, the question remained : why among various CNS neurons only striatal and hippocampal CA1 neurons were attacked by the toxin ?

In the striatum and hippocampus (especially CA1), there are massive glutamatergic inputs and NMDA receptor (NMDAR), thus the excitatory neurotoxicity would be easily induced. Distribution of glutamatergic inputs and NMDAR is much higher in the CA1 than in the striatum. From the point of glutamatergic neurotoxicity, thus the CA1 neurons should be attacked more easily and heavily than striatal neurons by 3-NPA. However, the result was opposite : the striatum was at first and CA1 the second. The real mechanism is

not known yet, but this discrepancy might be due to the vulnerability of the striatal artery [Nishino et al., 1995], massive dopaminergic input [Ben-Shachar et al., 1995] or a rather poor distribution of glutamate transporter in the striatum [Kiryu et al., 1995], resulting in a poor uptake mechanism for excessive glutamate during brain insult such as intoxication or ischemia.

When $[Ca^{++}]_i$ increased, Na^+-Ca^{++} exchanger pumps out Ca^{++} and pumps in Na^+ and tries to keep the $[Ca^{++}]_i$ at a resting level [Blaustein, 1988; Reuter, 1991]. Na^+-Ca^{++} exchanger is well coupled with Na^+-K^+ pump driven by high energy source (ATP). By 3-NPA intoxication Na^+-K^+ pump and Na^+-Ca^{++} exchanger would be disturbed and could not pump out $[Ca^{++}]_i$. Moreover, in a depolarized state given a loss of Na^+ gradient by the disturbance of Na^+-K^+ pump, Na^+-Ca^{++} exchanger might work reversibly [Takuma et al., 1995] (pumping out Na^+ and pumping in Ca^{++}) and would make the situation worse.

The present data indicate that although the hypothalamic BBB function is not so tight at rest, it remained quite intact even after the intoxication with 3-NPA, suggesting the stout viability of the structure to gain the vital physiological roles even in a critical situation.

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REFERENCES

Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R. and Hyman, B.T. (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J. Neurosci. 13, 4181-4192.

Ben-Shachar, D., Zuk, R. and Glinka, Y. (1995) Dopamine neurotoxicity : inhibiton of mitochondrial respiration. J. Neurochem. 64, 718-723.

Blaustein, M.P. (1988) Calcium transport and buffering in neurons. TINS 11, 438-443.

Czurko, A. and Nishino, H. (1994) Appearance of immunoglobulin G and complement factor C3 in the rat. Neurosci. Lett. 166, 51-54.

Gilbert, R.S. and Herschman, H.R. (1993) Transforming growth factor beta differentially modulates the inducible nitric oxide synthase gene in distinct cell types. Biochem. Biophys. Res.Commun. 195, 380-384.

Grossman, S.P. (1962) Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. Am. J. Physiol. 202, 872-882.

Hamilton, B.F. and Gould, D.H. (1987) Nature and distribution of brain lessions in rats intoxicated with 3-nitropropionic acid : a type of hypoxic (energy deficient) brain damage. Acta Neuropathol. 72, 286-297.

Hashimoto, P.H. and Hama, K. (1967) An electron microscope study on protein uptake into brain regions devoid of the blood-brain barrier. Med. J. Osaka Univ. 18, 331-346.

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

He, F., Zhang, S., Zhang, C., Qian, F., Liu, X. and Lo, X. (1990) Mycotoxin induced encephalopathy and dystonia in children. In : Basic Science in Toxicology (eds. Volans, G.N., Sims, J., Sullivan, F.M. and Turner, P.), pp. 596-604. Taylor and Francis, London.

Hu, W. (1986) The isolation and structure identification of a toxic substance, 3-nitropropionic acid, produced by Arthrinium from mildewed sugar cane. Chin. J. Prev. Med. 20, 321-323.

Kiryu, S., Yas, G.L. and Kiyama, H. (1995) Cloning of rat neuronal glutamate transporter as a nerve regeneration associated molecule by the differential display PCR. Neural Growth, Regen. Transpl. 7, 93-94.

Koizumi, K. and Nishino, H. (1976) Circadian and other rhythmic activity of neurons in the ventromedial nuclei and lateral hypothalamic area. J. Physiol. 263, 331-356.

Ludolph, A.C., He, F., Spencer, P.S., Hammerstad, J. and Sabri, M. (1991) 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. Can. J. Neurol. 18, 492-498.

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991) Nitric oxide : Physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109-142.

Nishino, H., Shimano, Y., Kumazaki, M. and Sakurai, T. (1995) Chronically administered 3-nitropropionic acid induces striatal lesions attributed to dysfunction of the blood-brain barrier. Neurosci. Lett. 186, 161-164.

Nishino, H., Shimano, Y., Kumazaki, M., Sakurai, T., Fukuda, A., Fujimoto, I., Inoue, K. and Hida, H. (1996a) iNOS expression and the specific striatal vulnerability for a mitochondrial toxin, 3-nitropropionic acid. J. Chem. Neuroanat. in press.

Nishino, H., Onizuka, K., Ungsuperkorn, C., Hida, H., Fujimoto, I., Isobe, Y., Furuyama, F. and Fukuda, A. (1996b) Dexamethasone attenuates the extravasation of serum proteins and has beneficial effect in protecting infarction in brain ischemia-reperfusion model rat. Neurosciences, in press.

Oomura, Y. (1973) Central mechanisms of feeding. In : Advances in Biophysics (ed. Kotani, M.), pp. 65-142. Tokyo Univ. Press, Tokyo.

Oomura, Y. (1980) Input-output organization in the hypothalamus relating to food intake behavior. In : Handbook of the Hypothalamus (eds. Morgane, J. and Panksepp, J.) Vol. 2, pp. 557-620. M. Dekker, New York.

Priitz, W.A. (1986) Nitro-tyrosine as promoter of free radical damage in a DNA model system. Free Rad. Res. Comms. 2, 77-83.

Reichlin, S., Baldessarini, R.J. and Martin, J.B. (1978) The Hypothalamus. Raven Press, New York.

Reuter, H. (1991) Sodium-calcium exchange : Ins and outs of Ca^{2+} transport. Nature 349, 567-568.

Shimano, Y., Kumazaki, M., Sakurai, T., Hida, H., Fujimoto, I., Fukuda, A. and Nishino, H. (1995) Chronically administered 3-nitropropionic acid produces selective lesions in the striatum and reduces muscle tonus. Obesity Res. in press.

Simpson, J.R. and Isacson, O. (1993) Mitochondrial impairment reduces the threshold for in vivo NMDA-mediated neuronal death in the striatum. Exp. Neurol. 121, 57-64.

Sluga, E. and Seitelberger, F. (1967) Zur Ultrastruktur der Kapillaren und ihrer Beziehung zu Nervenzellen im Bereich der neurosekretorischen Kerne des Hypothalamus. Z. Zellforsch. 78, 303-312.

Takuma, K., Matsuda, T., Asano, S. and Baba, A. (1995) Intracellular ascorbic acid inhibits the Na^{+}-Ca^{2+} exchanger in cultured rat astrocytes. J. Neurochem. 64, 1536-1540.

Wauquier, A. and Rolls, E.T. (1976) Brain-Stimulation Reward. North-Holland Publishing Co., Amsterdam.

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CENTRAL ENHANCEMENT OF TASTE PLEASURE BY INTRAVENTRICULAR MORPHINE

Peciña, S. and Berridge K. C.

Department of Psychology, University of Michigan,
525 E. University, Ann Arbor, MI 48109-1109, USA

Do centrally-administered opioid agonists stimulate feeding by enhancing the palatability of foods? This hypothesis has been supported by several lines of evidence, including previous 'taste reactivity' studies of the influence of systemic morphine on affective (hedonic and aversive) behavioral reactions to taste palatability. The present study examined whether opioid agonists enhance palatability by acting centrally on brain palatability systems. Here we report the effect of intraventricular microinjections of morphine (0, 12, 25, 50 nmols) on hedonic taste reactions to a 0.12 M sucrose solution. The effect on feeding was also assessed in order to determine whether feeding and palatability enhancement are linked, as would be required by the hypothesis that feeding is due to enhanced palatability. Both hedonic taste reactivity patterns and feeding were significantly increased together by morphine administration into the lateral ventricle. We conclude that opioid-induced enhancement of the hedonic palatability of food is a centrally mediated effect. Enhancement of food palatability may be an important psychological route by which intracranial administration of opioid agonists induce feeding.

Key Words: Feeding; Food Intake; Palatability; Taste reactivity; Lateral ventricle; Opioid; Morphine

INTRODUCTION

Opioid neural systems have long been implicated in the mediation of feeding behavior. For example, ingestion of palatable food naturally activates endogenous opioid brain systems [Blass et al., 1993; Blass & Hoffmeyer, 1991; Gosnell, 1987; Lieblich et al., 1983]. Further, both systemic and intracranial administration of opioid agonists stimulate feeding in animals [for reviews see Cooper et al., 1988; Gosnell, 1987; Levine et al., 1985; Morley et al., 1983; Reid, 1985]. Conversely, opioid antagonists suppress intake of palatable foods [Apfelbaum & Mandenoff, 1981; Calcagnetti et al., 1990; Cooper, et al., 1988; Gosnell, 1987; Gosnell & Majchrzak, 1990; Levine et al., 1982; Morley, et al., 1983; Reid, 1985; Rockwood & Reid, 1982; Rowland et

Correspondence should be addressed to: Susana Peciña or Kent Berridge
University of Michigan,
Department of Psychology,
Ann Arbor, MI 48109-1109, USA
E-mail: pesu@umich.edu or berridge@umich.edu

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al., 1980b; Stapleton et al., 1979]. Thus the degree of activation of brain opioid systems appears correlated with behavioral feeding under a variety of circumstances.

What is the *psychological process* by which opioid agonists stimulate feeding? Opioid agonists might increase food intake by increasing the perceived palatability of food [Cooper & Gilbert, 1984; Cooper & Kirkham, 1993; Cooper & Turkish, 1981; Evans & Vaccarino, 1990; Gosnell & Majchrzak, 1990; Oomura et al., 1986; Wise & Raptis, 1986], by inhibiting caloric satiety [Kirkham & Cooper, 1988], by reducing neophobia [Gosnell, 1987], or by acting on other processes involved in feeding. These hypotheses are not mutually exclusive, and it is likely that opioids enhance feeding by activating more than one psychological process.

In particular, the hypothesis that opioid-induced feeding is mediated at least in part by enhanced taste pleasure is supported by findings in animal studies that opioid agonists selectively increase the intake of palatable food or saccharin over that of ordinary food or water [Evans & Vaccarino, 1990; Milano et al., 1989; Shor-Posner et al., 1986], and by findings that opioid antagonists attenuate the preference for palatable foods or liquids [Apfelbaum & Mandenoff, 1981; Calcagnetti, et al., 1990; Gosnell & Majchrzak, 1990; Levine, et al., 1982; Rockwood & Reid, 1982; Rowland et al., 1980a; Stapleton, et al., 1979]. In this vein, studies of subjective reports of palatability in humans have produced suppression of subjective food pleasantness in some studies [Drewnowski et al., 1992; Fantino et al., 1986], but not in others [Hetherington et al., 1991]. Such discrepancies in findings from studies of human reports are difficult to interpret. They might result in part because human subjective reports of affect may sometimes be distorted by the cognitive processes of interpretation required for their generation [Nisbett & Wilson, 1978]. Under at least some conditions, subjective reports concerning taste pleasantness may fail to accurately reflect basic underlying affective events [Berridge, in press; Mook & Votaw, 1992; Wilson & Schooler, 1991]. For this reason, it is helpful to 'double-check' putative changes in taste palatability induced by opioid agents using a measure, such as affective reactions, that can be applied to animals.

Affective reactions elicited by taste palatability can be measured in animals using the taste-reactivity paradigm developed by Grill and Norgren [Grill & Norgren, 1978b]. The taste reactivity test measures species-typical hedonic and aversive reactions. Hedonic reactions, such as tongue protrusions, paw-licks, etc., are emitted by rats in response to preferred sweet tastes. Aversive reactions, such as gapes, head-shakes, etc., are emitted to bitter tastes. Many manipulations that alter feeding, such as caloric hunger or satiety, sodium hunger, benzodiazepine administration, conditioned taste aversions, brain lesion-induced aphagia, etc., change rat hedonic and aversive reaction patterns in the appropriate direction [Berridge, 1991; Berridge et al., 1984; Berridge & Peciña, 1995; Cabanac & Lafrance, 1990; Grill & Norgren, 1978a; Pelchat et al., 1983; Pfaffmann et

al., 1977]. These manipulations appear to control feeding in part by altering the affective perception of food palatability. A few manipulations change feeding without altering affective reaction patterns, such as electrical stimulation of the lateral hypothalamus and mesotelencephalic dopamine depletion [Berridge & Valenstein, 1991; Berridge et al., 1989]. These manipulations appear to control feeding by acting on a process that is separate from basic affective perceptions of palatability [Berridge, in press]. Thus, the taste reactivity paradigm can be used to discover whether a particular brain system mediates food pleasure and aversion, versus whether it controls feeding by some alternative psychological process [Berridge & Peciña, 1995; Berridge, in press; Grill & Berridge, 1985].

Using the taste reactivity measure of affective reactions, Parker and colleagues found that systemic morphine suppressed aversive reactions to a bitter quinine taste [Parker et al., 1992], and suggested that opioid activation reduced the aversiveness of the bitter taste. To further determine whether opioid activation actually enhances *hedonic* taste palatability, Doyle et al. examined the effect of systemic morphine (2 mg/kg), a dose that stimulates feeding, upon affective reactions to a sucrose-quinine solution directly infused into the mouth [Doyle et al., 1993]. Doyle et al. [1993] found that morphine selectively enhanced positive hedonic reactions to the bittersweet taste, without enhancing aversive reactions, at a time when the feeding increase would be maximal. The authors concluded from their results that opioid-induced feeding is at least partly mediated by an increase in the hedonic palatability of food.

Do opioid agonists enhance hedonic reactions by acting directly on brain systems that process food palatability? All taste-reactivity studies of opioid agents so far have used only systemic administration. By contrast, elicitation of feeding has been shown to result from central, as well as systemic, administration of opioid agonists. In order to determine whether palatability enhancement is produced by central administration, we investigated whether intraventricular opioid microinjections into the forebrain lateral ventricle, which have been shown to elicit feeding [Gosnell, 1987; Imura et al., 1986; Morley, et al., 1983; Robert et al., 1989], also are sufficient to enhance hedonic reactions to a sweet taste.

METHODS

Subjects

Seven Sprague-Dawley male rats (from the breeding colony in our laboratory) weighing 300 to 350 grams at the beginning of the experiment were housed in pairs on a 14-10 LD cycle and tested in individual cages. Rats had free access to food and water. All surgery and testing was performed during the light phase of the cycle. Experiments were conducted between 9:00 a.m. and 5:00 p.m.

Cannulae Implantation

Rats were pretreated with Atropine (1 mg/kg) and anesthetized with Ketamine (100 mg/kg) and Rompun (5 mg/kg). Each animal was surgically implanted with a unilateral intracranial cannula (22 gauge) aimed at either the right or the left lateral ventricle. With Bregma and Lambda in the same horizontal plane, skull holes were drilled and the dura was opened. A 22-gauge stainless steel guide cannula was placed using the stereotaxic coordinates from the atlas of Paxinos and Watson [Paxinos & Watson, 1982]. The coordinates used were A-P= -0.8 mm from bregma, L= $\pm$ 1.5 mm, and V=3.5 mm from skull surface. Following surgery, a dummy cannula was placed in the guide cannula to prevent occlusion. Rats were allowed to recover for at least one week before testing.

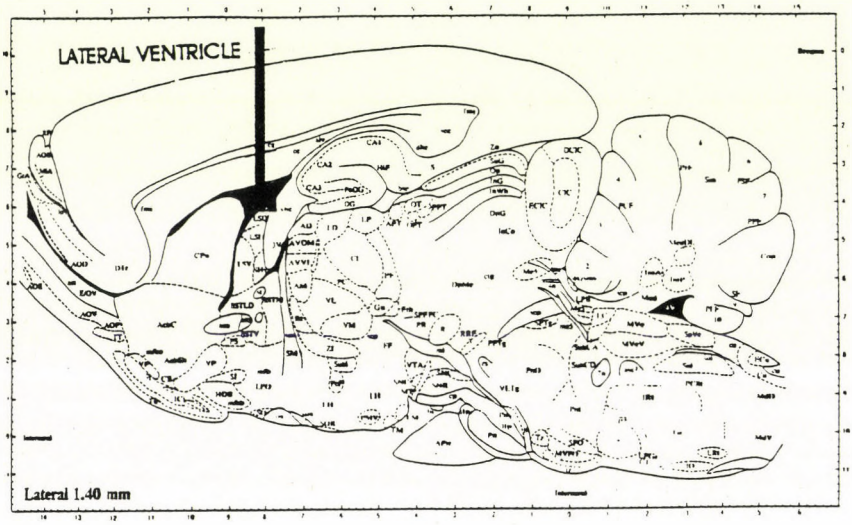

Fig. 1: Lateral ventricle targets. Adapted from Paxinos and Watson [Paxinos and Watson, 1982]

Each rat was also implanted with two bilateral chronic oral cannulae. The cannulae (heat-flared PE 100 tubing) entered the mouth just lateral to the first maxillary molar, ascended lateral to the skull, and exited the head at the dorsal part of the skull, where they were attached to a 19 gauge steel tubing. These cannulae do not interfere with the normal eating behavior of the animal, and they allow the direct infusion of solutions into the mouth. All cannulae were anchored with skull screws and acrylic cement.

Morphine doses and stimuli

Morphine sulfate (Sigma) was dissolved in bacteriostatic physiological saline. The order of drug administration (0, 12, 25, 50 nmols) was randomized across animals except for the first and the last injections which were always vehicle alone (0 nmols morphine). Morphine doses were administrated in a volume of 1.0 µl, and were chosen from the existing literature on morphine-induced stimulation of feeding and from pilot studies performed in our laboratory. The taste stimulus used for oral infusions was a 0.12 M sucrose solution, given in a 1 ml volume. The palatable food used for the feeding test was a cereal mash (1g commercial baby cereal/3 ml of water) given ad lib throughout the intake test.

Habituation

During the 10-day recovery period after surgery, rats were supplied with cereal mash in their home cages to reduce neophobia. In addition, during the last five days of this period, rats were mock-injected each day and exposed to the exact sequence of events that would later be exposed to during the real experiment. The purpose of this initial habituation phase was to familiarize animals with the experimental paradigm and ensure that stable baseline levels for both feeding and taste reactivity would be attained by the time microinjections began.

Feeding Tests

Testing began 10 days after surgery and continue every other day for 10 days. Rats were taken in individual cages to the testing room and food pellets were removed from the top of the cages. Rats were intracranially injected with either morphine sulfate or the saline vehicle, and left undisturbed for three hours. On each test day, a rat received either morphine or vehicle in a counterbalanced order. Since previous reports on opioid-induced feeding suggested that stimulation of feeding is greatest around two to three hours after the drug injection [Stanley, Lanthier, and Leibowitz, 1989; Woods and Leibowitz, 1985], both taste reactivity and feeding tests were administrated in that order beginning three hours after the microinjection.

Taste reactivity test

Three hours after administration of the drug, one of the rat's oral cannulae was connected to a stimulus delivery tube, consisting of PE 50 tubing attached to PE 10 fine tubing. The rat was then placed in a cylindrical test chamber. After a ten minute habituation period, a 1 ml volume of the 0.12 M sucrose solution was infused into the mouth of the animal at a constant rate (1 ml/min) during one minute. The behavior of the rat was videotaped during testing via a mirror mounted beneath the transparent floor, which allowed the camera to zoom up so that the face and the mouth of the rat filled the entire screen. The entire taste reactivity procedure took approximately 12 minutes.

Videoanalysis of taste reactivity records

The videotaped behavior of each rat was scored (frame-by-frame to 1/10 speed) for the occurrence of hedonic, aversive, and neutral reactivity components (see, [Berridge and Peciña, 1995; Grill and Berridge, 1985] for a discussion of taste reactivity analysis and taste reactivity components and classification). Hedonic actions were *paw licking; lateral tongue protrusions*, non-rhythmic protrusion past the lip followed by forward extension, lasting about 160 ms; and tongue protrusions, *rhythmic tongue protrusion* along the midline, with a cycle length of roughly 160 ms. Neutral components were *rhythmic mouth movements* at the same or lower frequency as rhythmic tongue protrusion; and *passive dripping,* the passive leaking of fluid from the mouth. Aversive actions were *gapes*, large openings of the mandible and retraction of the lower lips lasting about 125 ms; *chin rubbing*, bringing the mouth in direct contact with the floor and projecting the body forward; *face washing,* either a single wipe over the face with the paws or a bout of several wipes: *forelimb flails*, shaking of the forelimb with a frequency of greater than 60 HZ; head shaking, at greater than 60 HZ; and *paw treading*, planting of the limbs on the floor and alternating forceful strikes forward and back; and rapid locomotion around the chamber.

Videotapes were scored by an observer who was blind to microinjection condition in a slow motion analysis at 1/30 to 1/10 normal speed. For the purpose of quantifying the number of responses emitted, discrete actions such as lateral tongue protrusions, gapes, chin rubs, forelimb flails and head shaking, and bouts of face washing, paw treading, and locomotion were counted each time they occurred. Continuous actions that typically persist for relatively long periods were counted as follows: paw licks, mouth movements, passive dripping, face washing and locomotion were counted in 5 sec. bins (any occurrence of these behaviors up to 5 sec. in duration was counted as a single occurrence). Rhythmic tongue protrusions were scored in the same way in 2 sec. bins.

Verification of the cannulae placement

At the completion of the experiment, rats were deeply anesthetized with sodium pentobarbital (5 mg/kg). India ink was microinjected into the lateral ventricle, using the same procedure as for morphine. Th rats were perfused intracardially ten minutes later. The brains were removed and cut midsagitally. Th presence of ink in the lateral ventricle verified the correct location of the ventricular cannulae.

Statistical Analysis

The results were evaluated for statistical significance by a one-way ANOVA for repeated measure with dose of morphine sulfate as the main factor, and by post hoc Newman-Keuls tests for paired comparisons.

RESULTS

Feeding

Microinjections of morphine sulfate administration into the lateral ventricle significantly stimulate feeding, $F(3, 24)= 3.02$, $p<0.05$ (Fig.2). Individual post-hoc comparisons of the average amount of baby cere ingested showed that morphine sulfate significantly stimulated feeding at all the doses tested: 12 nmols ($p<0.0$ Newman-Keuls), 25 nmols ($p<0.01$) and 50 nmols ($p<0.05$). The intermediate dose used, 25 nmols, proved t be the most effective one for increasing intake. Under the effects of this dose, animals ingested nearly twice much cereal mash as they did after the vehicle alone.

Taste Reactivity

Microinjections of morphine into the lateral ventricle significantly enhanced the number of hedon responses elicited by the 0.12 M sucrose solution, $F(3,18)=3.24$, $p<0.05$; (Fig.3). Although morphine appeare to increase the number of hedonic reactions at all the doses tested, a Newman-Keuls comparison indicated th only the intermediate dose, 25 nmols, significantly increased the number of hedonic reactions over baseli levels.

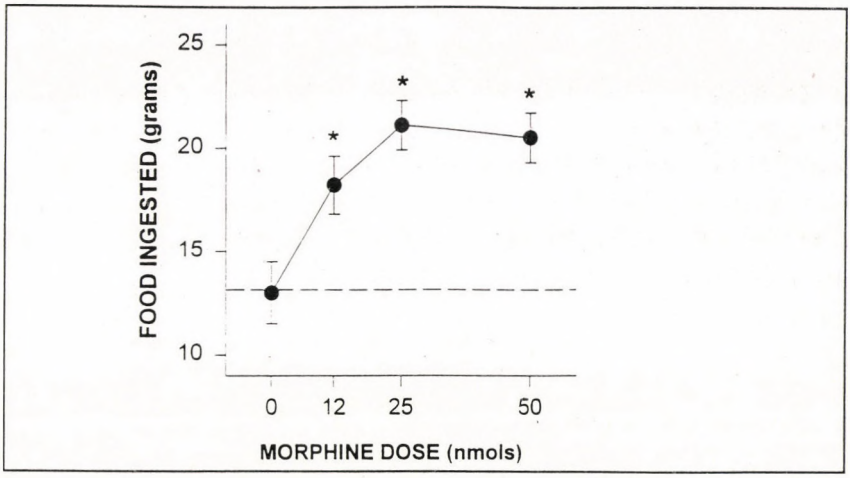

Fig. 2: Lateral ventricle microinjections of morphine enhance feeding. Consumption in a 1-h voluntary intake test of cereal mash after intraventricular morphine (ANOVA, $p < 0.05$)

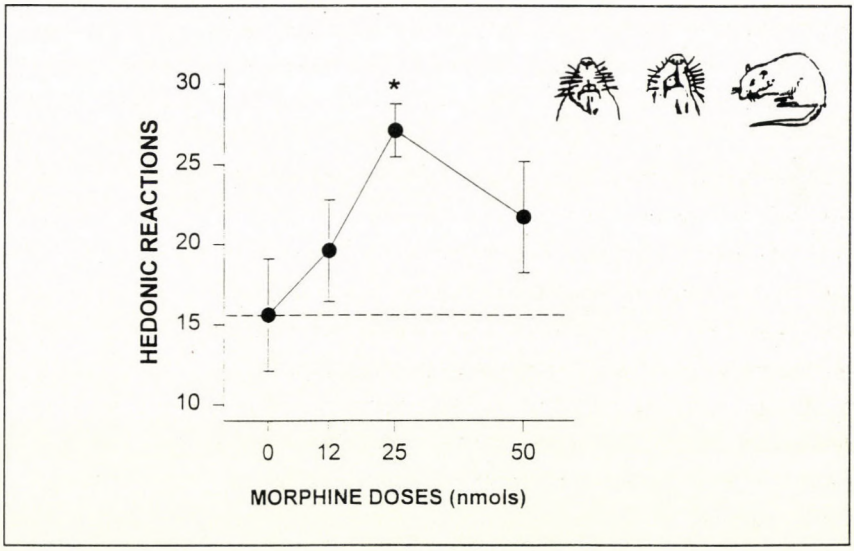

Fig. 3: Lateral ventricle microinjections of morphine enhance hedonic taste reactions. Hedonic taste reactions elicited by a 0.12 M sucrose solution after intraventricular morphine (ANOVA, $p < 0.05$)

DISCUSSION

Microinjections of morphine into the lateral ventricle stimulated intake of a cereal mash and simultaneously enhanced hedonic affective reactions emitted in response to a 0.12 M sucrose solution. These results support the hypothesis that opioid agonists stimulate feeding behavior by enhancing the hedonic perception of food palatability. These results provide the first direct evidence that opioid enhancement of hedonic taste reactivity is a central effect of the drug on brain systems. Morphine appears to activate brain systems that process hedonic taste palatability. The relative contribution of particular opioid receptor subtypes to palatability enhancement remains to be determined.

Of the doses tested (12, 25 and 50 nmols), the most effective dose for feeding enhancement was 25 nmols. Rats ingested almost twice the amount of cereal mash after this dose as they did after vehicle control microinjections. The same 25 nmol dose also produced the strongest enhancement of taste pleasure, as assessed by hedonic reaction patterns. Hedonic reactions to 0.12 M sucrose were nearly doubled by this dose compared to vehicle control levels. The similar magnitude of the effects on intake and on hedonic taste reactivity potentiation suggests that palatability enhancement may mediate the stimulation of feeding.

The anatomical site responsible for opioid-induced enhancement of taste pleasure is an important to question that remains to be answered by future studies. Many different brain sites have been shown to be able to support opioid-induced feeding. For example, food intake is increased by opioid microinjections into the ventral tegmentum, the paraventricular and ventromedial nuclei of the hypothalamus, the nucleus accumbens, and the amygdala [Badiani et al., 1995; Bakshi & Kelley, 1993a; Bakshi & Kelley, 1993b; Evans & Vaccarino, 1990; Gosnell, 1988; Hamilton & Bozartz, 1988; Jenck et al., 1986; Leibowitz & Hor, 1982; Majeed et al., 1986; McLean & Hoebel, 1983; Mucha & Iversen, 1986; Nencini & Stewart, 1990; Noel & Wise, 1993; Stanley et al., 1989]. Whether all of these structures are responsible for palatability enhancement, or only some of them, remains to be seen. Although it is possible that all sites which support feeding would also enhance hedonic palatability, it is equally possible that some sites stimulate feeding via other functions. For example, opioid agents have been reported to modulate hypothalamic neuronal responsiveness to glucose [Aou et al., 1984; Karadi et al., 1992; Nishino et al., 1987; Ono et al., 1982; Ono et al., 1985; Oomura et al., 1974]. Such an effect might or might not alter hedonic reactions to taste, since at least some brain manipulations that stimulate feeding increase intake without enhancing palatability [Berridge, in press; Berridge & Valenstein, 1991].

Although anatomical sites that support opioid-induced feeding provide the most likely candidates for the opioid substrates of palatability, it is also possible that opioid modulation of palatability might occur earlier in the processing of the taste signal itself. Opioid systems do exist in the primary gustatory pathway: for example in the nucleus of the solitary tract (first relay nucleus) and in the pontine parabrachial nucleus (second relay nucleus) [Carr et al., 1991; Herman & Novin, 1980; Mansour et al.,1987]. Benzodiazepine-induced hedonic

enhancement, which acts via $GABA_A$ neurotransmitter systems, has already been shown to act via brainstem systems [Berridge, 1988; Peciña & Berridge, 1992; Peciña & Berridge, submitted]. Although this may not be directly relevant to opioid-induced hedonic enhancement, it at least lends plausibility to the hypothesis that brainstem substrates are capable of mediating changes in palatability induced by pharmacological agents.

Future studies, which combine cannulae mapping with taste reactivity tests, will be needed in order to discover which among these various candidate brain structures contains the site(s) responsible for the central enhancement of hedonic taste palatability by opioid agonists. In any case, we can conclude from the present results that opioid agonists may stimulate feeding by enhancing the perceived palatability of food, and that palatability enhancement results directly from the action of opioid agonists on central brain systems.

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REFERENCES

Aou, S., Oomura, Y., Lenard, L., Inokuchi, A. and Minami, T. (1984) Behavioral significance of monkey hypothalamic glucose-sensitive neurons. Brain Res. 302, 69-74.

Apfelbaum, M. and Mandenoff, A. (1981) Naltrexone suppresses hyperphagia induced in the rat by a highly palatable diet. Pharmacol. Biochem. & Behav. 15, 89-91.

Badiani, A., Leone, P., Noel, M. B. and Steward, J. (1995) Ventral tegmental area opioid mechanisms and modulation of ingestive behavior. Brain Res. 670, 264-276.

Bakshi, V. P. and Kelley, A. E. (1993a) Feeding induced by opioid stimulation of the ventral striatum: role of opiate receptor subtypes. J. Pharmacol. Exp. Ther. 265, 1253-1260.

Bakshi, V. P. and Kelley, A. E. (1993b) Striatal regulation of morphine-induced hyperphagia: an anatomical mapping study. Psychopharmacology 111, 207-214.

Berridge, K. C. (1988) Brainstem systems mediate the enhancement of palatability by chlordiazepoxide. Brain Res.447, 262-268.

Berridge, K. C. (1991) Modulation of taste affect by hunger, caloric satiety, and sensory- specific satiety in the rat. Appetite 16, 103-120.

Berridge, K. C. (in press) Neural systems of wanting and liking in food reward: a taste reactivity analysis of process and brain substrates. Neurosci. Biobeh. Rev.

Berridge, K. C., Flynn, F. W., Schulkin, J. and Grill, H. J. (1984) Sodium depletion enhances salt palatability in rats. Behav. Neurosci. 98, 652-660.

Berridge, K. C. and Peciña, S. (1995) Benzodiazepines, appetite, and taste palatability. Neurosci. Biobehav. Rev.19, 121-131.

Berridge, K. C. and Valenstein, E. S. (1991) What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? Behav. Neurosci. 105, 3-14.

Berridge, K. C., Venier, I. L. and Robinson, T. E. (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. Behav. Neurosci. 103, 36-45.

Blass, E. M., Cramer, C. P. and Fanselow, M. S. (1993) The development of morphine-induced antinociception in neonatal rats: a comparison of forepaw, hindpaw, and tail retraction from a thermal stimulus. Pharm. Biochem. Behav.44, 643-649.

Blass, E. M. and Hoffmeyer, L. B. (1991) Sucrose as an analgesic for newborn infants. Pediatrics 87, 215-218.

Cabanac, M. and Lafrance, L. (1990) Postingestive alliesthesia: the rat tells the same story. Physiol. Behav. 47, 539-543.

Calcagnetti, D. J., Calcagnetti, R. L. and Fanselow, M. S. (1990) Centrally administered opioid antagonists, norbinaltorphimine, 16-methyl cyprenorphine and MR2266, suppress intake of a sweet solution. Pharmacol. Biochem. Behav. 35, 69-73.

Carr, K. D. (1991) Effects of parabrachial opioid antagonism on stimulation-induced feeding. Behav. Neurosci.545, 283-286.

Cooper, S. J. and Gilbert, D. B. (1984) Naloxone suppresses fluid consumption in tests of choice between sodium chloride solutions and water in male and female water-deprived rats. Psychopharmacology (Berlin) 84, 362-367.

Cooper, S. J., Jackson, A., Kirkham, T. C. and Turkish, S. (1988) Endorphins, opiates and food intake. In: Endorphins, opiates and behavioral processes (Eds.), pp. 143-186. Willey, Chichester.

Cooper, S. J. and Kirkham, T. C. (1993) Opioid mechanisms in the control of food consumption and taste preferences. In: Opioids, part I and II (Eds.), pp. 239-262. Springer-Verlag, Berlin.

Cooper, S. J. and Turkish, S. (1981) Food and water intake in the non-deprived pigeon after morphine and naloxone administration. Neuropharmacology 20, 877-883.

Doyle, T. G., Berridge, K. C. and Gosnell, B. A. (1993) Morphine enhances hedonic taste palatability in rats. Pharmacol. Biochem. Behav. 46, 745-749.

Drewnowski, A., Krahn, D. D., Demitrack, M. A., Nairn, K. and Gosnell, B. A. (1992) Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. Physiol. Behav. 51, 371-379.

Evans, K. R. and Vaccarino, F. J. (1990) Amphetamine- and morphine-induced feeding: evidence for involvement of reward mechanisms. Neurosci. Biobehav. Rev. 14, 9-22.

Fantino, M., Hosotte, J. and Apfelbaum, M. (1986) An opioid antagonist, naltrexone, reduces preference for sucrose in humans. Am. J. Physiol. 251, R91-96.

Gosnell, B. A. (1987) Central structures involved in opioid-induced feeding. Fed. Proc. 46, 163-167.

Gosnell, B. A. (1988) Involvement of mu opioid receptors in the amygdala in the control of feeding. Neuropharmacology 27, 319-326.

Gosnell, B. A. and Majchrzak, M. J. (1990) Centrally administered opioid peptides stimulate saccharin intake in nondeprived rats. Pharmacol. Biochem. Beh. 33, 805-810.

Grill, H. J. and Berridge, K. C. (1985) Taste reactivity as a measure of the neural control of palatability. In: Progress in Psychobiology and Physiological Psychology (Eds.), pp. 1-61. Academic Press, Orlando.

Grill, H. J. and Norgren, R. (1978a) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurological normal rats. Brain Res. 143, 263-279.

Grill, H. J. and Norgren, R. (1978b) The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats. Brain Res. 143, 281-297.

Hamilton, M. E. and Bozartz, M. A. (1988) Feeding elicited by dinorphin (1-13) microinjections into the ventral tegmental area in rats. Life Sci. 43, 941-946.

Hetherington, M. M., Vervaet, N., Blass, E. and Rolls, B. J. (1991) Failure of naltrexone to affect the pleasantness or intake of food. Pharmacol. Biochem. Behav. 40, 185-190.

Herman, B. H., Leslier, F. and Goldstein, A. (1980) Behavioral effects and in vivo degradation of intraventricularly administered dynorphin-(1-13) and D-Ala^2 dynorphin-(11) in rats . Life Sci. 42, 1705-1712.

Imura, H., Nakao, K., Yanaihara, N., Katsuura, G. and Nakamura, M. (1986) Potent action of leumorphine on consummatory behaviors in rats: comparison with other opioid peptides. Neuroendocrinology 44, 142-148.

Jenck, F., Gratton, A. and Wise, R. A. (1986) Effects of pimozide and naloxone on latency for hypothalamically induced eating. Brain Res. 375, 329-337.

Karadi, Z., Oomura, Y., Nishino, H., Scott, T. R., Lenard, L. and Aou, S. (1992) Responses of lateral hypothalamic glucose-sensitive and glucose-insensitive neurons to chemical stimuli in behaving rhesus monkeys. J. Neurophysiol.67, 389-400.

Kirkham, T. C. and Cooper, S. J. (1988) Attenuation of sham feeding by naloxone is stereospecific: Evidence for opioic mediation of oro-sensory reward. Physiol. Behav. 43, 845-847.

Leibowitz, S. F. and Hor, L. (1982) Endorphinergic and alpha-noradrenergic systems in the paraventricular nucleus: effects on eating behavior. Peptides 3, 421-428.

Levine, A. S., Morley, J. E., Gosnell, B. A., Billington, C. L. and Bartness, T. J. (1985) Opioids and consummatory behavior. Brain Res. Bull. 14, 663-672.

Levine, A. S., Murray, S. S., Kneip, J., Grace, M. and Morley, J. E. (1982) Flavour enhances the antidipsogenic effect of naloxone. Physiol. & Behav. 28, 23-25.

Lieblich, I., Cohen, E., Ganchrow, J. R., Blass, E. M. and Bergmann, F. (1983) Morphine tolerance in genetically selected rats induced by chronically elevated saccharin intake. Science 221, 871-873.

Majeed, N. H., Przewlocka, B., Wedzony, K. and Przewlocka, R. (1986) Stimulation of food intake following opioid microinjection into the nucleus accumbens septi in rats. Peptides 7, 711-716.

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. and Watson, S. J. (1987) Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. J. Neurosci. 7, 2445-2464.

McLean, S. and Hoebel, B. G. (1983) Feeding induced by opiates injected into the paraventricular hypothalamus. Peptides 4, 287-292.

Milano, W. C., Wild, K. D., Hui, Y., Hubbell, C. L. and Reid, L. D. (1989) PCP, THC, ethanol and morphine and consumption of palatable solutions. Pharmacol. Biochem. Behav. 31, 893-897.

Mook, D. G. and Votaw, M. C. (1992) How important is hedonism? Reasons given by college students for ending a meal. Appetite 18, 69-75.

Morley, J. E., Levine, A. S., Yim, G. K. and Lowy, M. T. (1983) Opioid modulation of appetite. Neurosci. Biobehav. Rev. 7, 281-305.

Mucha, R. F. and Iversen, S. D. (1986) Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. Brain Res. 397, 214-224.

Nencini, P. and Stewart, J. (1990) Chronic systemic administration of amphetamine increases food intake to morphine, but not to U50-488H, microinjected into the ventral tegmental area in rats. Brain Res. 527, 254-258.

Nisbett, R. W. and Wilson, T. D. (1978) Telling more than we can know: verbal reports on mental processes. Psychological Rev. 84, 231-259.

Nishino, H., Oomura, Y., Plata-Salaman, C. R. and Morimoto, M. (1987) Catecholaminergic mechanisms of feeding-related lateral hypothalamic activity in the monkey. Brain Res. 405, 56-67.

Noel, M. B. and Wise, R. A. (1993) Ventral tegmental injections of morphine but not U-50,488H enhance feeding in food-deprived rats. Brain Res. 632, 68-73.

Ono, T., Nishino, H., Fukuda, M., Sasaki, K., Muramoto, K. and Oomura, Y. (1982) Glucoresponsive neurons in rat ventromedial hypothalamic tissue slices in vitro. Brain Res. 232, 494-499.

Ono, T., Sasaki, K., Nakamura, K. and Norgren, R. (1985) Integrated lateral hypothalamic neural responses to natural and artificial rewards and cue signals in the rat. Brain Res. 327, 303-306.

Oomura, Y., Nishino, H., Aou, S. and Lenard, L. (1986) Opiate mechanisms in reward-related responses during operant feeding behavior of the monkey. Brain Res. 365, 335-339.

Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T. and Yamada, Y. (1974) Glucose inhibition of the glucose-sensitive neuron in the rat lateral hypothalamus. Nature 247, 284-286.

Parker, L. A., Maier, S., Rennie, M. and Crebolder, J. (1992) Morphine- and naltrexone-induced modification of palatability: analysis by the taste reactivity test. Behav. Neurosci. 106, 999-1010.

Paxinos, G. and Watson, C. (1982) The rat brain in stereotaxic coordinates. Academic Press, New York.

Peciña, S. and Berridge, K. C. (1992) Fourth ventricle microinjections of diazepam enhance hedonic reactions to taste. Soc. Neurosci. Abstr. 18, 1231.

Peciña, S. and Berridge, K. C. (submitted) Brainstem mediates benzodiazepine enhancement of taste palatability: comparison of diazepam microinjections into fourth versus lateral ventricles. Brain Res.

Pelchat, M. L., Grill, H. J., Rozin, P. and Jacobs, J. (1983) Quality of acquired responses to tastes by Rattus Norvegicus depends on type of associated discomfort. J. Comp. Psycho. 97, 140-153.

Pfaffmann, C., Norgren, R. and Grill, H. J. (1977) Sensory affect and motivation. Ann. N. Y. Acad. Sci. 290, 18-34.

Reid, L. D. (1985) Endogenous opioid peptides and regulation of drinking and feeding. Am. J. Cline. Nut. 42, 1099-1132.

Robert, J., Orosco, M., Rouch, C., Jacquot, C. and Cohen, Y. (1989) Effects of opiate agonists and antagonists on food intake and brain neurotransmitters in normophagic and obese "cafeteria" rats. Pharmacol. Biochem. Behav.34, 577-583.

Rockwood, G. A. and Reid, L. D. (1982) Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. Physiol. Behav. 29, 1175-1178.

Rowland, D. L., Perrings, T. S. and Thommes, J. A. (1980a) Comparison of androgenic effects on food intake and body weight in adult rats. Physiol. Behav. 24, 205-209.

Rowland, N., Marques, D. M. and Fisher, A. E. (1980b) Comparison of the effects of brain dopamine-depleting lesions upon oral behaviors elicited by tail pinch and electrical brain stimulation. Physiol. Behav. 24, 273-281.

Shor-Posner, G., Azar, A. P., Filart, R., Tempel, D. and Leibowitz, S. F. (1986) Morphine-stimulated feeding: Analysis of macronutrient selection and PVN lesions. Pharmacol. Biochem. & Behav. 24, 931-936.

Stanley, B. G., Lanthier, D. and Leibowitz, S. F. (1989) Multiple brain sites sensitive to feeding stimulation by opioid agonists: a cannula mapping study. Pharmacol. Biochem. Behav. 31, 825-832.

Stapleton, J. M., Lind, M. D., Merriman, V. J. and Reid, L. D. (1979) Naloxone inhibits diazepam-induced feeding in rats. Life Sci. 24, 2421-2425.

Wilson, T. D. and Schooler, J. W. (1991) Thinking too much: introspection can reduce the quality of preferences and decisions. J. Pers. Soc. Psychol. 60, 181-192.

Wise, R. A. and Raptis, L. (1986) Effects of naloxone and pimozide on initiation and maintenance measures of free feeding. Brain Res. 368, 62-68.

Woods, J. S. and Leibowitz, S. F. (1985) Hypothalamic sites sensitive to morphine and naloxone: effects on feeding behavior. Pharmacol. Biochem. Behav. 23, 431-438.

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BRAIN MECHANISMS OF SATIETY AND TASTE IN MACAQUES

Scott, T. R.[1], Yan, J.[1] and Rolls, E. T.[2]

[1]Department of Psychology and Program in Neuroscience, University of Delaware, Newark, Delaware, 19716, USA

[2]Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, England

SUMMARY

Flavor is the primary reinforcer of eating. As satiety is induced, the reinforcement of flavor is lost. Since flavor derives largely from taste, one might expect gustatory responsiveness to decline with increasing satiety. However, no such loss of sensitivity occurs in humans, even as the reinforcing value of taste declines with satiety. Thus, we explored the effect of satiety on taste responses at several levels of the macaque's nervous system to determine where its influence began. Taste-evoked activity in the NTS and primary taste cortex was unaffected by the induction of satiety through oral administration of glucose. Taste cortex projects to amygdala and orbitofrontal cortex (OFC). In amygdala, satiety reduced responsiveness by 58%; in OFC, neurons were fully suppressed. Both amygdala and OFC project to the hypothalamus, where taste responsiveness was also suppressed. Thus, the neural impact of food is reduced not in areas devoted to quality analysis, but in those concerned with motivation and reinforcement.

Key Words: taste, satiety, monkey, cortex, amygdala, hypothalamus

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INTRODUCTION

Flavor provides the primary inducement to eat. The rewarding value of flavor increases with food deprivation, and is suppressed by satiety. Where does this occur in the primate brain?

The suggestion from rodents is that satiety has its effects in the hindbrain. Chemicals that increase or decrease food intake are most effective when administered into the fourth ventricle, there to work on cells in caudal hindbrain. Moreover, factors that raise or lower the reward value of taste have been shown to influence activity in the nucleus tractus solitarius (NTS). Specifically, gastric distention (Glenn and Erickson, 1976), hyperglycemia (Giza and Scott, 1983), moderate hyperinsulinemia (Giza and Scott, 1987b), and elevated levels of pancreatic glucagon (Giza et al., 1993) all result in a reduced sensitivity to glucose in the NTS. In each case except the first, the manipulation resulted in an increase in the delivery of utilizable calories to the brain, a factor by which the taste system is presumably influenced. Since the NTS is an obligatory synapse for taste, a reduction in its sensitivity should lead to a lower perceived intensity for glucose. This is the case. Rats made hyperglycemic through an intravenous injection of 0.5g/kg glucose reacted to the taste of 1.0M glucose as if it were 0.58M, a loss of sensitivity that matches the reduction in neural response to glucose in the NTS (Giza and Scott, 1987a).

The situation is different in humans. The perceived intensity of glucose does not decrease significantly with satiety, even as its reinforcing value is reduced. Humans asked to evaluate the intensity of glucose solutions before and after a satiating glucose load report similar values (Rolls et al., 1981; Thompson et al., 1976). However, they experience less reward from the glucose following satiety and are not as motivated to consume it (Cabanac, 1971). To evaluate the relationship between sensitivity and reward value, we have recorded taste-induced single neuron activity from a progression of gustatory relays in the alert macaque before, during, and after the oral administration of a satiating glucose load.

MATERIALS AND METHODS

Subjects and Recording

Male cynomolgus monkeys weighing 4-6 kg were used for all recordings. Single neuron activity was isolated using glass-coated tungsten microelectrodes (Merrill and

Ainsworth, 1972) while the monkey sat in a primate chair with its head restrained to provide recording stability. Each animal was approximately 18-hr food deprived at the beginning of a recording session.

Stimuli and Delivery

Taste stimuli included fruit juice and representatives of the four basic taste qualities: 1.0M glucose, 0.3M sucrose or .01M aspartame (sweet), 0.3 or 1.0M NaCl (salt), 0.01 or 0.03M HCl (sour) and 0.001 or 0.01M quinine HCl (bitter). They were delivered through a syringe in quantities of about 0.5ml.

Requirements for Conducting a Satiety Experiment

In order to initiate a satiety experiment, three conditions had to be satisfied. (1) The neural response evoked by the stimulus on which the monkey would subsequently be satiated had to be robust; (2) isolation of the neuron's activity had to be sufficiently secure to give confidence that it would be maintained through the 60-min period during which satiety was induced; (3) the monkey had to demonstrate a strong motivation for the satiating chemical. An experiment was not initiated unless the monkey's behavior warranted a rating of at least +1.0 on a scale of +2.0 (avid acceptance) to -2.0 (active rejection) as evaluated by at least two experimenters. In practice this required an efficient search for a neuron in the relay under study and the achievement of a stable recording with a minimum of stimulus presentations so that the monkey was still hungry when the experiment started.

Protocol

If the criteria for conducting a satiety experiment were satisfied, the following protocol was invoked. (1) The gustatory neural response to each of the five sapid stimuli plus water was determined by application of 0.5ml of each solution. Each application was followed by a 1.0ml water rinse, and a minimum of 30 sec of rest. The stimulus series was then repeated. The total testing time was approximately 12 min., and the volume consumed was a maximum of 16ml. (2) The monkey's acceptance-rejection score for the satiating solution was determined by observing his response as 0.5ml was applied to the tongue. (3) The monkey was fed a 50ml aliquot of the satiating solution. This was generally fruit juice, sucrose, or glucose. All satiating solutions were delivered orally by a syringe. The duration of administration was approximately 2 min for the initial aliquot, and as much as 4 min for the last. (4) The monkey's acceptance-rejection score to the satiating solution was reassessed. (5) Steps (1)-(4) were repeated through as many cycles as were required to attain a behavioral score of -0.5 to -2.0. This typically involved five 50ml aliquots over a period of 60 min. Conventional satiety, defined by the stage at which the subject would stop working to obtain food, would normally correspond to a rating of 0.0 to -0.5. Thus the feeding used in these experiments was sufficient to produce complete satiety. After feeding had stopped, multiple

further measurements were taken of the neuronal responses to each of the sapid solutions and to water. Experiments were separated by at least two days so as to permit the effects of repletion to dissipate.

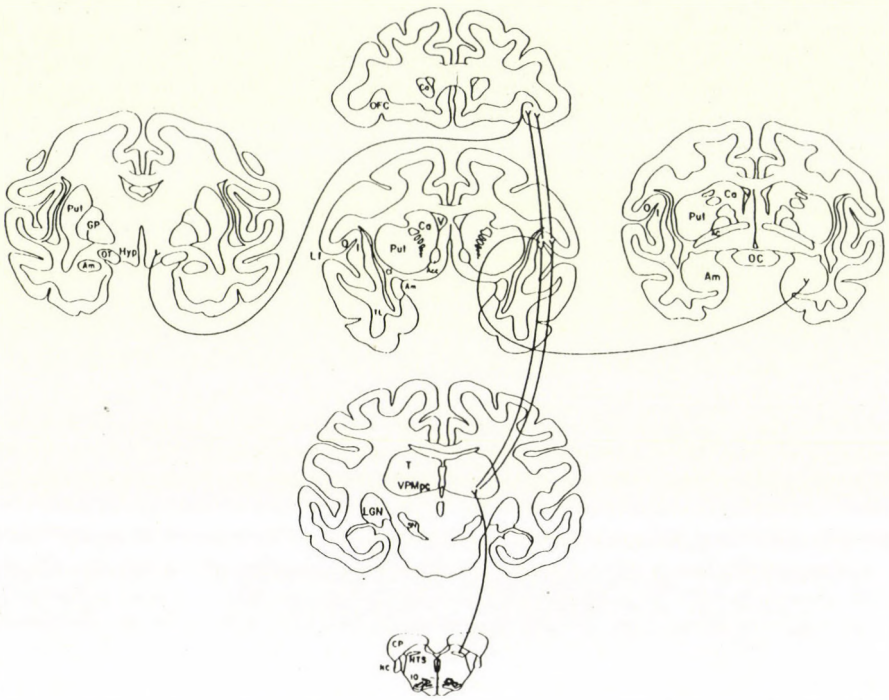

Fig.1: Transverse sections summarizing the afferent limb of central gustatory projections in the cynomolgus monkey. AC, Anterior commissure; Acc, nucleus accumbens; Am, amygdala; Ca, Caudate; Cl, claustrum; CP, cerebellar penduncle; GP, globus pallidus; Hyp, hypothalamus; I, insula; IO, inferior olive; LF, lateral fissure; LGN, lateral geniculate nucleus; NC, cuneate nucleus; NTS, nucleus of the solitary tract; O, Operculum; OC, optic chiasm; OFC, orbitofrontal cortex; OT, optic tract; Put, putamen; SN, substantia nigra; T, thalamus; TL, temporal lobe, V, ventricle; VPMpc, ventroposterior medial necleus of the thalamus, pars parvocellularis. Reprinted from Scott and Yaxley (1989) with permission

Recording sites

In Figure 1 we present a schematic diagram of the macaque's taste system. Peripheral taste nerves (VII, IX, and X) project to the rostral NTS (Beckstead and Norgren, 1979) from which second-order neurons send fibers to the thalamic taste area in the ventroposterior medial nucleus (VPMpc) (Beckstead et al., 1980). Third-order thalamic cells project to the primary taste cortex located in the frontal operculum and anterior insula (Pritchard et al., 1986). From here, fibers are sent both anteriorly to the orbitofrontal cortex (OFC) (Baylis et al., 1994) and ventromedially to the amygdala (Mesulam and Mufson, 1982). Not shown in the figure is the fact that OFC also projects to the amygdala (Potter and Nauta, 1979) and that the amygdala, as well as OFC, communicates reciprocally with the lateral hypothalamic area (LHA). The recordings described below were taken at each of these relays with the exception of the thalamic taste area.

RESULTS

In Figure 2 we show the effects of feeding monkeys to satiety on the responsiveness of NTS neurons in nine experiments (Yaxley et al., 1985). Responses evoked by the satiating chemical and the spontaneous discharge rate of the neuron under consideration are shown in the top part of each panel, while ratings of the behavioral reactions of the monkey are presented below. In no case did satiety abolish, or even seriously compromise the responsiveness of the neuron, even as the behavior shifted from avid acceptance to active rejection. Averaged over the nine experiments, the effect of satiety was to increase the response to the satiating stimulus by 4%. Similarly, responses to all other taste stimuli remained constant in the face of complete satiety. Therefore, there was no appreciable effect of satiety on the responses of these NTS neurons.

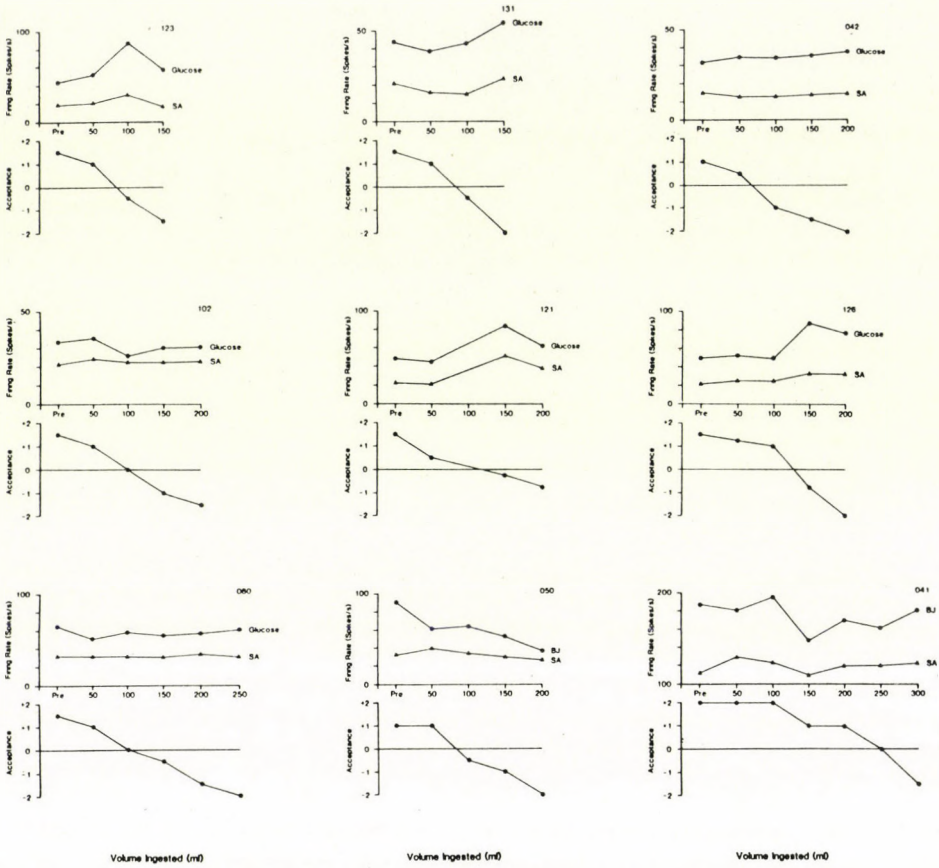

Fig. 2: Effects of feeding to satiety on the neural response (spikes/s) to the solution on which the monkey was satiated for 9 separate experimental runs. The spontaneous firing rate is also indicated (SA). Below the neural response data for each experiment, the behavioral measure of the acceptance or rejection of the solution on a scale from +2 to -2 is shown. The solution used to feed to satiety is indicated. The monkey was fed 50 ml of the solution at each stage of the experiment, until he was satiated as shown by whether he accepted or rejected the solution. BJ. blackcurrant juice. Reprinted from Yaxley et al., 1985

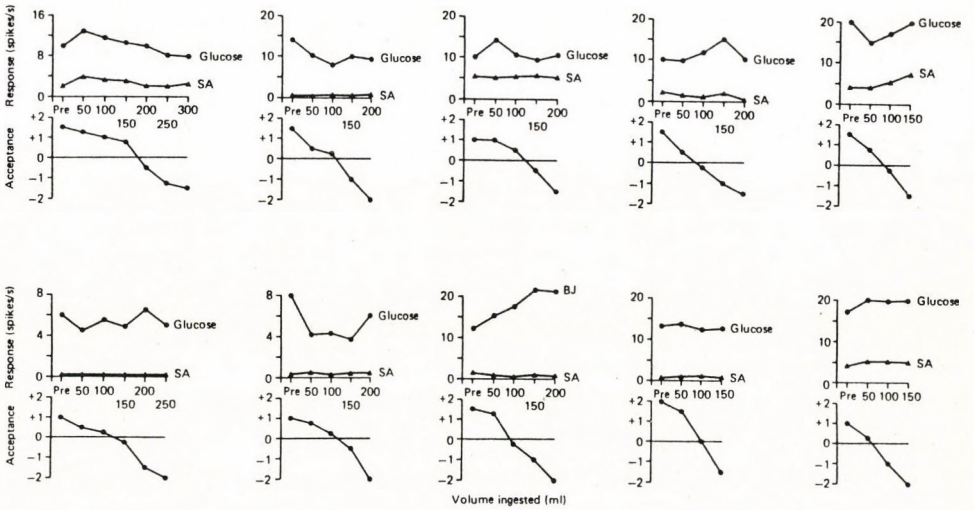

Fig. 3: Effects of feeding to satiety on the neural responses in opercular cortex to the solution on which the monkey was satiated, for ten separate experimental runs. The protocol is as in figure 2. Reprinted from Rolls et al., 1988

In Figure 3 we present corresponding results from neurons in the frontal operculum (Rolls et al., 1988) and anterior insula (Yaxley et al., 1989). As in NTS, these gustatory cells in the primary taste cortex were not significantly influenced by the induction of satiety. It is noteworthy that neural responses in primary taste cortex are most similar to those predicted by human psychophysical data (Smith-Swintosky et al., 1991). Thus, at the gustatory relay most closely associated with the analysis of taste quality, there is no modulation of responsiveness according to level of satiety or to the hedonic reaction to taste.

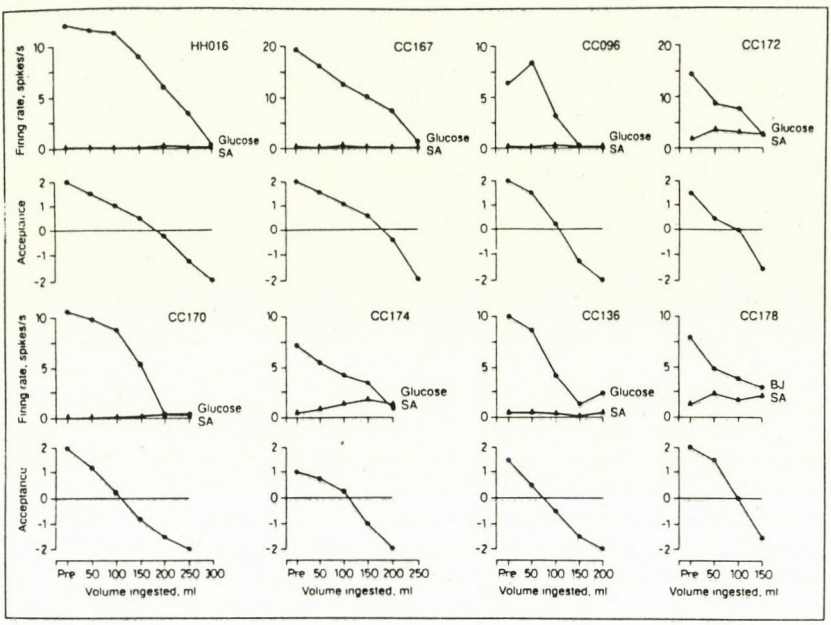

Fig. 4: The same format is used as in Fig. 2 & 3, except that responses are derived from single neurons in the lateral caudal orbitofrontal cortex of the monkey. At this level of processing, discharge rate is related to the level of satiety rather than the purely sensory aspects of the stimulus. SA, Spontaneous activity. Reprinted from Rolls et al. (1989) with permission

From this point on the situation reversed. In Figure 4 the responses of neurons in the orbitofrontal cortex are plotted as a function of satiety. As the monkey progressed from acceptance to rejection, the responsiveness of OFC cells to the satiating stimulus declined from

initially robust levels, to reach spontaneous rate when satiety was complete (Rolls et al., 1989). Thus these neurons are tuned to, and perhaps responsible for, the hedonic reaction to a taste. Responses elicited by other stimuli, even those with similar taste qualities to that of the satiating stimulus, were not significantly affected by satiety. In amygdala, to which primary taste cortex also projects, the effect was intermediate. The responses of some neurons were suppressed 100%, others not at all. The net effect across 14 amygdaloid cells was to decrease the response to the satiating stimulus by 58%, while leaving sensitivity to other chemicals essentially unaltered (Scott and Yan, 1994). Finally, in the lateral hypothalamus, responses were severely and selectively suppressed by satiating monkeys to glucose or to more complex foods such as peanuts and bananas (Fig. 5) (Rolls et al., 1976).

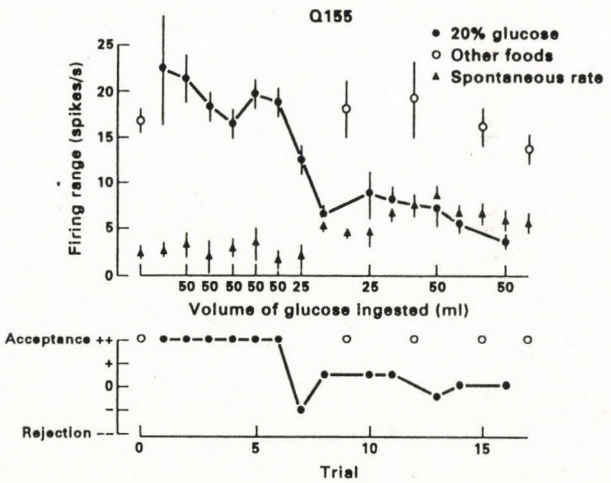

Fig. 5: The effect of feeding a monkey to satiety with 20% glucose solution on the responses of a hypothalamic neuron to the taste of glucose (filled circles) and to the taste of other foods (open circles). The willingness of the monkey to accept the glucose is shown below. Reprinted from Rolls et al., 1976

DISCUSSION

Humans fed to satiety report that the intensity of the satiating food was almost the same as when they were hungry, even though that food now offered them little reward. Data cited above imply that this perceptual consistency derives from the activity of taste neurons in the hindbrain and primary taste cortex, and almost certainly in the thalamus, whose relay is between them. Conversely, responses of neurons in the orbitofrontal cortex, amygdala, and lateral hypothalamus are modulated by level of satiety. It is presumably in these areas that neurons monitor, and perhaps determine, the hedonic value of foods and whether they should be eaten.

This progression — no effect of satiety on cells in primary taste cortex, a partial influence in amygdala, and full suppression in OFC and lateral hypothalamus — parallels that seen in the visual system. The inferotemporal cortex is a site of advanced processing of visual information in primates. Responses here to the sight of the syringe from which the monkey is to be fed are not affected by his level of satiety. Inferotemporal cortex projects to the amygdala, where visual responses to the syringe are partially suppressed by satiety (Sanghera et al., 1979), then to lateral hypothalamus where suppression is complete (Burton et al., 1976). Cells in OFC are also rendered unresponsive to the sight of the syringe by satiety. Thus in both vision and taste, sensory cortical areas are devoted to an analysis of stimulus quality and intensity, and only when that analysis is complete is the information imbued with hedonic value.

In primary taste cortex and inferotemporal cortex, cells respond almost exclusively to their assigned modality: taste and vision, respectively. However, in orbitofrontal cortex, amygdala and lateral hypothalamus, many neurons are bi- or trimodal for taste, vision, and olfaction (Rolls and Baylis, 1994; Scott et al., 1993). In most cases the multiple systems are in register for quality, i.e. if a cell is activated only by a sweet taste, it will also respond only to a sweet odor and to the sight of a syringe containing the sweet stimulus. When satiety is induced, the response is suppressed in each modality. Therefore the neuron is no longer activated by the taste, odor, or sight of a food that had driven its responses when the monkey was hungry. Sensory information processed in isolation is evaluated for its quality and intensity. Flavor, however, involves a combination of several sensory attributes, carried through taste, olfaction, vision, and perhaps somesthesis. It appears that representations of flavor are constructed in OFC, amygdala, and lateral hypothalamus, and that the appraisal of that flavor is subject to modification by the monkey's level of satiety.

References

Baylis, L.L., Rolls, E.T., and Baylis, G.D. (1994). Afferent connections of the orbitofrontal cortex taste area of the primate. *Neuroscience* **64**: 801-812.

Beckstead, R.M., and Norgren, R, (1979). An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey. *Journal of Comparative Neurology*, **184**, 455-472.

Beckstead, R.M., Morse, J.R., and Norgren, R. (1980). The nucleus of the solitary tract in the monkey: Projections to the thalamus and brainstem nuclei. *Journal of Comparative Neurology* **190**, 259-282.

Burton, M., Rolls, E. T., and Mora, F. (1976). Effects of hunger on the response of neurons in the lateral hypothalamus to the sight and taste of food. *Experimental Neurology* **51**, 668-677.

Cabanac, M. (1971). Physiological role of pleasure. *Science* **173**, 1103-1107.

Glenn, J.F., and Erickson, R.P. ((1976). Gastric modulation of gustatory afferent activity. *Physiology and Behavior* **16**, 561-568.

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

Giza, B.K., and Scott, T.R. (1987a). Blood glucose levels affect perceived sweetness intensity in rats. *Physiology and Behavior* **41**, 459-464.

Giza, B.K., and Scott, T.R., (1987b). Intravenous insulin infusions in rats decrease gustatory-evoked responses to sugars. *American Journal of Physiology* **252**, R994-R1002.

Giza, B.K., Scott, T.R., and VanderWeele, D.A. (1992). The effect of satiety factors on taste-evoked activity in the nucleus tractus solitarius of the rat. *Brain Research Bulletin* **28**, 637-639.

Mesulam, M.-M., and Mufson, E.J. (1982). Insula of the Old World monkey. III. Efferent cortical output and comments on function. *Journal of Comparative Neurology* **212**, 38-52.

Potter, H. and Nauta, W.J.H. (1979). A note on the problem of olfactory associations of the orbitofrontal cortex in the monkey. *Neuroscience* 4: 361-367.

Pritchard, T.C., Hamilton, R.B., Morse, J.R., and Norgren, R. (1986). Projections of thalamic gustatory and lingual areas in the monkey. *Macaca fascicularis. Journal of Comparative Neurology* **244**, 213-228.

Rolls, E.T., Burton, M.J. and Mora, F., (1976) Hypothalamic neuronal responses associated with the sight of food. *Brain Research* **111**, 53-66.

Rolls, B.J., Rolls, E.T., Rowe, E.A., and Sweeney, K.I. (1981). Sensory specific satiety in man. *Physiology and Behavior* **27**, 137-142.

Rolls, E.T., Scott, T.R., Sienkiewicz, Z.J., and Yaxley, S. (1988). The responsiveness of neurons in the frontal opercular gustatory cortex of the macaque monkey is independent of hunger. *Journal of Physiology* **397**, 1-12.

Rolls, E.T., Sienkiewicz, Z.J., and Yaxley, S. (1989). Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. *European Journal of Neuroscience* **1**, 53-70.

Rolls, E.T., et al., (1989) Sensory-specific satiety: Food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. *Brain Research* **368**, 79-86.

Rolls, E.T. and Baylis, L.L. (1994) Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. *Journal of Neuroscience* **14**, 5437-5452.

Sanghera, M.K., Rolls, E.T., and Roper-Hall, A., (1979) Visual responses of neurons in the dorsolateral amygdala of the alert monkey. *Experimental Neurology* 63, 610-626.

Scott, T.R., Karadi, Z., Oomura, Y., Nishino, H., Plata-Salaman, C.R., Lenard, L., and Giza, B.K. (1993) Gustatory neural coding in the amygdala of the alert macaque monkey. *Journal of Neurophysiology* **69**: 1810-1820.

Scott, T.R. and Yan, J. (1994). The responsiveness of gustatory neurons in the amygdala of the macaque monkey is independent of hunger. *Neuroscience Abstracts* 20: 1224.

Smith-Swintosky, V.L., Plata-Salaman, C.R., and Scott, T.R. (1991). Gustatory neural coding in the monkey cortex: Stimulus quality. *Journal of Neurophysiology* **66**, 1156-1165.

Thompson, D.R., Moskowitz, H.R., and Campbell, R.G. (1976). Effects of body weight and food intake on pleasantness for a sweet stimulus. *Journal of Applied Physiology* **41**, 77-83.

Yaxley, S., Rolls, E.T., Sienkiewicz, Z.J., and Scott, T.R. (1985). Satiety does not affect gustatory activity in the nucleus of the solitary tract of the alert monkey. *Brain Research* **347**, 85-93.

Yaxley, S., Rolls, E.T., and Sienkiewicz, Z.J. (1989). The responsiveness of neurones in the insular gustatory cortex of the macaque monkey is independent of hunger. *Physiology and Behavior,* **42**, 223-229.

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HETEROGENEOUS DEVELOPMENT OF VOLTAGE DEPENDENCY OF NMDA RECEPTOR-MEDIATED RESPONSE IN CENTRAL NERVOUS SYSTEM

Nabekura, J. and Horimoto, N.

Department of Physiology, Akita University, School of Medicine, Akita, 010, Japan

Summary: Developmental changes of voltage dependency of NMDA responses were investigated on nucleus solitarii (NTS) ventromedial hypothalamic (VMH) and visual cortical neurons acutely dissociated from rats aged between 19 days in gestation (E19) and 21 days after birth (P21) using a whole cell patch clamp recording. Sensitivity of NMDA response to extracellular Mg^{2+} developed by P3 in the NTS, by P6 in the VMH and by P12 in the cortex. Protein kinase C modulators could not modulate the voltage dependency of NMDA response in immature neurons. These findings suggest that a regional heterogeneity of developmental change of the sensitivity of NMDA response to extracellular Mg^{2+} exists. This regional difference in development of NMDA response might be related to the order of vital necessities after birth.

Key Words: NMDA response, voltage dependency, development, protein kinase C, rat

Correspondence should be addressed to: Junichi Nabekura, MD.
Department of Physiology, Akita University School of Medicine,
1-1-1 Hondou Akita, 010, Japan
Phone: 81-188-33-1166 ext. 3230 and Fax: 81-188-36-2604
E-mail: nabekura@ipc.akita-u.ac.jp

INTRODUCTION

N-methyl-D-aspartate (NMDA) receptor participates in neural and neuronal development in a number of brain areas (Bear et al., 1990; Carmignoto and Vicini, 1992). In addition, various characteristics of NMDA response itself alter during development. NMDA-induced current shows a characteristic voltage dependent suppression by extracellular Mg^{2+} (Mayer and Westbrook, 1987). At a resting membrane potential NMDA response is severely blocked by extracellular Mg^{2+}. Depolarization removes the Mg^{2+} block. Thereafter, the receptor agonists can activate the NMDA receptor-channel complex. Sensitivity of NMDA response to extracellular Mg^{2+} has been proved for some neurons (Chen and Huang, 1992, Markram and Segal, 1992) and oocyte injected with rat brain RNA (Urushihara et al., 1992). Therefore, there are two possibilities for the lower effectiveness of extracellular Mg^{2+} on the NMDA response in immature animals. One is due to change of intracellular protein kinase C activity during development. The other is due to expression of subunits comprising NMDA receptor which is less sensitive to Mg^{2+}.

In the present study, developmental change of characteristics of NMDA responses, regarding to its sensitivity to extracellular Mg^{2+}, was investigated on neurons freshly dissociated from the NTS in the brain stem, in the ventromedial hypothalamus and in the visual cortex. Considering the difference in developmental process of the sensitivity of NMDA response to extracellular Mg^{2+} among these regions, possible mechanisms influencing the characteristics of NMDA reponses are discussed.

METHODS

Wistar rats ranging in age from day 19 of gestation to postnatal day 21 were decapitated under ether anesthesia. The brain was removed and dissected into coronal slices (350 mm thick) using a microslicer. The slices containing the NTS, VMH or visual cortical region were incubated in a standard solution equilibrated with 95% O_2 at room temperature (23-25 °C) for 40 minutes. After the preincubation, the slices were treated with enzyme (dispase, 1000 unit/ml) for 60 minutes at 31 °C. Subsequently, the NTS, VMH or visual cortical region was punched out and the neurons were dissociated mechanically.

Electrical recordings were carried out with a nystatin perforated patch recording under a whole-cell configuration (Nabekura, et al., 1993). Both current and membrane potential were measured with a patch-clamp amplifier. The resistance between the recording electrode was 3-5 MΩ. Drugs were applied by 'Y-tube' method. All experiments were performed at room temperature (23-25 °C). We used a standard external solution, unless otherwise stated. Its composition was as follows (concentrations in mM): NaCl 150, KCl 5,

$MgCl_2$ 1, $CaCl_2$ 2, glucose, 10; glycine 1μM and N-2-hydroxyethylpiperadine-N'-2-ethansulfonic acid (HEPES) 10. The pH was adjusted to 7.4 with tris-hydroxymethyl aminomethane (Tris-base). In Mg^{2+}-free external solution, Mg^{2+} was subtracted. Patch pipettes were filled with internal solution containing 150 mM KCl, 10 mM HEPES and 150 μg/ml nystatin. The pH of pipette solution was adjusted to 7.2 with Tris-base.

RESULTS

Characteristic voltage dependency of NMDA response was observed in all NTS neurons examined (n=12) obtained from 2-week old rats in the presence of extracellular 1 mM Mg^{2+} (Fig. 1 A). The NMDA response was severely suppressed at a membrane potential more hyperpolarized than -60mV. However, Mg^{2+} block was less obvious in a number of fetal NTS neurons. The potency of Mg^{2+} block was varied from cell to cell (Fig. 1 B). Some neurons exhibited severe suppression by Mg^{2+}, while NMDA responses in other neurons were less influenced, indicating non-uniform maturation of sensitivity of NMDA response to Mg^{2+} among NTS neurons.

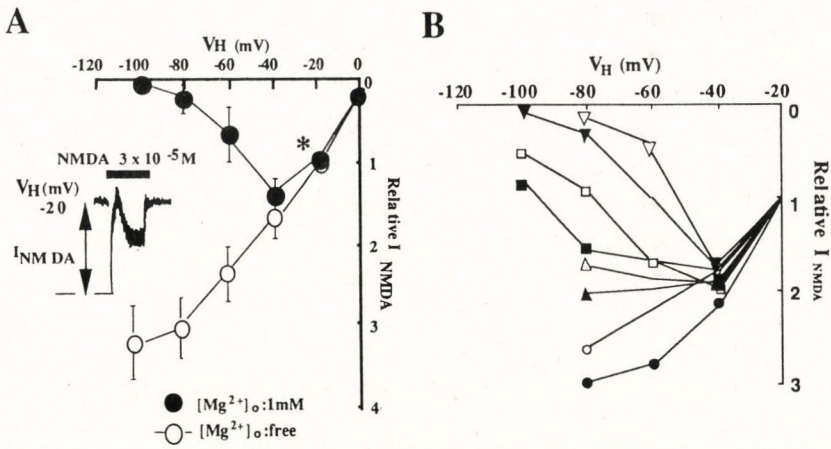

Fig. 1: Development of voltage dependency of NMDA response in NTS neurons. **A.** in the neurons obtained from rats older than P12, NMDA responses were severely suppressed at a holding potential (V_H) more hyperpolarized than -40 mV in the presence of 1 mM Mg^{2+}. Insertion shows the actual current response at a V_H of -20 mV. All peak current amplitudes were normalized to that obtained at a V_H of -20 mV. NMDA was applied at a concentration of $3x10^{-5}$ M. Open and closed circles indicate means of relative current amplitudes in the presence and absence of 1 mM Mg^{2+} in the perfusate, respectively. Vertical bars indicate ± s.e.m. of 7-12 NTS neurons. **B.** In fetal NTS neurons, the potency of extracellular Mg^{2+} (1 mM) on the NMDA response varies from cell to cell. Each symbol indicates holding potential - current relationship obtained from different cells

NTS neurons with a high sensitivity to extracellular Mg^{2+} gradually increased in number with age. The voltage dependency became apparent in all NTS neurons by P3 (Fig. 2 A). On the other hand, the development of the sensitivity of NMDA response to extracellular Mg^{2+} in the visual cortex was considerably delayed compared with that in the NTS. Visual cortical neurons acquired almost complete mature characteristics after P12 (Fig. 2 B). In addition, VMH neurons became mature by P6, regarding to the sensitivity of NMDA response to extracellular Mg^{2+}. These findings indicate that characteristics of NMDA response alter not uniformly but heterogenously with age among brain areas.

Recently, it has been reported that intracellular protein kinase C also enhances (Markram and Segal, 1992) and suppresses (Chen and Huang, 1992) the voltage dependency of NMDA responses. Therefore, the development of voltage dependency might be due to the change of intracellular protein kinase C activity. An application of phorbol ester (phorbol-12-myristate-13-acetate, $5x10^{-6}$ M) enhanced the amplitude of NMDA response at more hyperpolarized the -80 mM (317 $\pm$ 92% in amplitude, mean $\pm$ s.e.m., n=4, at a holding potential of -80 mV) on the NTS neurons of rats older than P12. However, in immature NTS neurons, neither phorbol ester ($5x10^{-6}$ M), nor staurosporine (10^{-7} M) affected the amplitudes of NMDA responses at any membrane potentials. Ratios of the amplitude of NMDA response in the presence of phorbol ester (10^{-5} M) and staurosporine (10^{-6} M) to that without these PKC modulators at a holding potential of -80 mV were 1.15 $\pm$ 0.16 and 1.07 $\pm$ 0.12 in the presence of 1 mM Mg^{2+}, respectively. This evidence indicates that appearance of voltage dependency in these neurons might not be due to developmental change of intracellular protein kinase C activity, but probably to alternation of combinations of subunits composing the NMDA receptor.

DISCUSSION

In a higher center, e.g., hippocampal CA1 (Bowe and Nadler, 1990) and CA3 (Ben-Ari, et al., 1988) and visual cortex (Fig. 2 A), the extracellular Mg^{2+} gives rise to a negative slope conductance of NMDA responses after the 2nd week after birth. On the other hand, NTS and VMH neurons acquired almost full potency of Mg^{2+} effect on NMDA response around P3 and P6, respectively. This finding indicates that NMDA response acquires mature characteristics at earlier stage in lower brain areas than in the higher ones. Regional discrepancy in the developmental process could be related to different maturation and vital necessities of behaviors, because the NTS is a direct control center to maintain life

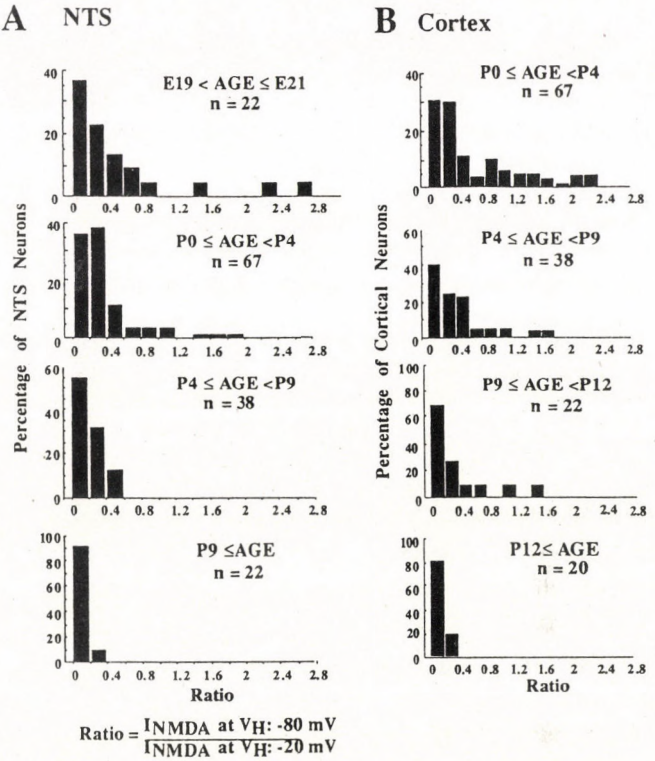

Fig. 2: Difference in developmental process of voltage dependency of NMDA response between NTS and visual cortex. Vertical bars indicate the amplitude ratio of NMDA response at a V_H of -80 mV to that at a V_H of -20 mV. Simply saying, neurons locating at righter side exhibit less sensitivity to extracellular Mg^{2+}

such as autonomic nervous system and respiration, and visual system is not definitely necessary immediately after birth. In addition, development of voltage-dependency of NMDA response varied among neurons in each area (Fig. 1 B and Fig. 2). The heterogeneity of developmental process among neurons in each brain area might also be related to variety of functions in which the same area plays a role.

The present data suggest that the development of sensitivity of NMDA response to extracellular Mg^{2+} is due not to the change of intracellular PKC activity but probably to alternation of receptor subunits comprising the NMDA receptor-channel complexes.

REFERENCES

Bear, M. F., Kleinschmidt, A., Gu, Q. A. and Singer, W. (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J. Neurosci. 10, 909-925.

Ben-Ari, Y., Cherubini, E. and Krjevic, K. (1988) Changes in voltage dependence of NMDA currents during development. Neurosci. Lett. 94, 88-92.

Bowe, M. A. and Nadler, J. V. (1990) Developmental increase in the sensitivity to magnesium of NMDA receptors on CA1 hippocampal pyramidal cells. Dev. Brain Res. 56, 55-61.

Carmignoto, G. and Vicini, S. (1992) Activity-dependent decrease in the NMDA receptor responses during development of the visual cortex. Science 258, 1007-1011.

Chen, L. and Huang, L. M. (1992) Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation. Nature 356, 521-523.

Markram, H. and Segal, M. (1992) Activation of protein kinase C suppresses responses to NMDA in rat CA1 hippocampal neurones. J. Physiol. 457, 491-501.

Mayer, M. L. and Westbrook, G. L. (1987) Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. J. Physiol. 394, 501-527.

Nabekura, J., Ebihara, S. and Akaike, N. (1993) Muscarinic receptor activation of potassium channels in rat dentate gyrus neurons. J. Neurophysiol. 70, 1544-1552.

Nabekura, J., Kawamoto, I. and Akaike, N. (1994) Developmental change in voltage dependency of NMDA receptor-mediated response in nucleus tractus solitarii neurons. Brain Res. 648, 152-156.

Urushihara, H., Tohda, M. and Nomura, Y. (1992) Selective potentiation of N-methyl-D-aspartate-induced current by protein kinase C in Xenopus oocytes injected with rat brain RNA. J. Biol. Chem. 267, 11697-11700.

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EFFECTS OF ARGININE-LYSINE MIXTURE, GLUCOSE AND ATP ON THE AUTONOMIC OUTFLOWS TO THE THYMUS AND SPLEEN

Niijima, A. and *Meguid, M. M.

Department of Physiology, Niigata University School of Medicine, Niigata, 951 Japan and *Department of Surgery, University Hospital, Syracuse, NY USA

Summary: Effect of arginine-lysine mixture, glucose and ATP on the efferent activities of the vagal thymic branch and splenic nerve were studied in urethane anesthetized rat. In male Wistar rats jugular vein was cannulated for drug administration. In one group the thymus was exposed and a nerve filament of the central cut end of vagal thymus was isolated. In another group a filament was isolated from that of the splenic nerve. The nerve filament was placed on electrodes to record efferent activity before and after administration of arginine-lysine mixture solution, glucose solution or ATP solution. Enhancement of efferent activity of vagal thymic nerve was observed after i.v. administration of arginine-lysine mixture, glucose and ATP solution. Suppression in efferent activity of splenic nerve was investigated following i.v. administration of arginine-lysine mixture, glucose and ATP solution. These results suggest that i.v. arginine-lysine, glucose and ATP increase thymic lymphocyte release, and that i.v. arginine-lysine, glucose and ATP enhance NK cell activity.

Key Words: arginine-lysine; glucose; ATP; vagal thymic efferents; splenic efferents; rat

Correspondence should be addressed to: Dr. Akira Niijima
31-2, Hamauracho,
Niigata City,
951 Japan

INTRODUCTION

There are numerous reports documenting enhanced immunity in a variety of animal models and in pathological conditions in patients by arginine given either orally or intravenously. In laboratory animals, the administration of supplemental arginine results in increased thymic size, weight and cellularity, enhanced lymphocyte proliferation to mitogen and alloantigen, augmented macrophage and NK-cell lysis of tumor cells, and increased lymphocyte interleukin-2 production and receptor activity (Barbul et al., 1978; Barbul et al., 1981; Reynolds et al., 1988).

To explain their immune enhancing mechanism, arginine, whether injected or provided as an oral supplement, would have to act directly on the thymus. On the other hand, data exist to demonstrate nutrient specific receptors in the hepato-portal region which via afferent vagal fibers interact with the hypothalamus (Niijima and Meguid, 1994). Furthermore, ablation or microinjection of different regions of the hypothalamus, significantly suppresses cytotoxic activity of splenic NK cells, abolished by prior splenic denervation, indicating neural control of an immune organ (Katafuchi et al., 1993). More recent works suggest that a direct and significant influence on the function of the immune organs may be mediated at least partly through the autonomic nervous system (Ichijo et al., 1994).

It has been reported that the thymus which is an organ of lymphocyte maturation and release, receives a large supply of nerve fibers from the vagus nerve (Bulloch, 1985). Since vagal stimulation increases the release of lymphocytes from the thymus and sectioning these fibers decreases it (Antonica et al., 1994), it suggests that the efferent vagal signals to the thymus play a role in the regulation of thymic function.

In this study we investigated the effects of a peripheral intravenous injection of arginine, lysine and a mixture of these amino acids (Arg-Lys), glucose and ATP on the efferent activity of vagal thymic nerve and sympathetic splenic nerve.

MATERIALS AND METHODS

Adult male Wistar rats, weighing about 300g, were used. They were kept in a room 24°C with illumination for 12 h a day (0700-1900). The animals were anesthetized by i.p. injection of 1g/kg urethane. For recording the vagal thymic efferent activity, a nerve filament was dissected from the central cut end of the small vagal nerve branch innervating the apical part of the right or left lobe of the thymus. To record the efferent activity of the

splenic nerve, a nerve filament was dissected from a splenic branch of the splanchnic nerve. The efferent activity was recorded with a pair of silver wire electrodes.

Nerve activity was amplified by means of a condenser coupled differential amplifier which led to an oscilloscope and a window discriminator connected with a rate meter. A reset time of 5 sec was used to observe the time course of nerve activity that was recorded on a pen recorder. The data of the nerve action potentials were stored with a digital magnetic tape recorder.

The left jugular vein or inferior vena cava was cannulated for administration of drugs. After having observed that the nerve activity was stabilized for at least for 30 min, the effect of i.v. administration of physiological saline (0.1 ml) was sometimes observed for about 30 min and then the injection of drugs was made. The drugs used were: l-arginine (Wako), l-lysine (Sigma), d-glucose (Wako), ATP (Oriental) and AMP (Oriental). All drugs were dissolved in physiological saline just before use and injected in a fluid volume of 0.5 ml at a rate of 0.1 ml/30 sec. The effects of drugs on the vagal thymic and splenic efferent activity were analyzed by comparing the mean frequency per 5 sec for over 50 sec (i.e. mean value of 10 continuous samples, $n=10$) just before and after the injection. All data were presented as mean $\pm$ SEM. Significance was determined by ANOVA ($p<0.05$).

RESULTS

Fig. 1 shows the effect of a solution of arginine and lysine mixture (10 mM, 0.5 ml) on the efferent activity of the vagal thymic nerve (Fig. 1, left) and splenic nerve (Fig. 1, right). A gradual increase in discharge rate was observed after i.v. administration of mixture solution which reached to peak about 90 min after injection (Fig. 1, left upper trace). The discharge rate just before and 30, 60 and 90 min after injection was 59.7 ± 2.3, 89.7 ± 2.2*, 112.8 ± 4.7* and 168.0 ± 14.7* (impulses/5 sec), respectively (*means significant increase) (Fig.1, left lower panel). The splenic nerve activity showed a gradual decrease in discharge rate after i.v. administration of the same dose of mixture solution which reach a nadir about 45 min after injection (Fig. 1, right upper trace).

The discharge rate of splenic nerve activity just before, 30 and 60 min after injection was 76.9 ± 2.3, 57.2 ± 2.3* and 54.1 ± 2.0* (impulses/5 sec) (*means significant decrease; $P<0.05$) (Fig.1, right lower panel). Four other observations on vagal thymic nerve activity demonstrated significant increase in discharge rate after injection of arginine-lysine mixture, and four other observations on splenic nerve activity presented similar suppressive responses after injection of the same mixture solution.

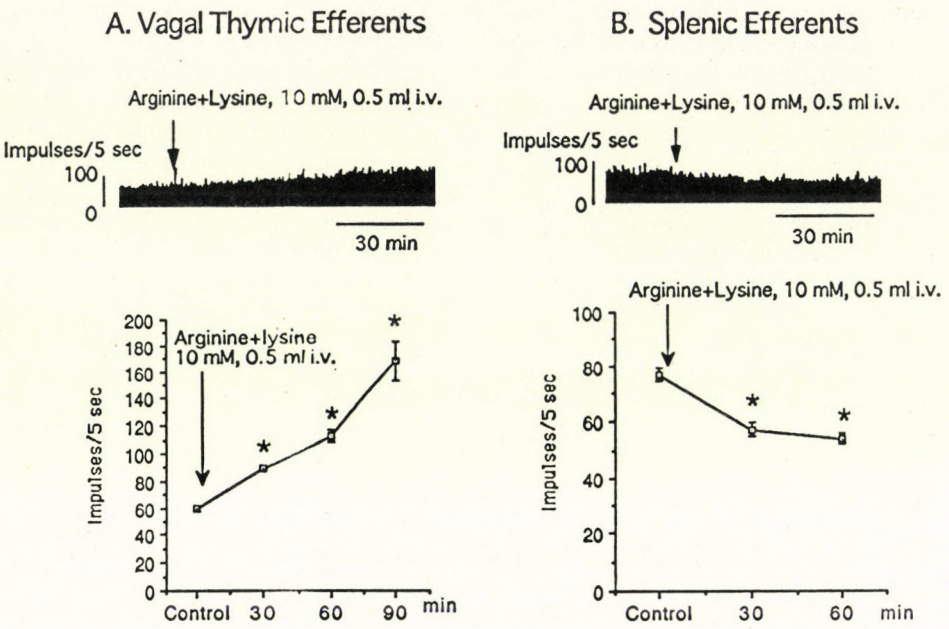

Fig. 1: Effect of mixture of arginine and lysine on the efferent activity of vagal thymic nerve and splenic nerve. Left trace and panel; vagal thymic nerve activity. Right trace and panel; splenic nerve activity

Fig. 2 shows the effects of i.v. administration of four different single amino acid solution (alanine, glycine, arginine, lysine; 10 **mM**, 0.5 ml **i.v.**) and a mixture solution of arginine and lysine (10 **mM**, 0.5 ml **i.v.**). As shown in the top trace of Fig. 2, injection of alanine and glycine caused no remarkable change in discharge rate. Administrations of single amino acid arginine or lysine caused a slight suppression, however, administrations of arginine-lysine mixture resulted in stronger and longer suppression in discharge rate (Fig. 2, middle and bottom trace).

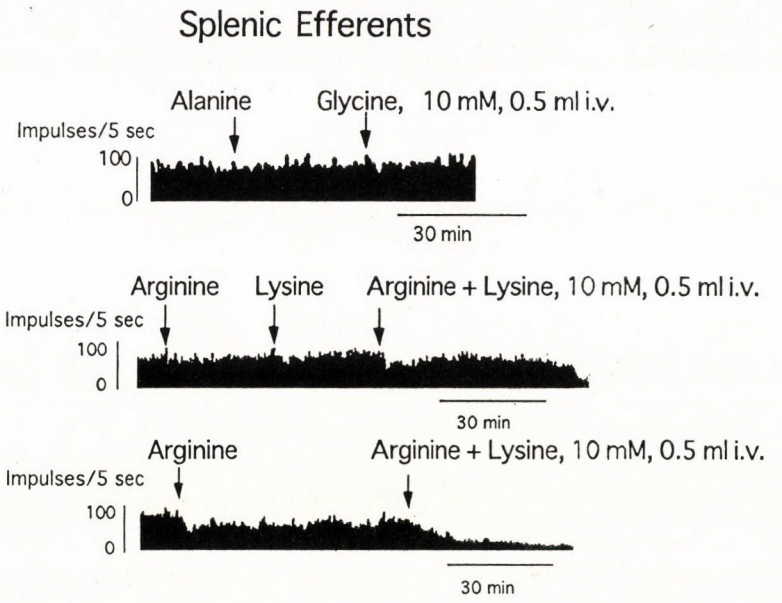

Fig. 2: Effect of alanine, glycine, arginine, lysine and mixture of arginine and lysine on efferent activity of splenic nerve

Fig. 3 demonstrates the effects of glucose on the efferent activity of the vagal thymic nerve (left traces) and that of splenic nerve (right traces). As shown in the upper trace, an i.v. injection of glucose solution caused a slight increase in discharge rate which lasted about 45 min. The discharge rate just before, and 30, 60 and 90 min after injection was 75.3±3.4, 81.5±2.6, 108.0±2.0* and 82.4±2.4 (impulses/5 sec), respectively. The larger dose of glucose (200 mg, 0.5 ml) resulted in stronger and longer increase in activity (left lower trace). Discharge rate just before and 30, 60, 90, 120, 150 min after administration was 68.7±1.5, 77.4±3.0*, 90.6±1.6*, 93.0±3.4*, 82.0±1.5* and 70.7±1.4 (impulses/5 sec), respectively.

The right upper trace presents the effect of subcutaneous injection (s.c.) of glucose (150 mg, 1 ml) on the splenic nerve activity. An injection of glucose solution caused a slow decrease in discharge rate. The discharge rate just before injection was 71.8±4.1 and that of 30, 60 and 90 min after injection was 52.1±3.6*, 31.9±2.2* and 20.1±1.5* (impulses/5 sec), respectively. I.v. injection of glucose in a dose of 200 mg caused a quick and strong suppression in splenic nerve activity and a dose related response for 50, 100 and 200 mg was also observed (right lower trace).

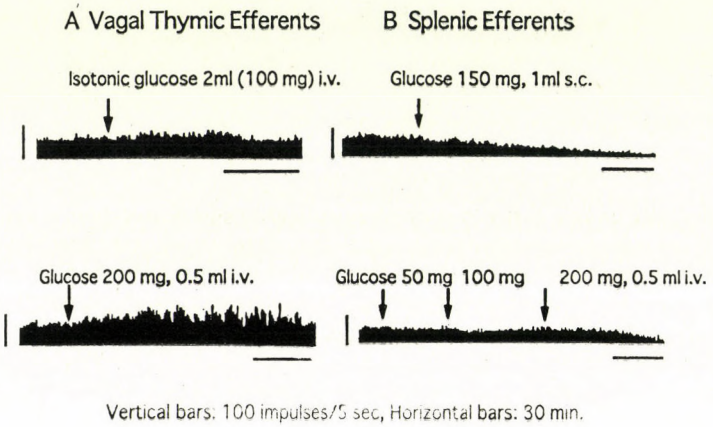

Fig. 3: Effect of glucose on the efferent activity of thymic nerve and splenic nerve. Left traces; vagal thymic nerve activity. Right traces; splenic nerve activity

Further, effects of ATP and AMP on the activity of vagal thymic nerve and that of splenic nerve were investigated (Fig. 4). Left upper trace shows the effect of AMP and ATP (1 mg, 0.1 ml i.v.) on the vagal thymic nerve activity. As shown in the trace an i.v. injection of ATP induced a slight increase in efferent activity, however, administration of AMP caused no noticeable change in nerve activity.

Efferent discharge rate just before injection of AMP and that of 15 min after injection was 68.4±2.5 and 66.4±1.8 (impulses/5 sec) indicating no significant difference

between these two values. The discharge rate just before injection of ATP and that of 15 min after injection was 60.2±0.5 and 83.1±3.0* (impulses/5 sec). The discharge rate 15 min after ATP injection demonstrated a significant increase. The left lower trace shows the effects of successive i.v. injections of ATP at a dose of 1 mg and 5 mg. The dose related increase in activity was observed. Thirty minutes after the first injection at a dose of 1 mg discharge rate increased from 68.8±4.0 to 78.9±2.7* (impulses/5 sec) and the second injection, which was made about 60 min after first injection, caused a significant increase in activity from 91.7+3.1 to 122.5+4.6* (impulses/5 sec) thirty minutes after second injection.

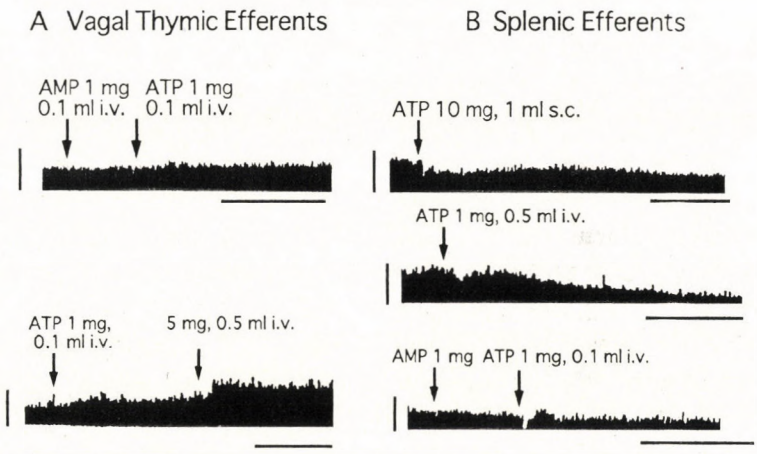

Fig. 4: Effect of ATP and AMP on the efferent activity of vagal thymic nerve and splenic nerve. Left traces; vagal thymic nerve activity. Right traces; splenic nerve activity

As shown in the right top trace, a subcutaneous (s.c.) administration of ATP (10 mg, 1 ml) resulted in a long lasting suppression in splenic nerve activity. I.v. injection of ATP (1 mg, 0.5 ml) caused a stronger inhibition in efferent activity. The discharge rate just before and 30, 60 and 90 min after injection was 67.3±2.8, 37.0±2.1*, 16.7±1.4* and 11.2±1.1* (impulses/5 sec), respectively (middle trace). Right bottom trace demonstrates that administration of ATP caused a long lasting suppression, however, injection of AMP was without effect.

DISCUSSION

The results of experiments indicate that administration of a mixture of arginine and lysine, glucose and ATP facilitate efferent activity of vagal thymic nerve activity and suppress splenic nerve activity and suggest an increase in immune function of the thymus and spleen. One possible explanation is that vagal stimulation increases the release of lymphocytes from the thymus (Antonica et al., 1994) and suppression in sympathetic activity to the spleen may facilitates NK-cell activity in the spleen because activation of sympathetic nerve to the spleen inhibits NK-cell activity in the spleen (Katafuchi et al., 1993). Other amino acids such as leucine and glycine, and AMP showed no effect.

There might be two pathways from periphery to the center of the autonomic nervous system in the hypothalamus which activates efferent signals to the thymus and suppresses efferent activity to the spleen. One possible pathway is from chemical sensors in the hepato-portal region, which sense Arg-Lys, glucose (Niijima, 1982) or ATP, to the hypothalamus via hepatic vagal afferents. A potentially different pathway might be via the circulation through the Organum Vasclosum Lamina Terminalis (OVLT) region in the hypothalamus or Area Postrema in the Medulla Oblongata to the hypothalamic center. Ablation of its medial portion (MPO) was shown by Katafuchi et al. to inhibit splenic NK-cell activity (Katafuchi et al., 1993).

It has been reported that an injection of glucose solution into the systemic vein or portal vein suppresses efferent activity of sympathetic adrenal nerve (Niijima, 1975; Niijima, 1980). Another report stated that there exists in the lateral hypothalamus (LHA) a chemoreceptor which controls the release of adrenaline (Himsworth, 1970). Further, it has been reported that systemic administration as well as direct application of glucose suppresses the activity of LHA neurons (Oomura et al., 1974). The depressive effect of glucose infusion on the activity of splenic nerve might be initiated by glucose sensitive LHA neurons and mediated through a pathway to the spleen because splenic nerve belongs to the sympathetic nerve as adrenal nerve does. In contrast to the response in the sympathetic efferents, an intravenous injection of glucose caused an activation of efferent discharge in the pancreatic branch of the vagus nerve (Niijima, 1975). The activation of vagal efferents to the thymus following administration of glucose can be explained as a vagal response. As a glucose related substance, ATP may cause the similar response as glucose in the sympathetic splenic and vagal thymic nerve.

REFERENCES

Antonica, A., Magni, F., Mearini, L. and Paolocci, N. (1994) Vagal control of lymphocyte release from rat thymus. J. Autonom. Nerv. Syst. 48, 187-197.

Barbul, A., Tettura, G., Leevenson, S. M. and Seiftec, E. (1978) Thymotrophic actions of arginine, ornithine, and growth hormone. Fed. Proc. 37, 264-268.

Barbul, A. (1986) Arginine: biochemistry, physiology and therapeutic implications. JPEN 10, 227-238.

Barbul, A., Rettura, G., Wasserkrug, H. L. and Efron, G. (1981) Arginine stimulates lymphocyte immune responses in healthy humans. Surgery 90, 244-251.

Bulloch, K. (1985) Neuroanatomy of Lymphoid tissues: a review. In : Guilmin, R. (Ed.) Neural modulation of Immunity. Raven Press: New York, N. Y. pp 111-141.

Himsworth, R. L. (1970) Hypothalamic control of adrenaline secretion in response to insufficient glucose. J. Physiol. 198, 451-465.

Ichijo, T., Katafuchi, T. and Hori, T. (1994) Central interleukin-1ß enhances splenic sympathetic nerve activity in rats. Brain Res. Bull. 34, 547-553.

Katafuchi, T., Take, S. and Hori, T. (1993) Roles of sympathetic nervous system in the suppression of cytotoxicity of splenic natural killer cells in the rat. J. Physiol. 465, 343-357.

Katafuchi, T., Ichijo, T., Take, S. and Hori, T. (1993) Hypothalamic modulation of splenic natural killer cell activity in rats. J. Physiol. 471, 209-221.

Niijima, A. (1975) The effect of 2-deoxy-D-glucose and D-glucose on the efferent discharge rate of sympathetic nerves. J. Physiol. 251, 231-243.

Niijima, A. (1975) The effect of glucose on the activity of the adrenal nerve and pancreatic branch of the vagus nerve in the rabbit. Neurosc. Lett. 1, 159-162.

Niijima, A. (1980) Glucose sensitive afferent nerve fibers in the liver and regulation of blood glucose. Brain Res. Bull. 5, Suppl. 4, 175-179.

Niijima, A. (1982) Glucose-sensitive afferent nerve fibers in the hepatic branch of the vagus nerve in the guinea-pig. J. Physiol. 332, 315-323.

Niijima, A. and Megiud, M. M. (1994) Parenteral nutrients in rat suppresses hepatic vagal afferent signals from portal vein to hypothalamus. Surgery 116, 294-301.

Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T. and Yamada, Y. (1974) Glucose inhibition of glucose-sensitive neurone in the rat lateral hypothalamus, Nature 247, 284-286.

Reynolds, J. V., Daly, J. M., Zhang, S., Evantash, E., Shou, J., Sigal, R. and Ziegler, M. M. (1988) Immunomodulatory mechanisms of arginine. Surgery 104, 141-151.

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THE HYPOTHALAMO-SYMPATHETIC NERVOUS SYSTEM MODULATES PERIPHERAL CELLULAR IMMUNITY

Hori, T., Katafuchi, T., Take, S., Kaizuka, Y., Ichijo, T. and Shimizu, N.

Department of Physiology, Kyushu University Faculty of Medicine, Fukuoka 812-82, Japan.

Summary: Our findings reviewed in this article have revealed that the stimulation of opioid receptors of the hypothalamic neurons by interferon α or β-endorphin synthesized in the brain or by stress causing the opioid-dependent analgesia suppresses the natural killer cytotoxicity, an important component of immunosurveillance, through an activation of the hypothalamic CRF-sympathetic nervous system.

Key Words: Hypothalamus, Immunosuppression, Natural killer cells, Splenic sympathetic nerve, Opioid receptors, Interferon α

INTRODUCTION

It is now apparent that the brain and the immune system may communicate with each other. While the brain may modulate the immune system by the neuroendocrine communication and the neural connections of autonomic nervous system (Felten et al., 1991), the immune system may signal the brain by means of chemical messengers like cytokines and some other signal molecules which are derived from immunological cells (Hori et al., 1991a). Several cytokines such as interleukin (IL)-1, IL-6 and interferonα (IFNα), which are synthesized in the brain as well as in the peripheral tissues (Higgins et al., 1991, Marcovitz et al., 1984), act on the brain cells to elicit a broad spectrum of CNS-mediated neuroendocrine,

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autonomic nervous and behavioral responses. A direct application of IL-1β or IFNα changes the activity of hypothalamic thermosensitive and glucose-responsive neurons in appropriate ways to explain the cytokines-induced fever and anorexia, respectively (Hori et al., 1988, 1991a; Nakashima et al., 1988, 1994; Kuriyama et al., 1990). While both the neuronal and febrile responses to IL-1β were mediated by prostanoids-dependent mechanisms, the IFNα-induced CNS responses including fever, analgesia and altered activity of hypothalamic neurons were attenuated or blocked by an opioid antagonist, suggesting the involvement of opioid receptor mechanisms. Indeed, IFNα was shown to bind to opioid receptors in the brain of rats (Menzies et al., 1992).

Recent studies in our and other laboratories have revealed that central injections of IL-1β and IFNα elicit the immunosuppressive responses, such as the inhibition of natural killer (NK) cytotoxicity and lymphocytes mitogenic activity, in rats and mice (Sundar et al., 1991; Take et al., 1992, 1993). The aim of this paper is to review our recent findings briefly on (1) the central and peripheral mechanisms of the inhibition of cytotoxicity of splenic NK cells which was induced by brain IFNα and IL-1β and (2) to present the evidence of the involvement of the hypothalamo-sympathetic nervous system in the central modulation of immunity.

RESULTS AND DISCUSSION

IFNα reduces the NK cytotoxicity by its action on brain opioid receptors

The effects of intracerebroventricular (ICV) injection of recombinant mouse (rm) IFNα on the cytotoxicity of splenic NK cells were studied in mice (Take et al., 1992). Thirty min after completing each injection, animals were deeply anesthetized with ether, sacrificed and splenectomized. The splenic NK activity was measured by a 4-hrs standard chromium release assay using 51Cr labeled YAC-1 murine lymphoma cells as targets. The ICV injection of rmIFNα at doses of 1000 and 2000 U significantly suppressed the splenic NK activity by about 30 % at an E/T ratio of 100:1. The reduced NK activity after central injection of rmIFNα at 1000 U was attenuated by pretreatment with naltrexone (NLTX).

A similar and dose-dependent inhibition of splenic NK activity was observed in the rat 30-120 min after an ICV injection of recombinant human (rh) IFNα (1000-

20000 U), but not after its intraperitoneal injection (Take et al., 1993). This inhibition is unlikely to be explained by the reduced number of NK cells in the spleen caused by their selective emigration to the general circulation, because the NK activity in the peripheral blood as well as in the spleen decreased to the same degree 30 min after injection of rhIFNα. Since colonic temperature did not change significantly within 30 min after the injection, the reduced NK activity was not caused by its pyrogenic action.

To determine the brain sites at which rhIFNα acts to inhibit splenic NK activity, we microinjected 200 U IFNα into the different sites of hypothalamus (the medial preoptic area (MPO), the lateral preoptic area, the ventromedial hypothalamus, the lateral hypothalamus, the paraventricular nucleus (PVN)), the neocortex and the thalamus in the rat and observed the splenic NK activity 30 min after injection (Take et al., 1995). IntraMPO injection of rhIFNα at 50 and 200 U reduced the NK activity to about 80 and 60 % of the vehicle-injected control, respectively. However, the injection of 200 U rhIFNα into the other brain sites had no effect.

Furthermore, it was found that the reduced NK activity after central injection of rhIFNα in the rat, like that in the mouse (Take et al., 1992), was abolished by pretreatment with NLTX (Take et al., 1993). This indicates that the brain rhIFNα suppresses the NK activity through an activation of opioid receptor mechanisms, and agrees well with the previous findings that an intracerebral, but not intravenous, injection of morphine or β-endorphin induced a suppression of splenic NK activity in a NLTX preventable way (Mori et al., 1989; Shavit et al., 1985). Shavit et al (1985) found that an inescapable type of footshock stress known to induce an opioid-dependent analgesia, but not that causing an equipotent but opioid-independent analgesia, inhibited the splenic activity and increased the growth rate of implanted tumor in a naloxone preventable way. In view of an important role of NK cells in immunosurveillance, these findings might offer an explanation for the vulnerability to diseases that is associated with some types of stresses.

The involvement of brain corticotropin releasing factor (CRF) in rhIFNα-induced decrease in the splenic NK activity

A neuropeptide CRF in the brain has been postulated to be a key mediator of stress-induced responses (Dunn & Berridge, 1990). While central administration of CRF may elicit a number of responses symptomatic of stress, α-helical CRF_{9-41} (a CRF antagonist) is demonstrated to attenuate many stress-induced responses. The inhibition of the splenic NK activity was also observed after an ICV injection of CRF in the rat (Irwin et al., 1988). When we pretreated with α-helical CRF_{9-41} (25 μg, ICV) which by itself had no effect on the NK activity, the rhIFNα-induced suppression of NK activity was completely abolished (Take et al., 1993). Furthermore, we found that the CRF-induced inhibition of NK activity was not affected by NLTX (50 μg, ICV) which could block the rhIFNα (10000 U, ICV)-induced immunosuppression. These results suggest that the reduced NK activity as a result of stimulation of opioid receptors by brain rhIFNα is dependent on the activity of central CRF systems.

Role of the splenic sympathetic nerve in the mediation of central rhIFNα–induced NK suppression

Subsequently, we found that bilateral adrenalectomy did not affect the reduced NK activity following both ICV and intraMPO administrations of rhIFNα (Take et al., 1993, 1995). This indicates that adrenal hormones do not play a major role in mediating the central rhIFNα-induced immunosuppression. Indeed, a recent study has revealed that an ICV injection of rhIFNα (1000 and 700000 U) had no effect on plasma concentration of corticosterone in the rat, although a much lower dose of rhIFNα (10 U) decreased it (Saphier et al., 1994). On the other hand, we observed that splenic denervation completely abolished the central rhIFNα-induced inhibition of splenic NK activity (Take et al., 1993, 1995). Since the splenic nerve is thought to consist mainly of sympathetic fibers (Bellinger et al., 1989), the rhIFNα-induced decrease of NK activity is suggested to be mediated predominantly through the splenic sympathetic innervation.

Relationship between the hypothalamic neuronal activity and the splenic sympathetic activity

Since the MPO was found to be the most sensitive site in the brain to microinjected rhIFNα in reducing the splenic NK activity, we further investigated the relationship between the MPO neuronal activity and the splenic sympathetic activity (Katafuchi et al., 1993b). When the MPO neuronal activity was enhanced by intraMPO microinjection of monosodium-L-glutamate (0.1 and 0.01 M) or electrical stimulation of the MPO in anesthetized rats, the firing rate activity of splenic sympathetic nerve filaments was depressed in association with a fall in blood pressure. Since hypertension evoked by an intravenous injection of phenylephrine (a peripherally acting vasoconstrictor) was accompanied by an inhibition of the splenic sympathetic activity, the inhibitory effect of the MPO stimulation on the splenic sympathetic activity was not a result of the induced hypotension. On the other hand, a bilateral lesion of the MPO produced a long-lasting (for more than 2 hours) increase in the splenic sympathetic activity and an inhibition of splenic NK activity that was completely abolished by prior splenic denervation (Katafuchi et al., 1993b). Furthermore, when the MPO neuronal activity was depressed by an intraMPO injection of rhIFNα (200 and 2000 U), which was known to suppress the activity of MPO neurons (Nakashima et al., 1988), the splenic sympathetic activity increased dose-dependently without any changes in blood pressure (Katafuchi et al., 1993b). These findings, taken together, the MPO neurons exert an inhibitory influence on the splenic sympathetic activity.

In contrast, microinjection of glutamate into the PVN of the hypothalamus increased the splenic sympathetic activity, suggesting that the PVN neurons in general exert an excitatory influence on the splenic sympathetic activity (Katafuchi et al., 1993b). Injection of rhIFNα (200 U) into the PVN had no effect on the splenic sympathetic activity, which was compatible with the finding that it failed to affect the splenic NK activity (Take et al., 1995).

The fact that the rhIFNα-induced inhibition of splenic NK activity is dependent on the CRF neurons suggests that modulatory actions of intraMPO rhIFNα on the splenic NK activity and the splenic sympathetic activity may involve

connections between the MPO neurons and the CRF-containing PVN neurons. An autoradiographical study has revealed a direct connection from the MPO to the PVN (Swanson, 1976). Electrical stimulation of the MPO has been demonstrated to suppress the multiple unit activity of the PVN in the rat (Saphier & Feldman, 1986), suggesting that the MPO has an inhibitory influence on the PVN activity. These findings, taken together, suggest that rhIFNα, which suppresses the MPO neuronal activity by its action on opioid receptors, may produce a disinhibition of the activity of PVN neurons, thereby inducing the enhancement of the splenic sympathetic activity.

The splenic sympathetic activity and the splenic NK activity

We further investigated whether the enhancement of the splenic sympathetic activity by its electrical stimulation actually inhibited the NK activity (Katafuchi et al., 1993a). The splenic NK activity after the laparotomy alone decreased to 69.3 ± 3.0 % (mean ± S.E.M.) of that in the control rats which did not receive any treatment except for anesthesia. The reduced NK activity then recovered a little bit (from 69.3 % to 80.3 ± 1.3 %), but significantly, when the splenic nerve was cut immediately after the laparotomy. When the peripheral cut end of the splenic nerve was subsequently stimulated, a further inhibition of NK activity (59.2 ± 2.4 % of the control) was observed. Furthermore, it was found that the inhibition of NK activity after the stimulation of the splenic nerve was completely abolished by an intravenous injection of nadolol (a peripherally acting β-adrenergic receptor antagonist), but not by that of prazosin (an α-antagonist). These findings indicate that the reduced NK activity after splenic nerve stimulation was caused predominantly by the action of noradrenaline (NA) on β-adrenergic receptors of splenic cells.

The above conclusion is further supported by our studies on the NA release in the spleen of conscious rats as determined by an in vivo microdialysis technique (Shimizu et al., 1994). An ICV injection of β-endorphin (10 and 30 μg) produced a dose dependent rise in the splenic NA release (2-3.5 times of the basal) which lasted for 90-120 min. Moreover, we recently found that an ICV injection of CRF (1-2.5 μg) increased both the splenic sympathetic activity (Ichijo et al., 1994) and the

NA release in the spleen (Shimizu, N. et al., 1994). The increases in the splenic sympathetic activity (Ichijo et al., 1994) and in the splenic NA release (Shimizu et al., 1994) after ICV and intraperitoneal injections of IL-1β, respectively, were attenuated by an ICV injection of α–helical CRF_{9-41}, indicating that these IL-1β-induced responses were mediated by the excitation of CRF-containing neurons in the brain.

The involvement of the sympathetic nervous system in the central modulation of the NK activity is further substantiated by the following observations. In addition to the abundant sympathetic innervation in the primary and secondary lymphoid organs and the presence of adrenergic receptors in the immunological cells (reviewed by Madden & Livnat, 1991), electron microscopic studies have revealed the close contact between the sympathetic nerve terminals and the immune cells in the spleen (Felten and Felten, 1991; Saito 1990). An application of adrenaline into the lymphocyte-target cell mixture in vitro inhibited the NK activity (Hellstrand et al., 1985). The inhibition of the splenic NK activity induced by a central injection of CRF (Irwin et al., 1988) and IL-1β (Sundar et al., 1991) were shown to be attenuated by chlorisondamine, a ganglionic blocker. Immobilization stress increased the release of NA in the spleen of rats which was associated with the reduced NK activity (Hori et al., 1991b). Both the NA release and reduced NK activity in the spleen were almost completely abolished by splenic denervation.

In conclusion, the stimulation of opioid receptors of the MPO neurons by IFNα or β-endorphin formed in the brain or by stress causing the opioid-dependent analgesia inhibits the splenic NK activity through an activation of the central CRF-sympathetic nervous system.

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REFERENCES

Bellinger, D.L., Felten, S.Y., Lorton, D. and Felten, D.L. (1989) Origin of noradrenergic innervation of the spleen in rats. Brain Behav. Immunity 3, 291-311.

Dunn and Berridge, C.W. (1990) Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses ? Brain Res. Rev. 15, 71-100.

Felten, D.L., Cohen, N., Ader, R., Felten, S.Y., Carlson, S. and Roszman, T.L. (1991) Central neural circuits involved in neural-immune interactions. In: Psychoneuroimmunology, 2nd Edition (ed. Ader, R., Felten, D.L. and Cohen, N.) pp.3-25, Academic Press, New York.

Felten, D.L. and Felten, S.Y. (1991) Innervation of lymphoid tissue. In: Psychoneuroimmunology, 2nd Edition (ed. Ader, R., Felten, D.L. and Cohen, N.) pp.27-61, Academic Press, New York.

Hellstrand, K., Hermdsson, S. and Strannegard. O. (1985) Evidence for a β-receptor-mediated regulation of human natural killer cells. J. Immunol. 134, 4095-4099.

Higgins, G.A. and Olschowka, J.A. (1991) Induction of interleukin-1β mRNA in adult rat brain. Mol. Brain Res. 9, 143-148.

Hori, T., Nakashima, T., Take, S., Kaizuka, Y., Mori, T. and Katafuchi, T. (1991a) Immune cytokines and regulation of body temperature, food intake and cellular immunity. Brain Res. Bull. 27, 309-313.

Hori, T., Shibata, M., Nakashima, T., Yamasaki, M., Asami, A., Asami, T. and Koga, H. (1988) Effects of interleukin-1 and arachidonate on the preoptic and anterior hypothalamic neurons. Brain Res. Bull. 20, 75-82.

Hori, T., Take, S., Mori, T., Kaizuka, Y., Shimizu, N. and Katafuchi, T. (1991b) Mechanisms of central opioids-induced suppression of rat peripheral cellular immunity. Soc. Neurosci. Abst. 17, 833.

Ichijo, T., Katafuchi, T. and Hori, T. (1992) Central interleukin-1β enhances splenic sympathetic nerve activity in rats. Brain Res. Bull. 34, 547-553.

Irwin, M., Hauger, R.L., Brown, M. and Britton, K.T. (1988) CRF activates autonomic nervous system and reduces natural killer cytotoxicity. Am. J. Physiol. 255, R744-R747.

Katafuchi, T,, Take, S. and Hori, T. (1993a) Roles of sympathetic nervous system in the suppression of cytotoxicity of splenic natural killer cells in the rat. J. Physiol. 465, 343-357.

Katafuchi, T., Ichijo, T., Take, S. and Hori, T. (1993b) Hypothalamic modulation of splenic natural killer cell activity in rats. J. Physiol. 471, 209-221.

Kuriyama, K., Hori, T., Mori, T. and Nakashima, T. (1990) Actions of interferonα and interleukin-1β on the glucose-responsive neurons in the ventromedial hypothalamus. Brain Res. Bull. 24, 803-810.

Madden, K.S. and Livnat, S. (1991) Catecholamine action and immunologic reactivity. In: Psychoneuroimmunology, 2nd Edition (ed. Ader, R., Felten, D.L. and Cohen, N.) pp.283-310. Academic Press, New York.

Marcovistz, R., Tsiang, H. and Hovanessian, A.G. (1984) Production and action of interferon in mice infected with rabies virus. Ann. Inst. Pasteur Paris 135E, 19-33.

Menzies, R.A., Patel, R., Hall, N.S.R., O'Grady, M.P. and Rier, S.E. (1992) Human recombinant interferon alpha inhibits naloxone binding to rat brain membranes. Life Sci. 50, PL227-PL232.

Mori, T., Kaizuka, Y, Hori, T., and Nakashima, T. (1989) Effects of intracerebral injection of β-endorphin on the cytotoxic activity of natural killer cells in the spleen. Jap. J. Physiol. 39(Suppl), S118.

Nakashima, T., Hori, T., Kuriyama, K. and Matsuda, T. (1988) Effects of interferon-α on the activity of preoptic thermosensitive neurons in tissue slices. Brain Res. 454, 361-367.

Nakashima, T., Murakami, T., Murai, Y., Hori, T., Miyata, S. and Kiyohara, T. (1995) Naloxone suppresses the rising phase of fever induced by interferon-α. Brain Res. Bull. 37, 61-66.

Saito, H. (1990) Innervation of the guinea pig spleen studied by electron microscopy. Am. J. Anatomy 189, 213-235.

Saphier, D. and Feldman, S. (1986) Effects of stimulation of the preoptic area on hypothalamic paraventricular nucleus unit activity and corticosterone secretion in freely moving rats. Neuroendocrinology 42, 167-173.

Saphier, D., Roerig, S.C., Ito, C., Vlasak, W.R., Farrar, G.E., Broyles, J.E. and Welch, J.E. (1994) Inhibition of neural and neuroendocrine activity by α-interferon: Neuroendocrine, electrophysiological, and biochemical studies in the rat. Brain, Behav. Immunity 8, 37-56.

Shavit, Y., Terman, G.W., Martin, F.C., Lewis, J.W., Liebeskind, J.C., and Gale, R.P. (1985) Stress, opioid peptides, the immune system, and cancer. J. Immunol 135, 834S-837S.

Shimizu, N., Hori, T. and Nakane, H. (1994) An interleukin-1-β-induced noradrenaline release in the spleen is mediated by brain corticotropin-releasing factor: an in vivo microdialysis study in conscious rats. Brain Behav. Immunity 7, 14-23.

Sundar, S.K., Cierpial, M.A., Kilts, C., Ritchie, J.C. and Weiss, J.M. (1991) IL-1-induced immunosuppression occurs through activation of both pituitary-adrenal axis and sympathetic nervous system by corticotropin-releasing factor. J. Neurosci. 10, 3701-3706.

Swanson, L.W. (1976) An autoradiographic study of the efferent connections of the preoptic region in the rat. J. Comp. Neurol. 167, 227-256.

Take, S., Mori, T., Katafuchi, T., Kaizuka, Y. and Hori, T. (1992) Central interferon α suppresses the cytotoxic activity of natural killer cells in the mouse spleen. Ann. N. Y. Acad. Sci. 650, 46-50.

Take, S,, Mori, T., Katafuchi, T. and Hori, T. (1993) Central interferonα inhibits the natural killer cytotoxicity through sympathetic innervation. Am. J. Physiol. 265, R453-R459.

Take, S., Uchimura, D., Kanemitsu, Y., Katafuchi, T. and Hori, T. (1995) Interferon-α acts at the preoptic hypothalamus to reduce natural killer cytotoxicity in rats. Am. J. Physiol. 268, in press.

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ROLES OF CYTOKINES IN THE NEURAL-IMMUNE INTERACTIONS: MODULATION OF NMDA RESPONSES BY IFN-α

Katafuchi, T., Take, S. and Hori, T.

Department of Physiology, Kyushu University Faculty of Medicine
Fukuoka, 812 Japan

Several lines of evidence have indicated that interferon-α (IFN-α) induces a variety of central actions; e.g., fever, anorexia, slow wave sleep and depression. However, little is known about the cellular mechanisms by which IFN-α affects neuronal activity. In the present article, the effects of recombinant human IFN-α on N-methyl-D-aspartate (NMDA)-induced responses of rat medial preoptic (MPO) neurons were examined by means of slice patch method as an *in vitro* model of neural-immune interactions. The results suggest that IFN-α suppresses the NMDA responses through its action on opioid receptors and the production of free radicals such as hydroxyl radicals and nitric oxide (NO) due to the neuron-glial cell interactions.

Keywords: neural-immune interaction; interferon-α; NMDA; hydroxyl radical; nitric oxide; glial cells

INTRODUCTION

It has been demonstrated that the central nervous system (CNS) and immune system have common ligand and receptor mechanisms by which the two systems communicate with each other. The most potent substances that mediate interactions between the nervous and immune systems are cytokines such as interferon-α (IFN-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Both these cytokines and receptors for them have been demonstrated to express in the CNS as well as in the immune system.

IFN-α is mostly produced by activated leukocytes and exerts antiviral, antitumor and immunomodulatory actions through its specific receptors [Pestka et al., 1987]. The interferon therapy is known to be accompanied by CNS-side effects, such as

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fever, anorexia, and depression. Furthermore, concentration of IFN-α in the cerebrospinal fluid is elevated in CNS infections with rabies virus [Marcovistz et al., 1984] and HIV [von Sydow et al., 1991], and in chronic fatigue syndrome [Lloyd et al., 1991], indicating that IFN-α is produced in the brain.

It has been reported that IFN-α affects EEG pattern when injected systemically [Smedley et al., 1983]. Furthermore, the firing rates of neurons in cerebral cortex, hippocampus [Dafny et al., 1985], and hypothalamus [Nakashima et al., 1988; Kuriyama, et al., 1990; Hori et al., 1991] were altered following direct application of IFN-α. These findings suggest that IFN-α might work as neurotransmitter/ neuromodulator in the CNS.

Glutamate is an excitatory transmitter substance used by a large number of neurons in the CNS. Furthermore, it is well known that NMDA subtype of glutamate receptors is involved in many physiological and pathological neuronal processes such as memory, learning, neurotoxicity, and epilepsy. In this article, based on our recent findings on the modulatory effects of IFN-α on the NMDA receptor-induced ion currents *in vitro* , possible mechanisms of neural-immune interactions will be discussed.

IFN-α in the brain

Since IFN-α has been produced by astrocytes in culture in response to polyribonucleotides [Tedeschi et al., 1986] and Newcastle disease virus [Lieberman et al., 1989], main source of IFN-α in the brain is considered to be astrocytes. Positive staining for IFN-α seen immunohistochemically in microgila and neurons [Akiyama et al., 1994] may reflect binding of IFN-α to the receptors in cell membranes. In fact, constitutive expression of receptors for IFN-α has been shown by immunoblotting in microglial cells in human brain tissues from normal as well as in those with Alzheimer's disease and cerebral infarction [Yamada and Yamanaka, 1995]. Since it is suggested that gangliosides are a part of the IFN-α/β receptors, neurons may also have the receptors, although the ganglioside binding of IFN-α does not produce the antiviral activity [Gupta et al., 1984]. Furthermore, astrocytes also may have IFN-α/β receptors since the IFNs induce class 1 antigens

of the MHC on an astrocyte subpopulation and thus render them susceptible to the lytic actions of cytotoxic T cells [Borgeson et al., 1989].

Concentration of IFN-α in the brain increases to the level of 1,000-2,000 U/ml in response to neurotropic viruses [Marcovistz et al., 1984] and Poly I:C [Cathala and Baron, 1970]. Intracerebroventricular injection of IFN-α at a dose of 1,000 U/ml, which was estimated to be about 300 U/ml in the parenchyma, was effective to produce a suppression of splenic natural killer cell activity in rats [Take et al., 1993]. Furthermore, the electrical activity of hypothalamic neurons in rat brain slice preparations was altered after application of 500-2,000 U/ml IFN-α [Nakashima et al., 1988]. Therefore, the dose of IFN-α of 100 U/ml, which we used in the present experiments, was considered to be comparable to or even lower than the concentration of this cytokine in the brain under pathological condition.

NMDA-induced responses in MPO neurons

Responses to glutamate or NMDA were recorded from rat medial preoptic (MPO) neurons by means of whole-cell slice-patch method [Edwards et al., 1989] Slices 120 μm in thickness were prepared from the hypothalamus including MPO of male 10-20 day old Wistar rats. Whole-cell recordings were made from MPO neurons visually identified under Nomarski optics with holding potential of -60 mV. To make the glutamate responses maximum, 1 μm glycine was added to the external solution and magnesium ions were removed from it. In addition, TTX (1 μm) was added. Glutamate or NMDA was applied by pressure ejection for 100 msec through an glass micropipette (tip diameter, 1 μm). An inward current induced by glutamate (100 μM) was profoundly attenuated by the preceding bath application of D-AP5, indicating an involvement of NMDA receptors in the glutamate-induced responses. An administration of NMDA(200 mM) itself elicited an inward current whose time course was essentially same as that induced by glutamate. Parton et al. [1991] have revealed that MPO neurons possess NMDA receptors, which mediate the synaptic transmission originated from the skin thermoreceptors.

IFN-α and brain opioid receptors

Bath application of human recombinant IFN-α (100 U/ml) for 2 min produced an attenuation the NMDA-induced inward current without changing the resting membrane current. The peak amplitude of the NMDA-induced responses were suppressed to about 50% of the pre-infusion level of control responses within a few minutes lasting for more than one hour.

Simultaneous application of an opioid antagonist, naloxone (10 μM) with IFN-α resulted in only 20% inhibition of the NMDA currents compared with those before application of IFN-α, suggesting an involvement of opioid receptors in the action of IFN-α. Several lines of evidence have indicated that IFN-α exerts some effects via opioid receptors in the brain. IFN-α inhibits the binding of [3H]naloxone to rat brain membranes [Menzies et al., 1992]. IFN-α reduces the abstinence syndrome in morphine-tolerant rats [Dafny, 1983], and analgesia and catatonia in the mouse [Blalock and Smith, 1981], fever in the rat [Nakashima et al., 1995], and suppression of splenic natural killer cell activity [Take et al., 1993] following intracerebral injection of IFN-α were attenuated by naloxone and naltrexone. Naloxone reversibly blocked the IFN-α-induced changes in neuronal activity of the rat hypothalamic neurons [Nakashima et al., 1988; Kuriyama et al., 1990]. Moreover, IFN-α inhibited forskolin-stimulated cAMP accumulation in human neuroblastoma cells which expressed predominantly μ-receptors [Saphier et al., 1994].

It has been reported that an activation of protein kinase C (PKC) induced by μ-opioid receptors potentiates the NMDA receptor-mediated responses [Chen and Huang, 1991] by reducing the voltage-dependent Mg^{2+} block of the receptors in rat trigeminal nuclei [Chen and Huang, 1992]. However, Snell et al. have demonstrated that in cerebellar granule cells NMDA-induced increases in intracellular calcium are attenuated by PKC activation. It is suggested that the inconsistency may be due to differences in PKC isozymes and/or the subunit composition of the NMDA receptors in different cell types [Snell et al., 1994].

Are prostaglandins involved in actions of IFN-α?

Since IFN-α stimulated prostaglandin E_2 (PGE_2) release from rabbit hypothalamic tissue in vitro [Dinarello et al., 1984], an involvement of PGE_2 as a mediator of the IFN-α-induced suppression of NMDA responses was examined. Application of sodium salicylate (SALC, 10 μM) immediately after or 40 min after an administration of IFN-α partially restored the suppression of NMDA responses. However, to our surprise, an application of PGE_2 (2 μM) itself did not modulate the NMDA currents at all.

Although both SALC and aspirin are said to be equally effective antiinflammatory agents, SALC is one-hundreds less potent than aspirin as an inhibitor of constitutive cyclooxygenase (COX-1) and only half as potent as an inhibitor of inducible cyclooxygenase (COX-2) [Mitchell et al., 1994]. Therefore, these findings and our results, taken together, suggest that the effects of IFN-α on NMDA responses might not be due to the synthesis of PGs.

Involvement of reactive oxygen intermediates and nitric oxide

It has been shown that salicylate can remove hydroxyl radicals from external solution [Chen and Gillis, 1991] by reacting with this radical species to form dihydroxybenzoic acid [Floyd et al., 1986]. Therefore, it was possible that the effects of SALC were mediated by its action as a free radical scavenger. In fact, direct application of hydrogen peroxide (1 mM) could suppress the NMDA-induced inward currents.

Hydroxyl radical in our system could be formed by iron-catalyzed Haber-Weiss interaction of superoxide and hydrogen peroxide. Thus, simultaneous application of superoxide dismutase (200 U/ml) with IFN-α blocked the suppressive effect of IFN-α on the NMDA currents. It has been shown that microglia are capable of releasing reactive oxygen intermediates such as superoxide and hydrogen peroxide [Piani et al., 1992] and that IFN-α modulates resting membrane potential and synaptic activity of CA3 pyramidal cells in rat hippocampal slice cultures at least in part through a mediation of reactive oxygen intermediates [Müller et al., 1993]. Furthermore, Aizenman [1995] has recently reported that whole-cell

voltage-clamped responses to NMDA decreased by hydroxyl radicals in rat cortical neurons in culture. He suggested that the suppression of NMDA responses was due to an oxidation of redox modulatory site of the NMDA receptor by hydroxyl radicals.

We found that concurrent application of a NOS inhibitor, N-ω-nitro-L-arginine (100 μM) with IFN-α also attenuated the IFN-α-induced suppression of the NMDA currents. It has been suggested that NO inhibits the NMDA responses by oxidizing the redox modulatory site of the NMDA receptor [Lei et al., 1992] as it is proposed in the action of hydroxyl radicals mentioned above. However, since NO has been shown to induce the formation of hydroxyl radicals via peroxynitrite formation [Hogg et al., 1992], modulation by NO may be mediated through the production of hydroxyl radicals. Furthermore, constitutive NO synthase (NOS) can dirctly generate superoxide at suboptimal concentration of L-arginine [Culcasi et al., 1994]. It has been demonstrated that IFN-β produces nitric oxide in the presence of LPS in macrophages [Zhang et al., 1994].

Conclusion

The present experiments demonstrated that IFN-α suppresses NMDA responses of rat MPO neurons, at least in part, by its action on opioid receptors and the production of reactive oxygen intermediates. Furthermore, since a NOS inhibitor could attenuate the action of IFN-α, an involvement of NOS producing NO and/or superoxide was also suggested. It is considered that there are at least four acting sites of IFN-α including neurons and glial cells (Fig. 1). First, IFN-α may act on opioid receptors on the neuronal membrane, modulating possibly through an activation of PKC (1). Second, IFN-α could act on the receptors for IFN-α of microglial cells to induce synthesis of superoxide and hydrogen peroxide which could easily produce hydroxyl radicals by Haber-Weiss reaction (2). It has been reported that constitutive NOS is present in astrocytes [Murphy et al., 1993] as well as neurons. Therefore, NO produced by astrocytes (3) and/or by neurons (4), and NOS itself may be involved in the actions of IFN-α through the direct action of NO and the production of superoxide, respectively. These free radicals would suppress the NMDA responses by their actions on redox modulatory sites of the

NMDA receptors (5). Thus, it is concluded that the modulatory effects of IFN-α on the NMDA responses may be attributed to the diverse actions of IFN-α through the neuron-glial cell interactions.

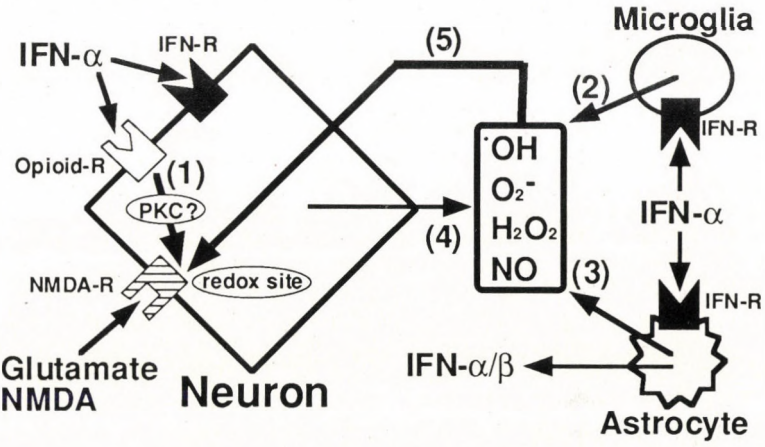

Fig. 1: Possible mechanisms of modulatory effects of IFN-α on NMDA responses

References

Aizenman, E. (1995) Modulation of N-methyl-D-aspartate receptors by hydroxyl radicals in rat cortical neurons in vitro. Neurosci. Lett. 189, 57-59.

Akiyama, H., Ikeda, K., Katoh, M., McGeer, E.G. and McGeer, P.L. (1994) Expression of MRP14, 27E10, interferon-a and leukocyte common antigen by reactive microglia in postmortem human brain tissue. J. Neuroimmunol. 50, 195-201.

Blalock, J.E. and Smith, E.M. (1981) Human leukocyte interferon (HuIFN-α): potent endorphin-like opioid activity. Biochem. Biophys. Res. Commun. 101, 472-278.

Borgeson, M., Tallent, M.W. and Keane, R.W. (1989) Astrocyte modulation of central nervous system immune responses. In: Neuroimmune Networks: Physiology and Diseases (Eds. Goetzl, E.J. and Spector, N.H.) pp. 51-55. Alan R. Liss, Inc. New York.

Cathala, F. and Baron, S. (1970) Interferon in rabbit brain, cerebrospinal fluid and serum following administration of polyinosinic-polycytidylic acid. J. Immunol. 104, 1355-1358.

Chen, L. and Huang, L-Y.M. (1991) Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a μ opioid. Neuron 7, 319-326.

Chen, L. and Huang, L-Y.M. (1992) Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation. Nature 356, 521-523.

Chen, X. and Gillis, C.N. (1991) Effect of free radicals on pulmonary vascular response to acetylcholine. J. Appl. Physiol. 71, 821-825.

Culcasi, M., Lafon-Cazal, M., Pietri, S. and Bockaert, J. (1994) Glutamate receptors induce a burst of superoxide via activation of nitric oxide synthase in arginine-depleted neurons. J. Biol. Chem. 269, 12589-12593.

Dafny, N. (1983) Interferon modifies morphine withdrawal phenomena in rodents. Neuropharmacol. 22, 647-651.

Dafny, N., Prieto-Gomez, B. and Reyes-Vazquez, C. (1985) Does the immune system communicate with the central nervous system? J. Neuroimmunol. 9, 1-12.

Dinarello, C.A., Bernheim, H.A., Duff, G.W., Le, H.V., Nagabhushan, T.L., Hamilton, N.C. and Coceani, F. (1984) Mechanisms of fever induced by recombinant human interferon. J. Clin. Invest. 74, 906-913.

Edwards, F.A., Konnerth, A., Sakmann, B. and Takahashi, T. (1989) A thin slice preparation for patch clamp recordings from neurons of the mammalian central nervous system. Pflügers Arch. 414, 600-612.

Floyd, R.A., Henderson, R., Watson, J.J. and Wong, P.K. (1986) Use of salicylate with high pressure liquid chromatography and electrochemical detection as a sensitive measure of hydroxyl free radicals in adriamycin treated rats. J. Free Radical Biol. Med. 2, 13-18.

Gupta, S.L., Raziuddin, a. and Sarkar, F.H. (1984) Receptors for human alpha interferon: are gangliosides involved? J. Interferon Res. 4, 305-314.

Hogg, N., Darley-Usmar, V.M., Wilson, M.T. and Moncada, S. (1992) Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. Biochem. J. 281, 419-424.

Hori, T., Nakashima, T., Take, S., Kaizuka, Y., Mori, T. and Katafuchi, T. (1991) Immune cytokines and regulation of body temperature, food intake and cellular immunity. Brain Res. Bull. 27, 309-313.

Kuriyama, K., Hori, T., Mori, T. and Nakashima, T. (1990) Actions of interferonα and interleukin-1β on the glucose-responsive neurons in the ventromedial hypothalamus. Brain Res. Bull. 24, 803-810.

Lei, S.Z., Pan, Z-H., Aggarwal, S.K., Chen, H-S.V., Hartman, J., Sucher, N.J. and Lipton, S.A. (1992) Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron 8, 1087-1099.

Lieberman, A.P., Pitha, P.M., Shin, H.S. and Shin, M.L. (1989) Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proc. Natl. Acad. Sci. USA 86, 6348-6352.

Lloyd, A., Hickie, I., Brockman, A., Dwyer, J. and Wakefield, D. (1991) Serum and cerebrospinal fluid cytokine levels in patients with chronic fatigue syndrome and control subjects. J Infect. Dis. 164, 1023-1024.

Marcovistz, R., Tsiang, H. and Hovanessian, A.G. (1984) Production and action of interferon in mice infected with rabies virus. Ann. Inst. Pasteur Paris 135E, 19-33.

Menzies, R.A., Patel, R., Hall, N.R.S., O'Grady, M.P. and Rier, S.E. (1992) Human recombinant interferon alpha inhibits naloxone binding to rat brain membranes. Life Sci. 50, 227-232.

Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J. and Vane, J.R. (1994) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc. Natl. Acad. Sci. USA 90, 11693-11697.

Müller, M., Fontana, A., Zbinden, G. and Gähwiler, B.H. (1993) Effects of interferons and hydrogen peroxide on CA3 pyramidal cells in rat hippocampal slice cultures. Brain Res. 619, 157-162.

Murphy, S., Simmons, M.L., Agullo, L., Garcia, A., Feinstein, D.L., Galea, E., Reis, D.J., Minc-Golomb, D. and Schwartz, J.P. (1993) Synthesis of nitric oxide in CNS glial cells. TINS 16, 323-328.

Nakashima, T., Hori, T., Kuriyama, K. and Matsuda, T. (1988) Effects of interferon-α on the activity of preoptic thermosensitive neurons in tissue slices. Brain Res. 454, 361-367.

Nakashima, T., Murakami, T., Murai, Y., Hori, T., Miyata, S. Kiyohara. T. (1995) Naloxone suppresses the rising phase of fever induced by interferon-α. Brain Res. Bull. 37, 61-66.

Parton, M.J., Gayton, R.J. and Taylor, D.C.M. (1991) The effect of glutamate receptor antagonists on rat thermoreactive hypothalamic neurones. J. Physiol. 438, 78P.

Pestka, S., Langer, JA., Zoon, KC., and Samuel, CE. (1987) Interferons and their actions. Annu. Rev. Biochem. 56, 727-777.

Piani, D., Spranger, M., Frei, K., Schaffner, A. and Fontana, A. (1992) Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. Eur. J. Immunol. 22, 2429-2436.

Saphier, D., Roerig, S.C., Ito, C., Vlasak, W.R., Farrar, G.E., Broyles, J.E. and Welch, J.E. (1994) Inhibition of neural and neuroendocrine activity by α-interferon: neuroendocrine, electrophysiological, and biochemical studies in the rat. Brain Behav. Immun. 8, 37-56.

Smedley, H., Katrak, M., Sikora, K. and Wheeler, T. (1983) Neurological effects of recombinant human interferon. Br. Med. J. 286, 262-265.

Snell, L.D., Iorio, K.R., Tabakoff, B. and Hoffman, P.L. (1994) Protein kinase C activation attenuates N-methyl-D-aspartate-induced increases in intracellular calcium in cerebellar granule cells. J. Neurochem. 62, 1783-1789.

von Sydow, M., Sonnerborg, A., Gaines, H. and Strannegard, O. (1991) Interferon-alpha and tumor necrosis factor-alpha in serum of patients in various stages of HIV-1 infection. (1991) AIDS Res. Hum Retroviruses 7, 375-380.

Take, S., Mori, T., Katafuchi, T. and Hori, T. (1993) Central interferon-a inhibits natural killer cytotoxicity through sympathetic innervation. Am. J. Physiol. 265, R453-R459.

Tedeschi, B., Barrett, J.N. and Keane, R.W. (1986) Astrocytes produce interferon that enhances the expression of H-2 antigens on a subpopulation of brain cells. J. Cell Biol. 102, 2244-2253.

Yamada, T and Yamanaka, I. (1995) Microglial localization of α-interferon receptor in human brain tissues. Neurosci. Lett. 189, 73-76.

Zhang, X., Alley, E.W., Russell, S.W. and Morrison, D.C. (1994) Necessity and sufficiency of beta interferon for nitric oxidd production in mouse peritoneal macrophages. Infect. Immun. 62, 33-40.

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EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR ON NEURONAL ACTIVITY OF THE PARVOCELLULAR PART IN RAT PARAVENTRICULAR NUCLEUS

Sasaki, K.[1], Oomura, Y.[2], Urashima, T.[1], Shiokawa, A.[1], Tsukada, A.[1], Kawarada, A.[1] and Yanaihara, N.[3]

[1]Department of Bio-Information Engineering, Faculty of Engineering, Toyama University, 3190 Gofuku, Toyama 930, [2]Institute of Bio-Active Science, Nippon Zoki Pharmaceutical Co. Ltd., Yashiro , Hyogo 673-14, and [3]Yanaihara Institute Inc., Fujinomiya, Shizuoka 418, Japan

The effects of acidic fibroblast growth factor (aFGF) and its amino-terminal and carboxyl-terminal fragments (aFGF(1-15) and aFGF(114-140), respectively) were examined on the neuronal activity in the parvocellular part of the paraventricular nucleus. As well known, this part contains a lot of corticotropin-releasing factor (CRF)-immunoreactive neurons. Application of 1 pg/ml and 2 pg/ml aFGF produced responses in 29.7% and 46.7% of neurons tested, respectively. Half or more than half of the responding neurons increased their discharge rate. Application of 0.2 ng/ml and 0.4 ng/ml aFGF(1-15) also elicited responses in 46.2% and 68.8% of neurons tested, respectively. Of these responding neurons, more than two third increased their firing rate. However, most of neurons tested for 0.67 ng/ml and 1.33 ng/ml aFGF(114-140) did not respond. Results suggest that aFGF and aFGF(1-15) promote the release of CRF through the activation of CRF-containing neurons.

acidic fibroblast growth factor, fragment, paraventricular nucleus, parvocellular part, corticotropin-releasing factor, slice, rat

INTRODUCTION

Acidic fibroblast growth factor (aFGF), which consists of a 140 amino acid sequence and has a molecular mass of about 16,000 Da, is a member of a large family of structurally related heparin-binding polypeptide growth factors [Baird and Walicke, 1989; Walicke, 1989; Baird and Böhlen, 1990]. aFGF stimulates the

Address: Kazuo Sasaki Ph.D.
Department of Bio-Information Engineering
Faculty of Engineering, Toyama University
3190 Gofuku, Toyama-city, Toyama 930
Japan
Phone: 81-764-41-1271 ext.2743
Fax: 81-764-41-8432
E-mail: sasaki@engl.eng.toyama-u.ac.jp

growth of mesenchymal cells such as fibroblasts, endothelial cells, vascular smooth muscle cells, and others, in vitro [Baird and Walicke, 1989; Walicke, 1989; Baird and Böhlen, 1990]. Recent studies also demonstrate neurotrophic effects of aFGF on neurons of the central nervous system (CNS) in vitro and in vivo. For example, aFGF promotes the survival and neurite outgrowth of various cultured CNS neurons [Walicke, 1988]. It also protects retinal ganglion cells from cell death after transection of the optic nerve [Sievers et al., 1987], and rescues hippocampal CA1 pyramidal cells from delayed neuronal death induced by transient forebrain ischemia [Sasaki et al., 1992; MacMillan et al., 1993]. These results suggest a possible function of aFGF as a neurotrophic factor.

Recently, cell surface, transmembrane tyrosine kinase receptors for FGFs have been identified [Ruta et al., 1989; Dionne et al., 1990; Keegan et al., 1991; Partanen et al., 1991]. These include FGF receptor (FGFR)-1 to FGFR-4. Of these, FGFR-1 mRNA is highly expressed in the neurons of the hippocampus and the hypothalamus such as the lateral hypothalamic area (LHA) and the paraventricular nucleus (PVN) [Wanaka et al., 1991; Itoh et al., 1994; Matsuo et al., 1994]. In agreement with this, it has been demonstrated that aFGF affects the neuronal activity in the hippocampus and the LHA. For example, aFGF modulates the efficacy in synaptic transmission in the hippocampus in vitro [Sasaki et al., 1994]. aFGF also produces an inhibition in the LHA neuronal activity, which leads to food intake suppression [Hanai et al., 1989; Oomura et al., 1992]. These findings suggest a possibility that aFGF has various physiological functions in the CNS, other than neurotrophic effects. Recently, we have found in our preliminary experiment that a microinfusion of aFGF into the third cerebral ventricle (3CV) induces an increase of plasma corticosterone, suggesting an involvement of corticotropin-releasing factor (CRF) in this effect. It has been reported that the parvocellular part of the PVN contains a lot of CRF-immunoreactive neurons [Swanson et al., 1983]. Thus, the effects of aFGF on the neuronal activity in the parvocellular part of the

PVN were examined in the present study. In addition, the effects of an amino-terminal aFGF fragment, aFGF(1-15), and a carboxyl-terminal aFGF fragment, aFGF(114-140), were also examined.

METHODS

Wistar male rats weighing 140-250 g were used. Immediately after decapitation, the brain was quickly isolated from the skull and frontal hypothalamic slices (400 μm thick) were cut by a microslicer. The slices including the PVN were selected and in each slice, only the PVN was separated from other tissues. The PVN slices were then preincubated at room temperature for more than 1.5 h in oxygenated (95% O_2-5% CO_2) Ringer solution containing 124 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.24 mM KH_2PO_4, 26 mM $NaHCO_3$ and 10 mM glucose.

Each slice was transferred into a recording chamber which was controlled at 36.0 ± 0.5 °C. The flow rate of the medium was 1 ml/min. Extracellular activity was recorded via a glass microelectrode filled with 3 M NaCl, and fed into an amplifier. The output of the amplifier was then monitored on an oscilloscope, and recorded on a magnetic tape. In addition, the signals were also passed through a pulse former and then into a computer. The computer calculated the mean firing rate of a neuron in 1 s intervals and displayed them on a screen.

Bovine aFGF was purchased from R & D Systems Inc. (Minneapolis, MN). aFGF fragments, aFGF(1-15) and aFGF(114-140), were synthesized as described previously [Iguchi et al., 1989]. aFGF and aFGF fragments were diluted to a concentration of 1 ng/ml and 400 ng/ml in distilled water, respectively, and small aliquots were stored at - 20 °C. One aliquot was taken in each experiment and then further diluted to the desired concentration by adding Ringer solution just before use. Application of these agents to slices was continued for 3 min.

RESULTS

For aFGF, the activity of 98 neurons was recorded in the parvocellular part of the PVN. Of these, the response to 1 pg/ml aFGF alone was tested in 38 neurons, that to 2 pg/ml aFGF alone in 34, and that to both 1 pg/ml and 2 pg/ml aFGF in remaining 26. Results are summarized in Table 1. When 1 pg/ml aFGF was applied to 64 neurons, 39 (60.9%) responded, and the remaining 25 (39.1%) were unchanged. Of the 39 responding neurons, 19 (29.7%) increased their discharge rate, and 3 (4.7%) showed an increase followed by a decrease (Fig. 1, B). Seventeen neurons (26.6%) simply decreased in discharge rate (Fig. 1, C). When 2 pg/ml aFGF

TABLE 1

Summary of aFGF effects on neurons in the parvocellular part of the PVN

Dose	No. of neurons	Effect of aFGF			
		E	E-I	I	N
1 pg/ml	n=64	19	3	17	25
2 pg/ml	n=60	28	6	8	18

E, excitation; E-I, excitation-inhibition; I, inhibition; N, no effect

was applied to 60 neurons, 42 (70.0%) responded, and the remaining 18 (30.0%) were unchanged. Of the 42 responding neurons, 28 (46.7%) increased (Fig. 1, A), 6 (10%) first increased then decreased, and 8 (13.3%) simply decreased their firing rate.

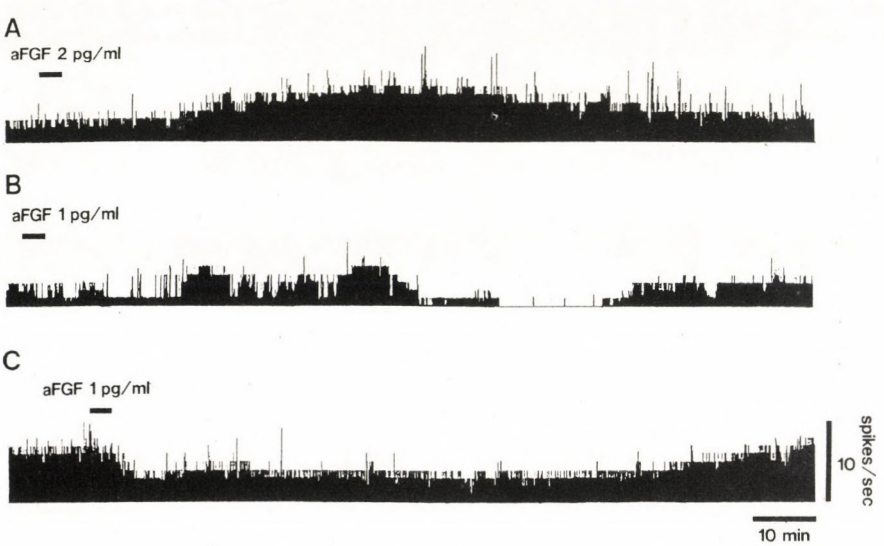

Fig. 1 Effects of aFGF on neurons in the parvocellular part of the PVN. A: excitation. B: excitation-inhibition. C: inhibition.

TABLE 2

Summary of aFGF(1-15) effects on neurons in the parvocellular part of the PVN

		Effect of aFGF(1-15)			
Dose	No. of neurons	E	E-I	I	N
0.2 ng/ml	n=26	12	4	2	8
0.4 ng/ml	n=16	11	1	0	4

E, excitation; E-I, excitation-inhibition; I, inhibition; N, no effect

The activity of 29 neurons was recorded for aFGF(1-15). Of these, the effect of 0.2 ng/ml aFGF(1-15) alone was tested in 13 neurons, that of 0.4 ng/ml aFGF(1-15) alone in 3, and that of both 0.2 ng/ml and 0.4 ng/ml aFGF(1-15) in 13. Table 2 shows summarized results. When 0.2 ng/ml aFGF(1-15) was applied to 26

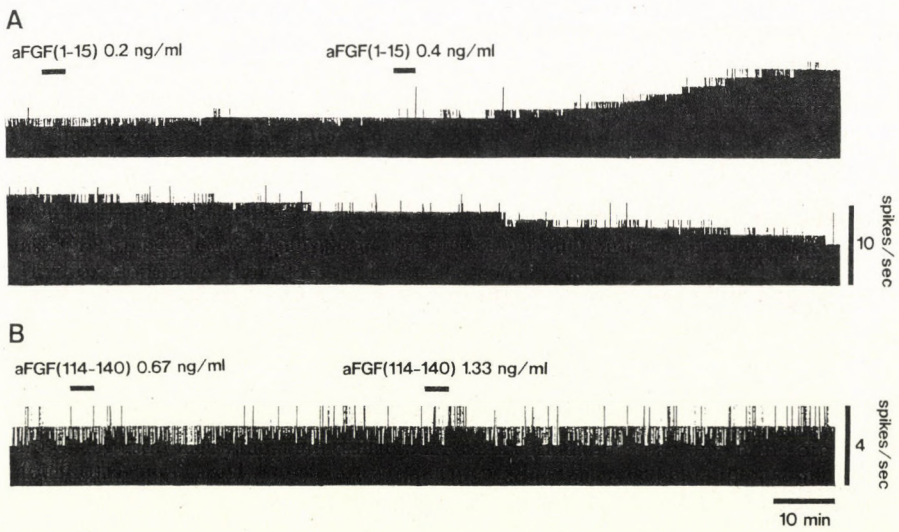

Fig. 2 Effects of aFGF(1-15) and aFGF(114-140) on neurons in the parvocellular part of the PVN. A: excitation by aFGF(1-15) in two doses. B: no effect by aFGF(114-140).

TABLE 3

Summary of aFGF(114-140) effects on neurons in the parvocellular part of the PVN

Dose	No. of neurons	Effect of aFGF(114-140)			
		E	E-I	I	N
0.67 ng/ml	n=19	3	0	2	14
1.33 ng/ml	n=13	2	0	0	11

E, excitation; E-I, excitation-inhibition; I, inhibition; N, no effect

neurons, 18 (69.2%) responded, and the remaining 8 (30.8%) were unchanged. Of the 18 responding neurons, 12 were excited (Fig. 2, A), 2 were inhibited, and the remaining 4 showed an excitation followed by an inhibition. Administration of 0.4 ng/ml aFGF(1-15) to 16 neurons excited 11 (68.8%) (Fig. 2, A), and did not affect 4 (25.0%). The remaining 1 (6.3%) neuron responded with an excitation-inhibition sequence.

The activity of 19 neurons was recorded for aFGF(114-140). Of these, the effect of both 0.67 ng/ml and 1.33 ng/ml aFGF(114-140) was tested in 13 neurons, and the remaining 6 had application of only 0.67 ng/ml aFGF(114-140). Results are summarized in Table 3. When 0.67 ng/ml aFGF(114-140) was applied to 19 neurons, 3 (15.8%) were excited, 2 (10.5%) were inhibited, and 14 (73.7%) were not changed (Fig. 2, B). When aFGF(114-140) in the higher dose (1.33 ng/ml) was applied to 13 neurons, 2 (15.4%) were excited, and the remaining 11 (84.6%) were unchanged (Fig. 2, B).

Latency of the effects of these agents was about 10 min to 28 min in average.

DISCUSSION

The PVN has a high density of CRF receptors [DeSouza et al., 1985; DeSouza, 1987], and contains a lot of CRF-immunoreactive

neurons, especially in the parvocellular part [Swanson et al., 1983]. A well-known role of the parvocellular part in the PVN is to mediate the release of adrenocorticotropic hormone (ACTH) via release of CRF. The release of ACTH also produces the secretion of adrenal steroids.

In the present study, 29.7% and 46.7% of neurons tested responded to application of 1 pg/ml and 2 pg/ml aFGF, respectively. Half or more than half of the responded neurons increased their firing rate. Application of 0.2 ng/ml and 0.4 ng/ml aFGF(1-15) also elicited responses in 46.2% and 68.8% of neurons tested, respectively. Of these responding neurons, more than two third increased the firing rate, as in the case of aFGF. In the present study, there is no evidence that the recorded neurons are CRF-containing neurons. However, the fact that many of randomly sampled neurons in the parvocellular part of the PVN increase their discharge rate by application of aFGF and aFGF(1-15) suggests that these agents elicit the release of CRF through the activation of CRF-containing neurons.

In contrast to aFGF(1-15), most of neurons tested for 0.67 ng/ml and 1.33 ng/ml aFGF(114-140) did not respond. In other studies, it has been demonstrated that infusion of aFGF(1-15) as well as aFGF into the 3CV suppresses food intake in rats [Sasaki et al., 1991: Oomura et al., 1992; Sasaki et al., 1994], and that perfusion of rat hippocampal slices with aFGF and aFGF(1-15) increases the magnitude of short-term potentiation (STP), transforms STP to long-term potentiation (LTP), and promotes the generation of LTP [Sasaki et al., 1994; Sasaki et al., 1995]. Even in these experiments, however, aFGF(114-140) had no effect. These results indicate that aFGF(1-15) has a biological effect that is similar to that of aFGF, and suggest that amino-terminal portion of aFGF is an active site for some physiological functions.

In our preliminary study, we have found that infusion of aFGF into the 3CV significantly elevates the level of plasma corticosterone. Taking the present result into consideration, it is possible that the elevation of plasma corticosterone level by

aFGF might be mediated by the activation of CRF-ACTH cascade triggered by the increase of discharge rate of CRF-containing neurons. To confirm it, further experiments will be required.

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REFERENCES

Baird, A. and Böhlen, P. (1990) Fibroblast growth factors. In: Peptide growth factors and their receptors II (eds Sporn, M.B. and Roberts, A.B.), pp. 369-472. Springer-verlag, Heidelberg.

Baird, A. and Walicke, P.A. (1989) Fibroblast growth factors. Br. Med. Bull. 45, 438-452.

DeSouza, E.B. (1987) Corticotropin-releasing factor receptors in the rat central nervous system: characterization and regional distribution. J. Neurosci. 7, 88-100.

DeSouza, E.B., Insel, T.R., Perrin, M.H., Rivier, J. Vale, W.W. and Kuhar, M.J. (1985) Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. J. Neurosci. 5, 3189-3203.

Dionne, C.A., Crumley, G., Bellot, F., Kaplow, J.M., Searfoss, G., Ruta, M., Burgess, W., Jaye, M. and Schlessinger, J. (1990) Cloning and expression of two distinct high-affinity receptors cross-reaction with acidic and basic fibroblast growth factors. EMBO J. 9, 2685-2692.

Hanai, K., Oomura, Y., Kai, Y., Nishikawa, K., Shimizu, N., Morita, H. and Plata-Salaman, C.R. (1989) Central action of acidic fibroblast growth factor in feeding regulation. Am. J. Physiol. 256, R217-R223.

Iguchi, K., Inoue, T., Ohshima, K. and Yanaihara, N. (1989) Immunochemical studies on acidic fibroblast growth factor (aFGF) using anti-synthetic peptide antisera. In: Peptide Chemistry 1989 (ed. Yanaihara, N.), pp. 285-290. Protein Research Foundation, Osaka.

Itoh, N., Yazaki, N., Tagashira, S., Miyake, A., Ozaki, K., Minami, M., Satoh, M., Ohta, M. and Kawasaki, T. (1994) Rat FGF receptor-4 mRNA in the brain is expressed preferentially in the medial habenular nucleus. Mol. Brain Res. 21, 344-348.

Keegan, K., Johnson, D., Williams, L.T. and Hayman, M. (1991) Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. Proc. Natl. Acad. Sci. USA 88, 1095-1099.

MacMillan, V., Walton-Roche, K. and Davis, J. (1993) Acidic fibroblast growth factor infusion reduces ischemic CA1 hippocampal damage in the gerbil. Can. J. Neurol. Sci. 20, 37-40.

Matsuo, A., Tooyama, I., Isobe, S., Oomura, Y., Akiguchi, I., Hanai, K., Kimura, J. and Kimura, H. (1994) Immunohistochemical localization in the rat brain of an epitope corresponding to the fibroblast growth factor receptor-1. Neuroscience 60, 49-66.

Oomura, Y., Sasaki, K., Suzuki, K., Muto, T., Li. A., Ogita, Z., Hanai,K., Tooyama, I., Kimura, H. and Yanaihara. N. (1992) A new brain glucosensor and its physiological significance. Am. J. Clin. Nutr. 55, 278S-282S.

Partanen, J., Mäkelä, T.P., Eerola, E., Korhonen, J., Hirvonen, H., Claesson-Welsh, L. and Alitaro, K. (1991) FGFR-4, a novel acidic fibroblast growth factor with a distinct expression pattern. EMBO J. 10, 1347-1354.

Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger, N., Kaplow, J., Crumley, G., Cionne, C., Jaye, M. and Schlessinger, J. (1989) Receptor for acidic fibroblast growth factor is related to the tyrosine kinase encoded by the fms-like gene (FLG). Proc. Natl. Acad. Sci. USA 86, 8722-8726.

Sasaki, K., Li, A., Oomura, Y., Muto, T., Hanai, K,, Tooyama, I., Kimura, H., Yanaihara, N., Yagi, H. and Hori, T. (1994) Effects of fibroblast growth factors and related peptides on food intake by rats. Physiol. Behav. 56, 211-218.

Sasaki, K., Oomura, Y., Figurov, A., Morita, N. and Yanaihara, N. (1995) An amino-terminal fragment peptide of acidic fibroblast growth factor modulates synaptic transmission in rat hippocampal slices. Brain Res. Bull. in press.

Sasaki, K., Oomura, Y., Figurov, A. and Yagi, H. (1994) Acidic fibroblast growth factor facilitates generation of long-term potentiation in rat hippocampal slices. Brain Res. Bull. 33, 505-511.

Sasaki, K., Oomura, Y., Suzuki, K., Hanai, K. and Yagi, H. (1992) Acidic fibroblast growth factor prevents death of hippocampal CA1 pyramidal cells following ischemia. Neurochem. Int. 21, 397-402.

Sasaki, K., Oomura, Y., Suzuki, K., Muto, T., Hanai, K., Tooyama, I., Kimura, H. and Yanaihara, N. (1991) Effects of fibroblast growth factors and platelet-derived growth factor on food intake in rats. Brain Res. Bull. 27, 327-332.

Sievers, J., Hausmann, B., Unsicker, K. and Berry, M. (1987) Fibroblast growth factors promote the survival of adult rat retinal ganglion cells after transection of the optic nerve. Neurosci. Lett. 76, 157-162.

Swanson, L.W., Sawchenko, P.E., Rivier, J. and Vale, W.W. (1983) Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36, 165-186.

Walicke, P.A. (1988) Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions.

J. Neurosci. 8, 2618-2627.
Walicke, P.A. (1989) Novel neurotrophic factors, receptors, and oncogenes. Ann. Rev. Neurosci. 12, 103-126.
Wanaka, A., Johnson, Jr., E.M. and Milbrandt, J. (1991) Localization of FGF receptor mRNA in the adult rat central nervous system by in situ hybridization. Neuron 5, 267-281.

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HYPOTHALAMUS REGULATES CALCIUM METABOLISM IN RATS

Aou, S., Shiramine, K., Ma, J., Matsui, H. and Hori, T.

Department of Physiology, Faculty of Medicine, Kyushu University, Fukuoka 812-82, Japan

Hypothalamic mechanisms of blood calcium homeostasis and their functional heterogeneity were investigated in rats. Electrical and chemical stimulation of the lateral hypothalamic area (LHA), the paraventricular nucleus (PVN) and the ventromedial nucleus of the hypothalamus (VMH) induced hypocalcemia. The hypocalcemic effect of PVN stimulation was suppressed by vagotomy of the thyroid/parathyroid branches, while that of LHA and VMH stimulation was eliminated by gastric vagotomy. Immobilization (IMB) stress elicited hypocalcemia through VMH-gastric vagal activation. Both IMB- and LHA stimulation-induced hypocalcemia was antagonized by muscarinic antagonist and histamine H2 blocker. The former was also blocked by α-blocker and gastrin release inhibitor, while the latter was antagonized by an β-blocker. The results suggest that the hypothalamic nuclei are involved in regulation of blood calcium homeostasis via the gastric or thyroid/parathyroid vagus. Muscarinic, histamine H2, adrenergic and gastrin receptors mediate the hypocalcemic effect of the hypothalamo-vagal activation depending on behavioral conditions and receptor subtypes.

Key Words: hypothalamus, calcium metabolism, gastric vagus, histamine

INTRODUCTION

In general, endocrine and metabolic functions are under the control of the central nervous system through the autonomic nervous system and/or

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the pituitary gland, however, little is still known about calcium homeostasis which is well known to be regulated by calciotropic hormones through their actions on peripheral organs such as the kidney, bone and intestine. Recently it has been shown that the hypothalamic neurons are sensitive to calciotropic hormones such as calcitonin [Gerber et al., 1985; Shimizu et al., 1986; Twery et al., 1985] and parathyroid hormone [Matsui et al 1995]. An intracerebroventricular injection of calcitonin reduce blood calcium levels [Goltzman and Tannnenbaum, 1987] while that of PTH reverses urethane-induced hypocalcemia in a dose dependent manner [Matsui et al, 1995]. These findings strongly suggest that the hypothalamus is involved in central regulation of calcium homeostasis. We recently found that an electrical stimulation of the lateral hypothalamic area (LHA), the paraventricular nucleus (PVN) or the ventromedial nucleus of the hypothalamus (VMH) decrease the concentration of blood calcium in anesthetized rats [Aou et al, 1993a; Ma et al, 1994a,b]. The calcium-lowering effects of electrical stimulation of the LHA and the VMH was blocked by bilateral gastric vagotomy, while PVN stimulation-induced hypocalcemia was suppressed by a vagotomy of the thyroid/parathyroid branches [Ma et al, 1994a,b]. In the present study, we further investigated the functional, anatomical and neurochemical heterogeneity of the calcium-regulating hypothalamo-vagal pathways in rats.

MATERIALS AND METHODS

Female Wistar rats weighing 150-250 g were used. All surgical treatments were made under pentobarbital anesthesia (40 mg/kg, ip). For the stimulation study, either a single monopolar/concentric bipolar stainless steel electrode or a 30 G stainless steel cannula with a 23 G guide cannula was stereotaxically implanted into the LHA (A6.44, L2.0, H1.5), the VMH (A6.44, L0.7, H0.6) or the PVN (A7.0, L0.5, H2.0), according to the atlas of Paxinos and Watson (1986). In some rats, a

selective vagotomy of the thyroid/parathyroid branches or the gastric branches was also performed. For the lesion study, bilateral electrical lesions of the hypothalamic nuclei were made by passing an anodal DC current of 1 mA for 20s. In the pharmacological study, muscarinic, adrenergic (α and β) and histamine H2 receptor antagonists, and inhibitors of gastrin release were injected either intravenously or intraperitoneally 5 or 20 min before hypothalamic stimulation or immobilization, respectively.

The rats in the immobilization-stressed groups were immobilized in the supine position by tying down all four limbs with adhesive tape on a plastic board for 2 h. The rats subjected to the water-restrained stress were immobilized and then immersed in water at a depth reaching the xiphoid for 20 h to obtain a steady phase of gastric lesions [Aou et al., 1994; Ma et al., 1994c].

The concentration of the ionized calcium in the whole blood taken from the heart or the jugular vein, was measured by a Ca^{2+}-selective electrode (634 Ca^{2+}/pH Analyzer, Ciba Corning). Sites of stimulation or lesions were histologically examined. Only the data obtained from the animals that were found to have the appropriate hypothalamic stimulation or lesions were used for the analyses. Student's *t*-test or one way analysis of variance with a post hoc Bonferroni's test was used for the statistical analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS

Effects of hypothalamic stimulation on the blood calcium levels

Unilateral electrical stimulation (0.1 mA, 0.5 ms, 30 Hz, 60 min, monopolar) of the PVN and the LHA, but not the VMH, induced a significant decrease (0.05-0.07 mM) in the blood ionized calcium in the pentobarbital anesthetized rats as shown in Table 1. Using different stimulation parameters (0.5 mA, 2ms, 10 Hz, 60 min, bipolar), which have previously been shown to be effective for eliciting gastric acid secretion [Ishikawa et al., 1983], not only LHA- and PVN-stimulation but also VMH stimulation were found to be effective for inducing hypocalcemia. The hypocalcemic effect of PVN-stimulation was suppressed by a vagotomy of the thyroid/parathyroid branches, while that of LHA-and VMH-stimulation was eliminated by a gastric vagotomy (Table 1).

Table 1. The effects of a selective vagotomy on hypocalcemia induced by electrical stimulation of the hypothalamus

Area	Surgery	Control	Stimulated
PVN	Intact	1.40 ± 0.02 (11)	1.33 ± 0.02 (8)*
	Thy/parath vagx	1.45 ± 0.02 (6)	1.40 ± 0.04 (4)
	Gastric vagx	1.41 ± 0.02 (6)	1.33 ± 0.01 (6)*
LHA	Intact	1.43 ± 0.02 (10)	1.37 ± 0.01 (14)*
	Thy/parath vagx	1.42 ± 0.01 (7)	1.35 ± 0.02 (7)*
	Gastric vagx	1.39 ± 0.03 (8)	1.39 ± 0.02 (8)
VMH	Intact (monopolar)	1.38 ± 0.02 (10)	1.37 ± 0.02 (6)
	Intact (bipolar)	1.39 ± 0.02 (7)	1.32 ± 0.02 (6)**
	Thy/parath vagx	1.43 ± 0.01 (5)	1.36 ± 0.01 (7)**
	Gastric vagx	1.39 ± 0.03 (4)	1.39 ± 0.01 (6)

The blood concentration of ionized calcium was measured just after 60 min of stimulation in stimulated groups or at corresponding time in non-stimulated control groups. LHA: lateral hypothalamic area; PVN: paraventricular hypothalamic nucleus; VMH: ventromedial hypothalamic nucleus. Thy/parath vagx, vagotomy of thyroid/parathyroid branches. Monopolar: 0.1 mA, 0.5 ms, 30 Hz, 60 min, monopolar stimulation; Bipolar: 0.5 mA, 2 ms, 10 Hz, 60 min, bipolar stimulation using a concentric bipolar stimulation electrode. $*p < 0.05$, $**p < 0.01$.

Electrical stimulation activates not only neuronal cell bodies but also passing fibers, therefore we injected bicuculline into the hypothalamic nuclei to examine whether the hypocalcemia is induced by activation of hypothalamic neurons in normal awake condition. The injection of bicuculline methiodide (0.12 mM, a $GABA_A$ antagonist) into these hypothalamic nuclei (LHA, 0.5 μl; VMH, 0.3 μl; PVN, 0.2 μl; 0.1 μl/min) elicited hypocalcemia (0.04-0.1 mM) in freely moving rats (Fig. 1). The blood calcium level significantly decreased 15 min after the start of the injection (in comparison with that of the Ringer-injected control group), then the difference reached a peak 30 min after the injection. It thereafter returned to the basal level within 60 min after the injection.

The hypocalcemic effect of the bicuculline-injection was observed when the cannula tips were located within the LHA, PVN and VMH. When the injection sites were outside of these areas, the hypocalcemic effects were either greatly attenuated or disappeared altogether.

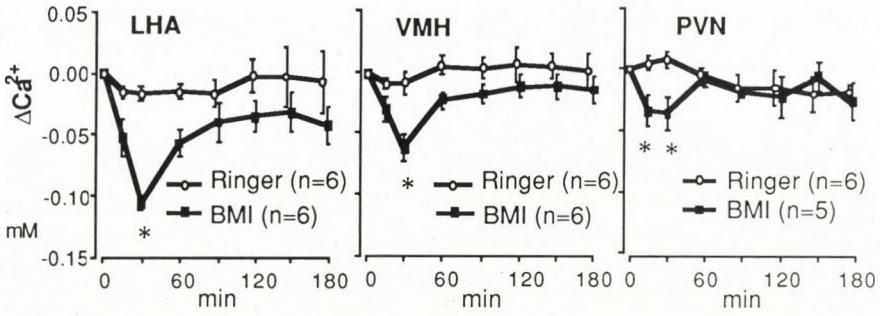

Fig. 1. Hypocalcemic effects of chemical stimulation (bicuculline methiodide, BMI) of the lateral hypothalamic area (LHA), ventromedial nucleus (VMH) and paraventricular nucleus (PVN) of the hypothalamus. Bicuculline methiodide (BMI) was injected to each hypothalamic nuclei at time 0. $*p < 0.05$

Effect of hypothalamic lesions on stress-induced hypocalcemia

Immobilization (IMB) has been shown to elicit hypocalcemia [Hofmann et al., 1979; Morimoto et al., 1986] and the gastric vagus mediates IMB-induced hypocalcemia [Ma et al., 1993]. We examined the effects of lesions of the PVN, the VMH and the LHA on IMB-induced hypocalcemia [Aou et al 1993]. The IMB stress induced a significant decrease in the blood ionized calcium level from 1.45 $\pm$ 0.01 to 1.40 $\pm$ 0.01 mM (n = 17, $p < 0.01$) in the rats undergoing sham surgery. Bilateral lesions of the PVN or the LHA did not eliminate IMB-induced hypocalcemia. The concentration of blood calcium significantly decreased from 1.48 $\pm$ 0.01 to 1.42 $\pm$ 0.01 mM (n = 14, $p < 0.001$) in the PVN lesioned rats and from 1.46 $\pm$ 0.02 to 1.42 $\pm$ 0.01 mM (n = 15, $p < 0.01$) in the LHA lesioned rats. In contrast, bilateral lesions of the VMH completely eliminated the

IMB-induced hypocalcemia. There was no difference in the mean concentration of blood calcium before (1.46 ± 0.03 mM) and after IMB (1.47 ± 0.03 mM) in the VMH lesioned rats (n = 9).

Furthermore, the VMH lesions eliminated the water-restraint stress-induced hypocalcemia and gastric ulcers, while the PVN lesions exacerbated them [Aou et al., 1994]. The basal levels of blood ionized calcium before the stress showed no significant differences among the hypothalamus-lesioned and the sham-operated animals (VMH-sham: 1.34 ± 0.02 mM, n = 11; VMH-lesioned: 1.34 ± 0.01 mM, n = 10; PVN-sham: 1.34 ± 0.02 mM, n = 12; PVN-lesioned: 1.36 ± 0.01 mM, n = 15). After water-restraint for 20 h, a highly significant decrease in the concentrations of blood calcium was observed in all the sham-operated animals (VMH-sham: 1.23 ± 0.02 mM; PVN-sham: 1.24 ± 0.02 mM, $p < 0.001$, respectively, paired t-test) as well as in the PVN-lesioned rats (1.19 ± 0.03 mM, $p < 0.001$, paired *t*-test). In contrast, the VMH-lesioned rats did not show any significant decrease (1.33 ± 0.03 mM) in the concentration of blood calcium. After the water-restraint stress, the blood calcium levels of the VMH-lesioned animals were significantly higher than those of the VMH-sham rats ($p < 0.05$, Bonferroni's test), whereas the concentration of blood calcium was significantly lower in the PVN-lesioned animals than in the PVN-sham rats ($p < 0.05$, Bonferroni's test).

Effect of pharmacological treatments on immobilization- and LHA stimulation-induced hypocalcemia

Because the gastric vagal activation has been shown to promote release of acetylcholine, gastrin and histamine, we examined the effects of antagonists or release inhibitors of these chemicals on the hypocalcemic responses in awake rats. The hypocalcemia induced by bicuculline injection to the LHA and IMB-induced hypocalcemia were antagonized by atropine methyl bromide (6mg/kg, ip), peripherally acting muscarinic

antagonist, and ranitidine (5mg/kg, ip), histamine H2 blocker. There was no significant difference in the mean concentration of blood calcium before (1.38 ± 0.01 and 1.35 ± 0.01 mM) and 30 min after (1.36 ± 0.01 and 1.32 ± 0.02 mM) bicuculline injection to the LHA in the atropine- and ranitidine-treated rats (n = 6, both), respectively. The blood calcium levels of IMB-stressed rats treated with atropine (1.52 ± 0.01 mM, n = 10) or ranitidine (1.49 ± 0.02 mM, n = 8) did not differ from those of non-stressed control rats (atropine treated, 1.51 ± 0.01 mM, n = 10; ranitidine treated, 1.49 ± 0.01 mM, n = 8).

Although both muscarinic and H2 antagonists showed similar antagonizing effects on hypocalcemic responses to the LHA stimulation and IMB, secretin (6μg/kg), an inhibitor of gastrin release, differently affected these responses. As shown in Table 2, it antagonized calcium lowering effect of IMB but not that of LHA stimulation.

It has been shown that the gastric vagus nerves contain adrenergic fibers (Graffner et al 1984). Thus, we investigated the possible involvement of adrenergic mechanism. The hypocalcemia induced by LHA stimulation was blocked by peripherally acting β-adrenergic antagonist (nadolol, 1 mg/kg, ip) but not by α-blocker (phenoxybenzamine, 3 mg/kg, ip), while the IMB-induced hypocalcemia was antagonized by phenoxybenzamine but not by nadolol (6 mg/kg, ip)(Table 2).

Table 2. The effects of various pharmacological treatments on LHA stimulation- and immobilization (IMB)-induced hypocalcemia

Inhibitor/ antagonist	LHA stimulation		IMB	
	Before	After (30 min)	Control	Stress (2h)
Vehicle	1.40±0.01	1.31±0.01 (7)***	1.47±0.01 (10).	1.39±0.02 (10)**
Secretin	1.35±0.01	1.28±0.01 (5)**	1.49±0.02 (7)	1.52±0.02 (7)
PBZ	1.34±0.01	1.28±0.01 (6)**	1.48±0.02 (10)	1.51±0.02 (10)
Nadlol	1.38±0.01	1.37±0.01 (7)	1.49±0.03 (7)	1.40 ± 0.02 (7)*

LHA was stimulated by bicuculline methiodide (0.12 mM, 0.5 μl). PBZ: phenoxybenzamine. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

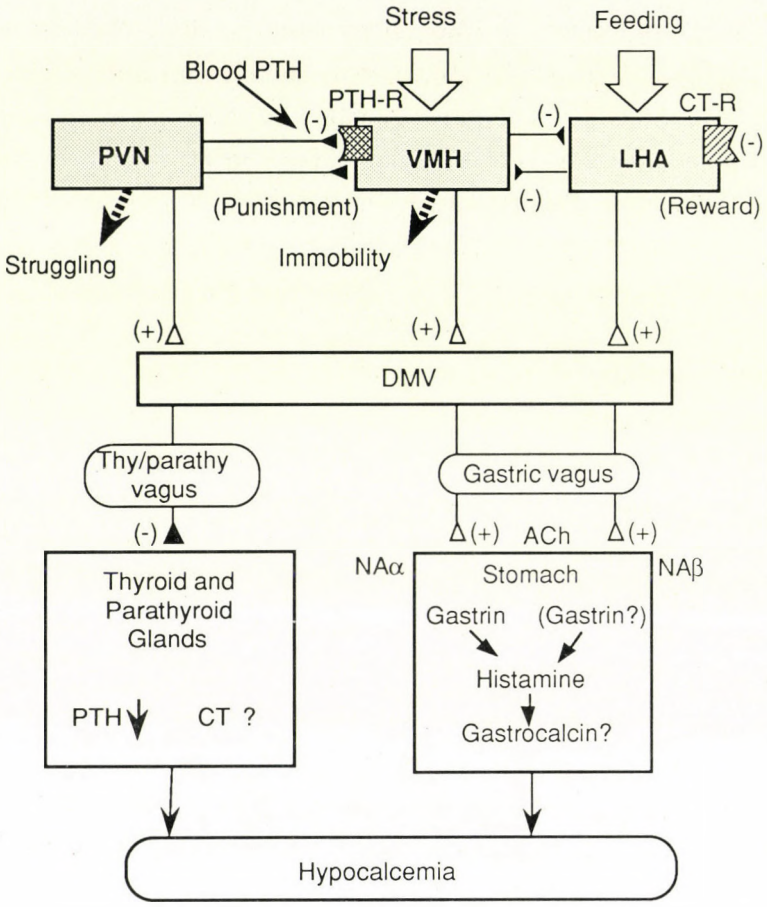

Fig. 2. Schematic diagram of hypothalamo-vagal pathways which regulate blood calcium homeostasis. See text

DISCUSSION

The present study demonstrated that the hypocalcemic functions of the LHA and the VMH were mediated by the gastric vagus, while that of the PVN was promoted by the vagus nerves innervating the thyroid and parathyroid glands (Fig. 2). All these hypothalamic areas have been shown

to send excitatory inputs to neurons of the dorsomotor nucleus of the vagus (DMV) [Nishimura and Oomura, 1987]. An electrical stimulation of the unilateral vagal trunk resulted in a suppression of the secretory activity of the parathyroid gland [Isono et al., 1982], while gastric vagal stimulation is well known to facilitate gastric acid secretion through acetylcholine, gastrin and histamine releases. The muscarinic and histamine H2 receptors may be common mediators for the gastric vagus-mediated hypocalcemia induced by LHA and VMH stimulation. Besides the muscarinic and histamine receptors, gastrin and α-adrenoceptors are also involved in hypocalcemia induced by IMB, while β-adrenoceptors play a role in LHA stimulation-induced hypocalcemia (see Table 2).

It has been shown that food intake elicits transient hypocalcemia during the initial phase of food intake along with the secretion of gastrin, which is a hypocalcemic agent [Hakanson et al., 1990]. We found that LHA stimulation increased the serum gastrin levels (our unpublished data). The LHA, which is known to be the "feeding center" as well as reward-related area, may thus play some role in the feeding-related calcium regulation. In contrast, the VMH is involved in calcium regulation during stress conditions as suggested by the present study. VMH and LHA neurons are sensitive to PTH and calcitonin, respectively [Matsui et al., 1995; Shimizu et al., 1986] and PVN neurons show parathyroid hormone (PTH) immunoreactivity [Pang et al., 1988]. Therefore, both the previous and present findings suggest that hypothalamic neurons receive calciotropic hormone-mediated signals and regulate blood calcium homeostasis via the autonomic nervous system (Fig. 2).

Although the VMH and PVN are well known to be involved in stress responses and aversive/aggressive behaviors, our lesion studies have revealed that lesions of the PVN and the VMH oppositely altered the susceptibility of the animals to stress-induced hypocalcemia and gastric damage. Since a similar increase in body weight was found in the VMH- and PVN-lesioned rats [Aou et al., 1994], the effects of these hypothalamic

lesions on stress-responses seem to be due to their direct actions rather than the secondary effects of changes in food consumption and calcium uptake through their digestive organs. We have recently found that these gastric responses to stress conditions are intimately linked with each other as well as with the behavioral activities in a forced-swimming test [Ma et al., 1994c]. The behavior observed during the forced-swimming test differed between the VMH- and PVN-lesioned animals. Lesions of the VMH decreased the duration of immobility (index of depressive behavior) and increased that of struggling, whereas lesions of the PVN elicited the opposite effect on VMH-lesions [Aou et al., 1994]. The possible linkage between depression and stress ulcer has been well investigated in human patients as well as in animals [Feldman et al., 1986; Pare, 1989]. A similar linkage, that is a significant correlation between the severity of depressive symptoms and the decreased plasma calcium level, has also been found in the acute phase of human depressed patients [Linder et al., 1989]. Further studies to evaluate the possible hypothalamic involvement in affective disorders as well as the possible role of hypocalcemia as an index of lowered mood may therefore provide a better understanding of the neural and humoral mechanisms of affective diseases and related physical dysfunctions, such as stress ulcer.

Although the hypothalamus is well known to be deeply involved in neurohumoral control of motivated behaviors as well as autonomic, endocrine and visceral functions under normal and stress conditions, its precise physiological and behavioral roles are still not well understood. We found that calcium metabolism is also under the control of the hypothalamus. Both the LHA and the VMH have gastric vagus-mediated hypocalcemic funtion but their physiologivcal significance seems to be quite different. The findings, which demonstrate the alleviating effects of VMH lesions on gastric pathology and depressive behavior suggest that the VMH may be one of the etiological brain regions that elicit these stress-related pathological and psychological changes. In contrast, PVN-

lesions aggravate stress-induced gastric pathology and behavioral despair. This finding supports the hypothesis that the PVN is involved in some defensive functions to cope with stress.

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REFERENCES

Aou, S., Ma, J. and Hori, T. (1993a) Hypothalamic involvement in stress-induced hypocalcemia in rats. Neurosci. Lett. 158, 197-200.

Aou, S., Ma, J., Hori, T. and Tashiro, N. (1994) Hypothalamic linkage in stress-induced hypocalcemia, gastric damage, and emotional behavior in rats. Am. J. Physiol. 267, R38-R43.

Aou, S., Ma, J., Shiramine, K. and Hori, T. (1993b) The stomach is the etiologic organ for immobilization-induced hypocalcemia in rats. Am. J. Physiol. 265, R1376-R1379.

Feldman, M., Walker, P., Green, J. L. and Weingarden, K. (1986) Life events stress and psychosocial factors in men with peptic ulcer disease. Gastroenterology 91, 1370-1379.

Gerber, U., Felix, D., Felder, M. and Schaffner, W. (1985) The effects of calcitonin on central neurons in the rat. Neurosci. Lett. 60, 343-348.

Goltzman, D. and Tannenbaum, G.S. (1987) Induction of hypocalcemia by intracerebroventricular injection of calcitonin: evidence for control of blood calcium by the nervous system. Brain Res. 416, 1-6.

Graffner, H., Ekelund, M., Hakanson, R., Oscarson, J., Rosengren, E. and Sundler F (1984) Effects of upper abdominal sympathectomy on gastric acid, serum gastrin, and catecholamines in the rat gut. Scand. J. Gastroenterol.19, 711-716.

Hakanson, R., Persson, P. and Axelson, J. (1990) Elevated serum gastrin after food intake or acid blockade evokes hypocalcemia. Regul. Pept. 28, 131-136.

Hofmann, P., Schwill, P. O. and Thun, R. (1979) Hypocalcemia during restraint stress in rats. Res. Exp. Med. Berlin 175, 159-168.

Ishikawa, T., Nagata, M. and Osumi, Y. (1983) Dual effects of electrical stimulation of ventromedial hypothalamic neurons on gastric acid secretion in rats. Am. J. Physiol. 245, G265-G269.

Isono, H., Shoumura, S., Ishizaki, N., Emura, S., Hayashi, K., Iwasaki, Y. and Kitamura, Y. (1982) Effects of electrical stimulation of the vagus nerve on the ultrastructure of the rabbit parathyroid gland. Okajimas Folia Anat. Jpn., 58, 453-466.

Linder, J., Brismar K., Beck-Friis, J., Saaf, J. and Wetterberg, L. (1989) Calcium and magnesium concentrations in affective disorder: difference between plasma and serum in relation to symptoms. Acta Psychiatr. Scand. 80, 527-537.

Ma, J., Aou, S. and Hori, T. (1994a) Hypothalamic stimulation induces vagally mediated hypocalcemia in the rat. Brain Res. Bull., 33, 65-69.

Ma, J., Aou, S. and Hori, T. (1994b) Ventromedial hypothalamus mediates stress-induced hypocalcemia via the gastric vagus in rats. Brain Res. Bull. 34, 41-45.

Ma, J., Aou, S., Hori, T. and Ding, J. (1994c) Linkage of stress-induced hypocalcemia, gastric lesions, and emotional behavior in Wistar-Kyoto rats. Am. J. Physiol. 266, R960-R965.

Ma, J., Aou, S., Matsui, H. and Hori, T. (1993) Gastric vagus mediates immobilization-induced hypocalcemia in rats. Am. J. Physiol. 265, R609-R614.

Matsui, H., Aou, S., Ma, J. and Hori, T. (1995) Central actions of parathyroid hormone on blood calcium and hypothalamic neuronal activity in the rat. Am. J. Physiol. 268, R21-R27.

Morimoto, S., Fausto, A., Birge, S. J. and Avioli, L. V. (1986) Effect of short- and long-term stress on plasma calcium and calcitonin in the rat. Horm. Metabol. Res. 18, 818-820.

Nishimura, H. and Oomura, Y. (1987) Effects of hypothalamic stimulation on activity of dorsomedial medulla neurons that respond to subdiaphragmatic vagal stimulation. J. Neurophysiol. 58, 655-675.

Pang, P. K. T., Kaneko, T. and Harvey, S. (1988) Immunocytochemical distribution of PTH immunoreactivity in vertebrate brains. Am. J. Physiol. 255, R643-R647.

Pare, W. P. (1989) "Behavioral despair" test predicts stress ulcer in WKY rats. Physiol. Behav. 46, 483-487.

Paxinos, G. and Watson, C. (1986) The rat brain in stereotaxic coordinates. 2nd ed. San Diego: Academic press, 1986.

Shimizu, N. and Oomura, Y. (1986) Calcitonin-induced anorexia in rats: evidence for its inhibitory action on lateral hypothalamic chemosensitive neurons. Brain Res. 367, 128-140.

Twery, M.J. and Moss, R.L. (1985) Calcitonin and calcitonin gene-related peptide alter the excitability of neurons in rat forebrain. Peptides 6, 373-378.

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OSMORESPONSIVENESS OF THE RAT SUPRAOPTIC NUCLEUS *IN VIVO* DEPENDS ON GLUTAMATERGIC INPUTS

Dyball, R. E. J., McKenzie, D. N. and Thomas, G. P. L.

Department of Anatomy, University of Cambridge, Cambridge CB2 3DY

Summary: Intracellular recording from supraoptic nucleus (SON) neurones in hypothalamic slices revealed that a 40 mOsm change in osmolality had little effect on membrane conductance. However, intracellular recordings *in vivo* revealed a significant increase in EPSP frequency after the plasma osmolality had been raised by approximately 10 mOsm. We recorded extracellularly *in vivo* from 18 antidromically identified SON neurones in urethane-anaesthetised male Wistar rats, while hypertonic saline was infused intravenously (1.05M NaCl, 50 µl/min for 35 minutes). In 9 experiments an intracerebroventricular (ICV) injection of 5µl 10mM kynurenic acid was given 5 minutes prior to the start of the infusion. Kynurenate significantly reduced the osmotic response ($P<0.001$, Student's t-test); the rate of increase in spike frequency was reduced from 0.089 ± 0.004 Hz/min ($n=9$) to 0.035 ± 0.003 Hz/min ($n=9$). Kynurenic acid did not reduce the basal firing rate of SON neurones, but in osmotically stimulated neurones, it reduced the firing rate to basal levels. Hence the osmoresponsiveness of SON neurones depends on a glutamatergic input that is independent of the mechanisms that maintain basal electrical activity.

Key words: supraoptic nucleus; neurosecretory cells; *in vivo*; osmotic stimulation; kynurenate.

INTRODUCTION

Verney (1947) demonstrated that a rise in plasma osmolality above the normal value of 290-300 mosm/kg increased AVP secretion. Numerous more recent studies support this, revealing enhanced AVP and oxytocin (Gardiner *et al.* 1985) release under hyperosmotic conditions and reduced release in hypoosmotic states. In addition the firing rate of magnocellular neurosecretory cells (MNCs) has been shown, *in vivo* in the rat, to increase in response to a systemic hyperosmotic stimulus (Brimble and Dyball, 1977).

MNCs are intrinsically osmosensitive. *In vitro*, rat SON neurones are depolarised by hyperosmotic stimuli presented in low Ca^{2+}, high Mg^{2+} solutions (Mason, 1980). Conversely, in solutions with reduced osmolality rat SON neurones hyperpolarise and show a reduction in membrane conductance (Oliet and Bourque, 1993). It appears that a non-

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selective cation conductance through the cell membranes is raised by hyperosmotic solutions and depressed by hypo-osmotic stimuli. However, these studies used large changes in osmolality (30 mOsm), which are probably greater than those encountered after 2 days dehydration *in vivo*. Also the experiments were carried out on acutely isolated MNCs, in which only about 20% of the cell membrane is present. Hence it is unlikely that intrinsic properties alone can account for the osmotic response of MNCs.

Areas other than the SON and PVN have been identified as possible central osmoreceptor sites. These include the circumventricular nuclei of the diencephalon the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) of the ventral AV3V region. The majority of the neurones in the AV3V region display spike behaviour that is inhibited by low osmolality solutions and excited under hyperosmotic conditions. This sensitivity is intrinsic to these cells and remains apparent during synaptic transmission block (Richard and Bourque, 1992). The OVLT is thought to lack a blood brain barrier making it a likely osmoreceptor site (Honda *et al.*, 1990). Electrolytic lesions of this site impair osmotically induced release of AVP and oxytocin and reduce the osmosensitivity of neurones in the PVN (Honda *et al.*, 1989) and SON (Chaudry *et al.*, 1989).

Neuroanatomical studies using retrograde tracers indicate that direct projections exist from the OVLT to the SON (Tribollet *et al.*, 1985) and to the PVN (Tribollet and Dreifuss, 1981). Sawchenko and Swanson (1983) have demonstrated that the neurones of the Median Preoptic Nucleus (MnPO) project directly to the SON. Electrophysiological studies by Honda *et al.* (1990) found excitatory efferents from the SON that project to the osmoresponsive cells of the OVLT. These OVLT cells send axons to the MnPO where they synapse and relay projections back to the SON. Honda *et al.* proposed that osmoreceptive neurones in the OVLT, MnPO and SON respectively, cooperate to mutually increase their firing rates in hyperosmotic conditions.

The supraoptic and paraventricular MNCs may also receive peripheral osmoreceptor information from splanchnic osmoreceptors directly via the nucleus tractus solitarius (NTS) (Kobashi and Adachi, 1985). The NTS also projects to the MnPO (Tribollet *et al.*, 1985) and Parabrachial Nucleus, which in turn project to the MNCs (Bourque *et al.*, 1994).

The evidence for extensive extrinsic osmoreceptor inputs to the MNCs of the SON and PVN is supported by *in vitro* intracellular recording studies using the hypothalamic explant preparation. These demonstrate that osmotic stimuli produce changes in the rate of spontaneous excitatory post synaptic potentials (sEPSPs) (Bourque *et al.*, 1994) occurring on the MNC membrane. Hyperosmotic stimulation of the OVLT dramatically enhances sEPSP frequencies in MNCs but does not affect the MNC membrane potential (Richard and Bourque, 1992).

Excitatory Amino Acids, notably glutamate, have been strongly implicated in the generation of sEPSPs at the MNCs (Gribkoff and Dudek, 1988). Kynurenic acid, a broad spectrum EAA antagonist (Watkins *et al.*, 1987), *in vitro*, eliminated sEPSPs in supraoptic MNCs. These sEPSPs were generated either spontaneously or from electrical stimulation of the dorsolateral hypothalamus (Richard and Bourque, 1992). Kynurenic acid, *in vitro*, reversibly abolished MNC sEPSPs evoked by OVLT hyperosmotic stimulation. *In vivo*,

local pressure application of kynurenic acid eliminated spontaneous spike activity in phasic cells in the SON.

In this study we aimed to investigate the extent to which glutamate receptors are responsible for generating the response of MNCs to increased systemic osmotic pressure. Nearly all the experiments were carried out *in vivo* since a great diversity of systems integrate in the intact animal and we did not wish to compromise the function of the osmoreceptor complex.

We injected kynurenic acid, a broad spectrum glutamate antagonist (Watkins *et al.*, 1987), into the lateral ventricle of the rat brain. Kynurenic acid has been used in many past studies of EAA activity in rat SON (Gribkoff and Dudek, 1988; Richard and Bourque, 1992; Nissen *et al.*, 1994) and its effects in the rat brain are well characterised, notably in the hippocampus and spinal cord (Ganong *et al.*, 1983). The hypertonic stimulation involved an intravenous (jugular vein) infusion of hypertonic saline. Our results imply that the osmoresponsiveness of MNCs is, to a large extent, dependent on glutamatergic inputs.

MATERIALS AND METHODS

The study was conducted on 12 male Wistar rats (250g-400g) anaesthetised with urethane (1.3g/kg, IP). We used the ventral surgical approach to the hypothalamus (Leng, 1980). Recording electrodes were filled with 0.5M Na Acetate for extracellular recording (resistance 10-20MΩ) and 4M potassium acetate for intracellular recording (resistance 80-100MΩ). The electrodes were inserted into the SON through a slit in the dura. Signals were conventionally amplified, displayed on an oscilloscope and recorded onto digital audio tape. Action potentials were counted using a voltage gated window discriminator connected to a computer programmed for on-line spike train analysis. *In vivo*, supraoptic MNCs were identified by antidromic activation following stimulation of their axons with a bipolar electrode placed on the neural stalk. The non-specific glutamate receptor antagonist kynurenic acid (10 mM in isotonic saline) was injected into the third ventricle of the rat brain via a cannula inserted through a guide tube cemented to the skull on the dorsal side (Ennis *et al.*, 1992). The dose was 5µl injected at a rate of <0.1µl/sec. Kynurenic acid blocks the AMPA and kainate glutamate-receptor subtypes, and the NMDA receptor complex at the glycine binding site (Watkins *et al.*, 1987). By way of a control, ICV injections of vehicle were made to confirm that it had no effect. Slices containing the SON (350-450 µm thick) used for *in vitro* experiments were cut from a block of tissue containing the hypothalamus, and perfused with Yamamoto's medium (124 mM NaCl, 26 mM $NaHCO_3$, 5 mM KCl, 1.3 mM $MgSO_4$, 1.24 mM KH_2PO_4, 2.4 mM $CaCl_2$, 11 mM glucose; saturated with 95% O_2, 5% CO_2 at 35 C); hypertonic solutions were made by adding NaCl to the required osmolality. To assess EPSP frequency, ten intracellular traces were collected, and printed onto unmarked sheets. The sheets were then scored for EPSP frequency by five experienced electrophysiologists and the values given as mean $\pm$ SEM. The scoring was carried out as a blind trial, with the scorers having no indication of which traces were taken under control or hypertonic conditions.

RESULTS

The effect of hypertonic stimulation on MNC membrane resistance measured *in vitro*

To determine whether raising the osmolality of the perfusing medium altered the membrane characteristics of individual MNCs, the membrane resistance of 4 magnocellular cells was determined *in vitro*. The recordings were made conventionally from cells with an input resistance of at least M. It can be seen from Fig. 1 that raising the osmolality of the perfusing medium marginally reduced the membrane resistance of the cells tested but the difference was not significant, confirming the results of Richard and Bourque (1992).

The effect of hypertonic stimulation on EPSP frequency recorded from MNCs *in vivo*

If it is true that excitatory input from other regions of the CNS excites MNCs synaptically during osmotic stimulation, it should be possible to confirm this by monitoring EPSP frequency during intracellular recording in osmotically stimulated animals. It is difficult to make intracellular recordings *in vivo* but an example of one is illustrated in Fig. 2 in which the depolarising PSP frequency under control conditions is compared with that 10 min after an IP injection of 1ml of 1.5M NaCl solution. Such an injection raises plasma osmolality by approximately 10 mosmoles (Brimble and Dyball, 1977). There was a clear increase in PSP frequency (from 5.06±0.56 Hz to 8.81±0.47 Hz) which was statistically significant ($P<0.01$, Student's t-test).

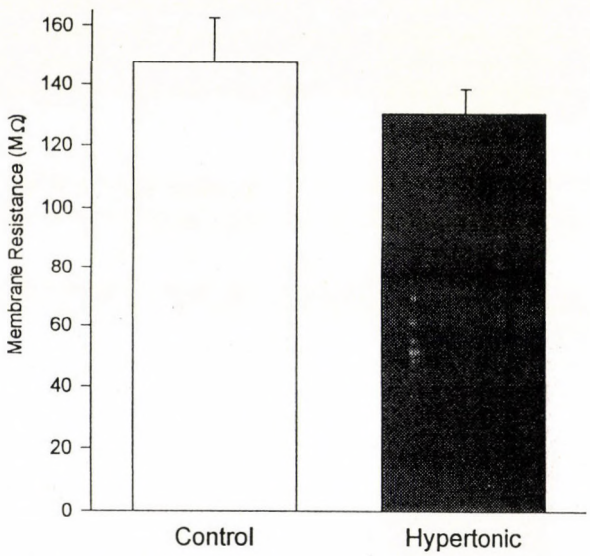

Fig. 1: The effect of hypertonic saline on membrane resistance of MNCs *in vivo*.
Mean (±SEM) input resistance of 4 magnocellular neurones measured in rat hypothalamic slices, under isotonic (control) and hypertonic (+40 mOsm) conditions. The difference is not significant

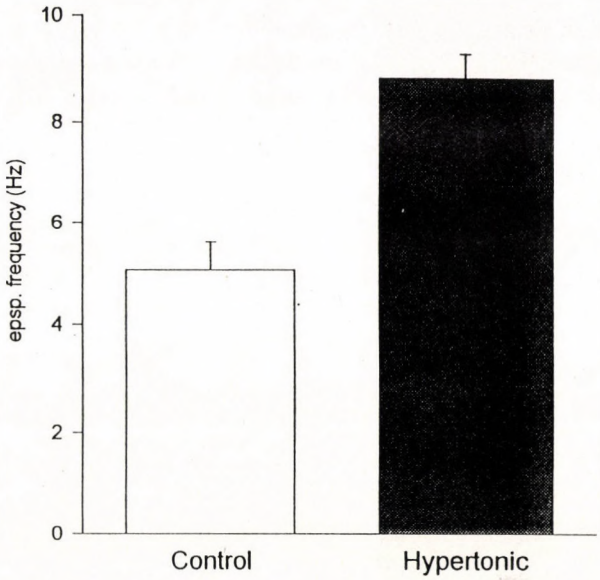

Fig. 2: The effect of hypertonic conditions on EPSP frequency in MNCs *in vivo*.
Mean (±SEM) EPSP frequency recorded from amagnocellular neurone in the supraoptic nucleus of a urethane-anaesthetised rat, before (control), and after plasma osmolality was raised by ~10 mOsm. The difference in EPSP frequency is significant ($P < 0.01$, Student's t-test)

The effect of kynurenic acid on MNC spike activity during osmotic stimulation.

In response to hypertonic infusion a group of 9 control cells recorded extracellularly showed a clear increase in spike frequency 10 minutes after the start of the infusion and continued to increase until the end of the infusion. The mean spike frequency rose from 3.96 ± 0.10 Hz ($n=9$) to 6.66 ± 0.46 Hz ($n=9$), 40 minutes later. This increase occurred in a linear manner and a straight line was fitted to it using the method of least squares. This revealed a rate of increase in mean spike frequency of 0.089 ± 0.004 Hz/min.

In 9 experiments a single ICV injection of kynurenic acid was given 5 minutes prior to the infusion. This group of cells showed a decrease in spike frequency immediately after the injection but the firing rate again increased in response to hypertonic infusion. This rise was, as in controls, a linear function of time. A straight line was fitted to it using the method of least squares to give a rate of increase in mean spike frequency of 0.035 ± 0.003 Hz/min. This rate of increase was significantly lower ($P < 0.001$, Student's t-test) than that observed in the control group. After the infusion the mean spike frequency of the kynurenic acid treated population (4.78 ± 0.44 Hz, $n=9$) was significantly less ($P < 0.02$, Student's t-test) than the mean spike frequency of controls (6.66 ± 0.46 Hz, $n=9$).(see Fig. 3)

The kynurenic acid injection significantly reduced the response of MNCs to rising systemic osmotic pressure.

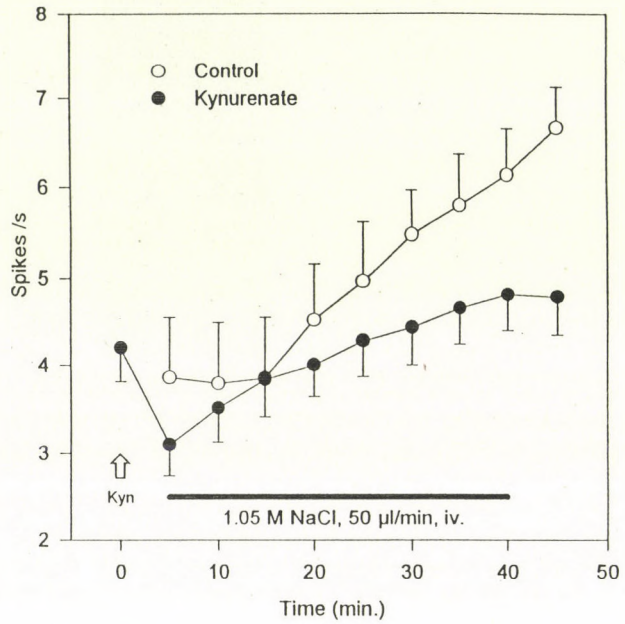

Fig. 3: The effect of kynurenic acid on the osmotic response of supraoptic neurones *in vivo*. The response of supraoptic neurones to an intravenous infusion of 1.05 M NaCl at 50 µl/min (bar) was measured under control conditions, and following an intracerebroventricular injection of kynurenic acid (arrowed). All points are mean ±SEM, n=9

The effect of kynurenic acid on MNC firing rates in hypertonic and isotonic conditions.

A control group of 7 cells recorded in animals that had previously received no hyperosmotic stimulus showed a small but not significant change in mean spike frequency in response to ICV kynurenic acid injection.

A second group (9 cells) were given an injection of kynurenic acid after an infusion of hypertonic saline (as described above). In these cells, kynurenic acid produced a significant reduction in spike frequency (a change of 1.29 ± 0.21 Hz, $P < 0.01$) for the

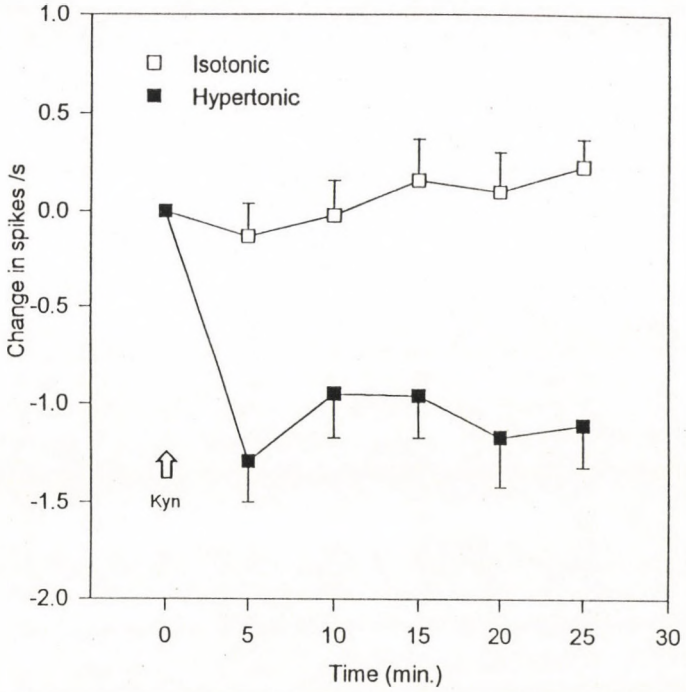

Fig.4: The effect of kynurenic acid on the firing rates of supraoptic neurones *in vivo*. An intracerebroventricular injection of kynurenic acid was given at 0 minutes (arrowed) while recording from 7 cells in control animals and 9 in hypertonically stimulated animals. There was a significant and prolonged reduction in the firing rates of the cells in the hypertonic group but no significant change in the control group.

period immediately after injection of kynurenic acid ICV. This effect was profound and prolonged (>20 minutes; see Fig. 4). Kynurenic acid decreased the mean spike frequency from an elevated level of 5.66±0.87 Hz (due to the raised osmotic pressure) to a mean spike frequency of 4.29±0.70 Hz, 5 minutes after kynurenic acid injection. The latter firing rate was not significantly different from the firing rate (3.61±0.51 Hz) of MNCs observed in isotonic conditions (see Fig. 4).

Kynurenic acid did not affect the spontaneous spike activity of MNCs under isotonic conditions. In hypertonically stimulated MNCs kynurenic acid decreased spike activity to values similar to those recorded MNCs in isotonic conditions.

DISCUSSION

It is clear from the recording studies *in vitro* that raising the osmolality of the perfusing medium did not significantly alter the membrane characteristics of individual MNCs deprived of input from cells outside the SON. The membrane resistance of the 4 magnocellular cells tested was marginally reduced by raising the osmolality of the medium by 40 mosmole but the difference in membrane resistance of the cells was not significant, confirming the results of Richard and Bourque (1992).

Although it is difficult to make long-term intracellular recordings *in vivo* it is equally clear that in the example shown in Fig. 2, the depolarising PSP frequency under control conditions was significantly raised after an IP injection of 1ml of 1.5M NaCl solution which would have raised plasma osmolality by approximately 10 mosmoles.

It seems likely therefore that the osmotic responsiveness of MNCs *in vivo* depends critically on synaptic input from elsewhere. We found that kynurenic acid effectively blocked the increase in MNC spike frequency in response to rising systemic osmotic pressure. Kynurenic acid injection into animals already subject to a hyperosmotic stimulus significantly reduced the firing rate of MNCs. We propose that the rise in MNC spike activity in response to a hyperosmotic stimulus *in vivo* is generated by a kynurenic acid sensitive osmosensitive mechanism.

This places in context evidence from *in vitro* slice studies using intracellular recording techniques, which demonstrated that post-synaptic glutamate receptors are important in generating sEPSPs in MNCs. Richard and Bourque (1992) have shown that bath application of kynurenic acid at a concentration of 200μM reversibly abolished OVLT mediated hyperosmotic activation of supraoptic MNCs. Local repetitive stimuli (10-40Hz) to the dorsolateral hypothalamus evoked EPSPs in supraoptic MNCs. These sEPSPs were eliminated by perfusion of kynurenic acid at high doses(1-3mM) and reduced by low kynurenic acid concentrations (250-500μM) (Gribkoff and Dudek, 1988). A fast excitatory connection between the OVLT and supraoptic MNC has been proposed in which electrical stimulation of the OVLT elicits CNQX-sensitive compound EPSPs in MNCs, implicating the AMPA sub-type of glutamate receptors (Yang *et al.*, 1994; Richard and Bourque, 1992). This evidence suggests that kynurenic acid blocks AMPA glutamate receptor dependent transmission of extrinsic osmosensitive information to the SON. The *in vitro* studies (Bourque *et al.*, 1994; Gribkoff and Dudek, 1988) all reveal that glutamate antagonists have a potent effect in eliminating EPSPs occurring at the MNC. These glutamate antagonists did not alter MNC membrane resistance or potential (Bourque *et al.*, 1993).

Leng *et al.* (1985) suggested that the intrinsic osmosensitivity of the MNC reduces membrane potential as extracellular osmolality rises. This combines with the increased frequency of sEPSPs generated by extrinsic osmosensitive afferents and increases the probability of action potential discharge. We suggest that the generation of sEPSPs at the MNC is kynurenic acid-sensitive. Thus *in vivo* the transmission of extrinsic osmosensitive information is blocked by kynurenic acid.

There are numerous potential post synaptic glutamate receptor sites responsible for the kynurenic acid mediated block of the supraoptic MNC osmoresponsiveness. The effects

of intravenous administration of ketamine (Nissen *et al.* 1994) suggest that the SON might be a site of glutamate dependent transmission of extrinsic osmosensitive information. Anatomical evidence (see introduction) for an osmoreceptor complex involving excitatory connections between the SON and other diencephalic nuclei (Honda *et al.*, 1990) suggests other potential sites. In particular projections from the SON to the OVLT and to the ventral AV3V, and the ventral AV3V to MnPO may well be glutamate dependent as some at least are known to be excitatory (Honda *et al.*, 1990)

In hypothalamic slice studies, most of the afferents from these nuclei to the SON are destroyed. Their preservation in our *in vivo* work may account for discrepancies between our observations and the results *in vitro*. In contrast to *in vitro* studies we were unable to abolish entirely the osmoresponsive component involved in increases in MNC spike activity. Furthermore, *in vivo* experiments which used local pressure application of large doses of kynurenic acid (Day *et al.*, 1990) or intravenous administration of ketamine (Nissen *et al.*, 1994) instead of ICV injection, demonstrated a transient inhibition of spontaneous spike activity in phasic cells. We did not observe this cessation in spontaneous activity in the cells we recorded under similar conditions. With reference to the results of Nissen *et al.* (1994) we suggest that in our work the effective concentration of kynurenic acid at the SON was insufficient to provide total glutamate receptor block. Thus the reduction of the extrinsic osmosensitive inputs to the SON seen after kynurenic acid injection can be accounted for by kynurenic acid blocking glutamatergic transmission throughout the hypothesized osmoreceptor complex.

After an ICV injection, the concentration of drug is greatest at the site of injection, namely the lateral ventricle. The osmoreceptor complex nucleus closest to this site is the OVLT. Richard and Bourque (1992) have shown that stimulating the OVLT osmotically generates sEPSPs at the SON. Honda *et al.* (1990) demonstrated reciprocal excitatory projections from the SON to the OVLT. We suggest that spike activity at the OVLT is dependent on extrinsically derived sEPSPs and an intrinsically modulated change in membrane properties (as hypothesized to occur at the supraoptic MNC). It is plausible that the generation of sEPSPs at the OVLT is glutamate dependent (as at the MNCs) and derived primarily from the SON (Honda *et al.*, 1990). The effect of post synaptic glutamate receptor block at the OVLT would be to reduce spike activity in neurones there and thus sEPSP generation at the SON and MnPO. This interruption of the proposed osmoreceptor circuit (Honda *et al.*, 1990) would greatly reduce osmosensitive generated sEPSPs arriving at the SON. It would explain the reduction by kynurenic acid of the increase in MNC spike activity as plasma osmotic pressure increased, even if there was incomplete block of glutamate receptors at the SON itself. An important assumption in this hypothesis is that spontaneous MNC activity in isotonic conditions, which was not inhibited by ICV kynurenic acid injection, is generated by a mechanism distinct from that responsible for extrinsic osmosensitive inputs.

Effects of kynurenic acid on basal activity in supraoptic MNCs

Our results show (Fig. 4) that in animals that have not been given hyperosmotic infusions, basal spike activity in supraoptic MNCs is not significantly reduced by kynurenic

acid. Our results indicate that this is not because there is insufficient kynurenic acid reaching the hypothalamus, since under identical experimental conditions, but in hyperosmotically stimulated animals, kynurenic acid profoundly and highly significantly reduced MNC spike frequency. This suggests that while supraoptic MNCs receive important glutamate-dependent osmoreceptive inputs that drive the MNC response to hypertonic stimuli, MNC spontaneous basal activity is not dependent upon the same glutamatergic drive.

In contrast, Nissen *et al.* (1994) have demonstrated *in vivo* that intravenous administration of ketamine (subanaesthetic dose) at the SON transiently inhibits basal activity in phasic, putative AVP secreting, MNCs. Non NMDA channel antagonists, e.g. CNQX, have no such effect. Nissen *et al.* concluded that NMDA-type post synaptic glutamate receptors are important in sustaining basal spike activity in phasic cells. They were unable to eliminate spontaneous basal activity in continuous firing, putative oxytocin, MNCs, and suggested that the neurones may not express cell surface NMDA receptors.

Our results indicating that a glutamatergic input is not necessary for establishing or maintaining basal activity in MNCs are based mainly on data from oxytocin cells, 6/7 cells. It may be that the mechanisms maintaining spontaneous activity in oxytocin and AVP MNCs differ. The spontaneous activity of AVP MNCs may be dependent upon tonic glutamatergic inputs that act at post synaptic NMDA receptors. But the spontaneous activity of oxytocin MNCs may not be dependent on such an input.

We conclude that there is a glutamate-dependent osmoresponsive input to MNCs in the rat SON that plays an important role in elevating spike frequency during hypertonic stimulation *in vivo*. In oxytocin cells at least, this input appears to be distinct from the mechanisms which maintain spontaneous firing rates in MNCs before osmotic stimulation.

REFERENCES

Bourque C.W., Oliet S.H.R., Kirkpatrick K., Richard D., Fisher T.E. (1993) Extrinsic and intrinsic modulatory mechanisms in regulating the electrical activity of supraoptic neurones. *Ann. NY. Acad. Sci.* **689**, 512-519.

Bourque C.W., Oliet S.H.R., Richard D. (1994) Osmoreceptors, Osmoreception and Osmoregulation. *Front. Neuroendocrinol.* **15**, 1-47.

Bourque C.W., Richard D. (1992) Glutamatergic excitation of rat supraoptic neurones following osmotic stimulation of the OVLT. *Soc. Neurosci. Abst.* **18**,

Brimble M.J., Dyball R.E.J. (1977) Characterisation of the responses of oxytocin and vasopressin secreting neurones in the supraoptic nucleus to osmotic stimulation. *J. Physiol.* **271**, 253-271.

Chaudry M.A., Dyball R.E.J., Honda K., Wright N.C. (1989) The role of interconnection between supraoptic nucleus and anterior third ventricle region in osmoreception in the rat. *J. Physiol.* **410**, 123-135.

Day T.A., Renaud L.P., Sibbald J.R. (1990) Excitation of supraoptic vasopressin cells by stimulation of the A1 noradrenaline group: failure to demonstrate role for established adrenergic or amino acid receptors. *Brain Res.* **516**, 91-98.

Ennis M., Aston-Jones G., Shiekhattar R. (1992) Activation of the locus coerulus neurones by nucleus paragigantocellularis or noxious sensory stimulation is mediated by intracoerular excitatory amino acid neurotransmission. *Brain Res.* **598**, 185-195.

Ganong A.H., Lanthorn T.H., Cotman C.W. (1983) Kynurenic acid inhibits synaptic and acidic amino acid induced responses in the rat hippocampus and spinal cord. *Brain Res.* **273**, 170-174.

Gardiner T.W., Verbalis J.G., Strickner E.M. (1985) Impaired secretion of vasopressin and oxytocin in rats after lesions of the nucleus medialis. *Am. J. Physiol.* **248**, 224-230.

Gribkoff V.K., Dudek F.E. (1988) The effects of the excitatory amino acid antagonist kynurenic acid on synaptic transmission to supraoptic neuroendocrine cells. *Brain Res.* **442**, 152-156.

Honda K., Negoro H., Higuchi T., Takano S. (1989) The role of the anterior third ventricle area in the osmotic control of paraventricular neurosecretory cells. *Exp. Brain Res.* **76**, 497-502.

Honda K., Negoro H., Dyball R.E.J., Higuchi T., Takano S. (1990) The osmoreceptor complex in the rat: evidence for interactions between the supraoptic and other diencephalic nuclei. *J. Physiol.* **431**, 225-241.

Kobashi M., Adachi A. (1985) Convergence of hepatic osmoreceptive inputs on sodium responsive units within the nucleus of the solitary tract of the rat. *J. Neurophysiol.* **54**, 212-219.

Leng G. (1980) Rat supraoptic neurones: the effects of locally applied hypertonic saline. *J. Physiol.* **304**, 405-414.

Leng G., Dyball R.E.J., Mason W.T. (1985) Electrophysiology of Osmoreceptors. In: Vasopressin (ed. Schrier R.W.) pp. 333-342. Raven Press, New York.

Mason W.T. (1980) Intracellular recordings from osmosensitive neurones in the rat supraoptic nucleus *in vitro*. *J. Physiol.* **303**, 56-57.

Nissen R., Hu B., Renaud L.P. (1994) NMDA receptor antagonist ketamine selectively attenuates spontaneous phasic activity of supraoptic vasopressin neurones *in vivo*. *Neurosci.* **59**, 115-120.

Oliet S.H.R., Bourque C.W. (1993) Mechanosensitive channels transduce osmosensitivity in supraoptic neurones. *Nature* **364**, 341-343.

Richard D., Bourque C.W. (1992) Synaptic activation of rat supraoptic neurones by osmotic stimulation of the organum vasculosum lamina terminalis. *Neuroendocrinol.* **55**, 609-611.

Sawchenko P.E., Swanson L.E. (1983) The organisation of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. *J. Comp. Neurol.* **218** 121-144.

Tribollet E., Armstrong W.E., Dubois-Dauphin M. (1985) Extrahypothalamic afferent inputs to the supraoptic nucleus area of the rat as determined by retrograde and anterograde tracing techniques. *Neurosci.* **15**, 135-148.

Tribollet E., Dreifuss J.J. (1981) Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat - a horseradish peroxidase study. *Neurosci.* **6**, 1315-1328.

Verney E.B. (1947) The antidiuretic hormone and the factors which determine its release. *Proc. R. Soc. Lond. (Biol.)* **135**, 25-106.

Watkins J.C., Evans R.H., Mewett K.N., Olverman H.J., Pook P.C. (1987) Recent advances in the pharmacology of excitatory amino acids. In Excitatory Amino Acids (eds. Hicks T.P., Lodge D., McLennan H.) pp. 1-26, Liss, New York.

Yang C.R., Senatorov V.V., Renaud L.P. (1994) Organum vasculosum lamina terminalis-evoked postsynaptic responses in rat supraoptic neurones *in vitro*. *J. Physiol.* **477** 59-74.

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INHIBITION OF NITRIC OXIDE SYNTHASE ATTENUATES OSMOTIC THIRST IN THE RAT

H. Kannan, K. Iki, T. Kunitake, A. Shimokawa, M. Saita, Y. Ishizuka, T. Hanamori

Department of Physiology, Miyazaki Medical College, Miyazaki, Miyazaki 889-16, Japan

Summary

Changes in water intake after intraperitoneal injection of a nitric oxide synthase (NOS) inhibitor was studied in the rat. Administration of N^{w}-nitro-L arginine methyl ester (L-NAME) at a dose of 50 mg/kg attenuated osmotic thirst induced by intraperitoneal injection of hypertonic saline, but did not affect spontaneous intake of water and thirst induced by subcutaneous injection of angiotensin II. Pretreatment with L-arginine significantly attenuated the inhibition of osmotic thirst evoked with subsequent L-NAME. Administration of N^{w}-nitro-D-arginine methyl ester (D-NAME) altered neither the spontaneous nor the osmotic drinking behavior. These findings suggest that NO may affect the osmotically induced drinking.

Key Words :

Nitric oxide, nitric oxide synthase, drinking behavior, N^{w}-nitro-L arginine methyl ester, rat

Introduction

Nitric oxide (NO) has been implicated as a physiological intercellular/intracellular messenger in many areas of the central and peripheral nervous system [Dawson et al, 1992; Garthwaite, 1991]. NO may have a physiological role in the regulation of water balance. The presence of mRNA for the synthetic enzyme protein, NO synthase (NOS) [Stuehr and Griffith, 1992] has been identified in the forebrain, including the circumventricular organs, which is involved in the control of body water balance [Bredt et al, 1991; Dawson et al, 1991; Grossman et al, 1994; Vincent and Kimura, 1992].

Since drinking is regulated primarily by the osmotic pressure and volume of body fluid, we examined whether NO specifically affected these factors in the rat. The aim of the present study was to examine the role of NO in spontaneous and evoked drinking by using an inhibitor of NOS, N^{w}-nitro-L-arginine methyl ester (L-NAME) [Dwyer et al, 1991; Rees et al, 1990].

Materials and Methods

Male Wistar rats weighing approximately 250 g were individually housed under controlled light/dark conditions (lights on : 0700-19.00) with room temperature regulated at 22 - 24 °C. During the experiments, food was removed from their cages to avoid the possible influence of feeding behavior on drinking.

N^{w}-nitro-L-arginine methyl ester (L-NAME) and N^{w}-nitro-D-arginine methyl ester (D-NAME) which were purchased from Sigma, St. Louis were dissolved in sterile physiological saline before use and intraperitoneally injected at a dose of 50 mg/ml/kg. Experiments were performed in the morning (0900 to 1000) unless otherwise noted.

Experiment 1

Fifteen minutes after administration of L-NAME or D-NAME, drinking behavior was induced by one of the following challenges. (a) Osmotic thirst : water intake after intraperitoneal injection of 20 ml/kg hypertonic (5 %) saline. Hypertonic saline was mixed with 0.8 mg/ml lidocaine to reduce local irritation [Grossman and Grossman, 1982]. (b) Angiotensin II-induced thirst : water intake after subcutaneous injection of 100 μg/kg angiotensin II. Angiotensin II is increased in the systemic circulation during hypovolemia. Water intake was measured to the nearest 0.1 ml by weighing the water bottle.

Experiment 2

We studied daily water intake, urine output, food intake, and body weight after administration of NAME at a dose of 50 mg/kg using metabolic cages. Ad libitum daily intake of water and food was measured to the nearest 0.1 g by weighing the water bottle and the food basket, respectively. The volume of urinary output (±0.1 ml) and body weight (± 1 g) were also measured.

Urinary sodium and potassium concentrations were measured with a flame photometer. Total sodium and potassium excretions were calculated as urinary sodium and potassium concentration × urinary volume. The daily measurements started at least 4 days before and continued for 7 days after the administration of NAME. The measurements were made between 0900 and 1000 h. NAME was intraperitoneally administered at 1630.

All results are expressed as the means ± S.E.M.. Statistical analyses were performed by analysis of variance and post hoc Fisher's least significant difference test. Statistical significance was set at $p<0.05$.

Results

Osmotic Thirst

Pretreatment with L-NAME attenuated water intake induced by an osmotic load (Fig. 1). Water intake for 30 min after administration of hypertonic saline was 4.1 ± 0.7 ml in rats pretreated with L-NAME at a dose of 50 mg/kg. This amount was significantly ($p<0.001$) less than that consumed by rats pretreated with D-NAME (11.0 ± 1.4 ml). Similarly, water

intake of rats pretreated with L-NAME for 4 hr after administration of hypertonic saline was significantly less than that consumed by rats pretreated with D-NAME.

Changes in osmotic thirst in response to i.p. injection of hypertonic saline in the rats pretreated with L-NAME plus L-arginine were also examined. Before i.p. injection of hypertonic saline, there were no significant differences in daily water intake among the groups pretreated with D-NAME, L-NAME, and L-NAME plus L-arginine. In response to the injection of hypertonic saline, the increase in osmotic thirst in the rats pretreated with L-NAME-plus L-arginine was greater, than that in the rats pretreated with L-NAME alone. This indicates that pretreatment with L-arginine blocked the decrease in osmotic thirst induced by L-NAME.

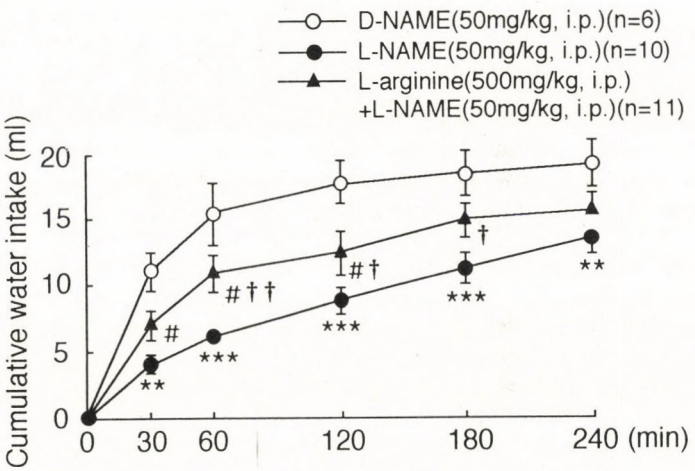

Fig. 1. Cumulative water intake by rats after intraperitoneal injection of 5 % NaCl solution at a dose of 20 ml/kg following pretreatment with L- or D-NAME at a dose of 50 mg/kg. Asterisks show water intake significantly different from that of rats injected with D-NAME. **p<0.01, ***p<0.001. Numbers of rats are shown in parentheses. All values are means ± S.E.M.

Angiotensin II-Induced Thirst

Pretreatment with L-NAME had no statistically significant effect on angiotensin II-induced thirst (Fig. 2). Rats pretreated with L-NAME at a dose of 50 mg drank 3.6 ± 1.1 ml of water for 30 min after administration of angiotensin II, while rats pretreated with D-NAME drank 5.3 ± 0.9 ml of water for 30 min. There were no significant differences between the groups up to 4 h after injection of angiotensin II.

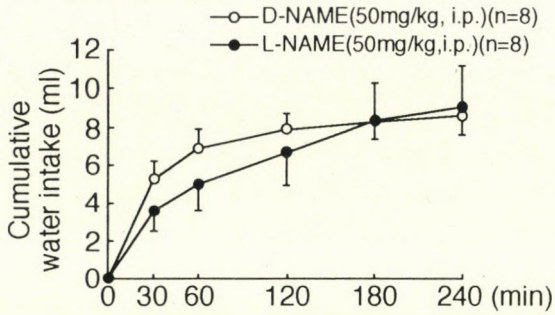

Fig. 2. Cumulative water intake by rats after subcutaneous injection of 100 μg/kg angiotensin II following pretreatment with 50 mg/kg L- or D-NAME. Although the mean water intake was less in L-NAME-treated rats than in D-NAME-treated rats, there no significant differences in water intake within the groups. Numbers of rats are shown in parentheses. All values are means ± S.E.M.

Daily water intake

Fig. 3 shows the daily water intake, urine output, food intake, and body weight after i.p. injection of 50 mg/kg NAME. They drank similar amounts of water on the day before injection; 28.8 ± 0.5 ml in the L-NAME-injected rats, and 28.7 ± 1.1 ml in D-NAME injected rats. Intraperitoneally administered L-NAME did not affect daily water intake, food intake, body weight, and urine volume. In addition, urinary sodium and potassium excretion were also not affected by injection of L-NAME.

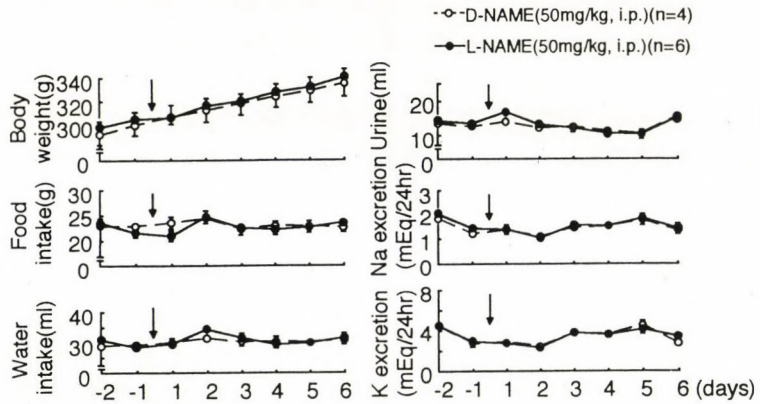

Fig. 3. Effects of the intraperitoneal injection of L- or D-NAME (50 mg/kg) on daily metabolism measurements in conscious rats. Arrows show day of injection. Numbers of rats are shown in parentheses. All values are means ± S.E.M.

Discussion

The present findings confirm the previous report that NO is involved in the complex processes that initiate drinking behavior induced by an osmotic stimulus [Kadekaro et al, 1994]. The administration of L-arginine alone did not modify any of the parameters measured (unpublished results), but it effectively prevented the inhibition in osmotic thirst induced by the injection of 50 mg/kg L-NAME, thus suggesting that the drinking induced by osmotic stimuli is mediated by the production of NO.

The presence of nitric oxide synthase has been demonstrated in magno-cellular neurons of the supraoptic and paraventricular nuclei as well as in the posterior pituitary gland by the NADPH-diaphorase histochemical technique and by immunocytochemistry [Alonso et al, 1992; Pow, 1992; Sagar and Ferriero, 1987]. Colocalization of nitric oxide synthase and vasopressin has been observed in magnocellular neurons of the supraoptic and paraventricular nuclei. These observations suggest that nitric oxide participates in the regulation of vasopressin release. In this regard, administration of NOS inhibitors has been reported to increase plasma vasopressin in conscious rabbits [Goyer et al, 1994]. Thus rats treated with L-NAME would excrete more concentrated urine than rats treated with D-NAME because of the increase in reabsorption of water in the kidney. This may explain why the rats drank less in response to hypertonic saline. On the other hand, L-NAME has a pressor effect [Gardiner et al, 1990], which may also inhibit thirst [Robinson and Evered, 1987]. Thus the pressor effect may account for the decrease in water intake after administration of hypertonic saline. However, administration of L-NAME attenuated osmotic thirst, but affected neither water intake in response to angiotensin II nor spontaneous water intake. Therefore, nonspecific inhibition of thirst, either by the pressor effect or by vasopressin, could not fully account for the phenomena. Moreover, the decrease in water intake after administration of L-NAME is not secondary to feeding behavior, since food was not available in the present experiment. These findings suggest that administration of L-NAME did not suppress general behavior but inhibited osmotic thirst.

Our findings suggest that NO promotes rehydration by regulating neural mechanisms to promote drinking behavior. NOS has been identified in the subfornical organ (SFO) and NADPH-diaphorase activity has been demonstrated in the organum vasculosum laminae terminals (OVLT) [Pasqualotto et al, 1991; Summy-Long et al, 1984]. These two circumventricular organs, devoid of the blood-brain barrier, have a physiological role in the regulation of drinking behavior in response to osmotic or angiotensin II stimulation [Gardiner and Stricker, 1985; Simpson et al, 1978; Thrasher et al, 1982]. Potentially, therefore, NO

could be produced at these sites and influence water intake during conditions of decreased intracellular volume.

In contrast with the decreased water intake caused by an i.p. administration of L-NAME, i.c.v. administration of L-NAME did antagonize the antidipsogenic action of L-arginine in water deprived rats but, by itself, did not increase thirst [Calapai et al, 1992]. This indicates that NO acts as an inhibitor when thirst is stimulated by water deprivation. However, Kadekaro et al.have reported that i.c.v. administration of NO synthase inhibitor (L-NMMA) to conscious rats attenuates drinking induced by water deprivation for 24 hr [Kadekaro et al, 1994], which is compatible with the present result. The reason for the discrepancy remains to be elucidated.

Previous studies have demonstrated that the earliest measurable renal effect produced by L-NAME consists of a significant decrease in urine volume and urinary sodium excretion with no change in any other renal parameter, thus suggesting a tubular mechanism [Denton and Anderson, 1994; Lahera et al, 1991]. A higher dose of L-NAME elicited diuresis, natriuresis, renal blood flow and glomerular filtration rate, and was associated with an elevation of mean arterial pressure. In the present study, no significant changes in daily urine volume, urinary sodium and potassium excretion were observed following the administration of L-NAME. This difference might be due to experimental arrangement such as sampling time and dose of L-NAME used. Further experiments are required to elucidate the roles of the NO system in the control of renal electrolyte excretion.

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References

Alonso, J. R., Sánchez, F., Arévalo, R., Carretero, J., Vázquez, R. and Aijón, J. (1992) Partial coexistence of NADPH-diaphorase and somatostatin in the rat hypothalamic paraventricular nucleus. Neurosci. Lett. 148, 101-104.

Bredt, D. S., Glatt, C. E., Hwang, P. M., Fotuhi, M., Dawson, T. M. and Snyder, S. H. (1991) Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 7, 615-624.

Calapai, G., Squardrito, F., Altavilla, D., Zingarelli, B., Campo, G. M., Cilia, M. and Caputi, A. P. (1992) Evidence that nitric oxide modulates drinking behavior. Neuropharmacology 31, 761-764.

Dawson, T. M., Bredt, D. S., Fotuhi, M., Hwang, P. M. and Snyder, S. H. (1991) Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc. Natl. Acad. Sci. USA 88, 7797-7801.

Dawson, T. M., Dawson, V. L. and Snyder, S. H. (1992) A novel neuronal messenger molecule in brain: the free radical, nitric oxide. Ann. Neurol. 32, 297-311.

Denton, K. M. and Anderson, W. P. (1994) Intrarenal haemodynamic and glomerular responses to inhibition of nitric oxide formation in rabbits. J. Physiol. 475, 159-167.

Dwyer, M. A., Bredt, D. S. and Snyder, S. H. (1991) Nitric oxide synthase: irreversible inhibition by L-N^G-nitroarginine in brain *in vitro* and *in vivo*. Biochem. Biophys. Res. Commun. 176, 1136-1141.

Gardiner, S. M., Compton, A. M., Kemp, P. A. and Bennett, T. (1990) Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. Br. J. Pharmacol. 101, 625-631.

Gardiner, T. W. and Stricker, E. M. (1985) Impaired drinking responses of rats with lesions of nucleus medianus : circadian dependence. Am. J. Physiol. 248, R224-R230.

Garthwaite, J. (1991) Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci. 14, 60-67.

Goyer, M., Bui, H., Chou, L., Evans, J., Keil, L. C. and Reid, I. A. (1994) Effect of inhibition of nitric oxide synthesis on vasopressin secretion in conscious rabbits. Am. J. Physiol. 266, H822-H828.

Grossman, A. B., Rossmanith, W. G., Kabigting, E. B., Cadd, G., Clifton, D. and Steiner, R. A. (1994) The distribution of hypothalamic nitric oxide synthase mRNA in relation to gonadotrophin-releasing hormone neurons. J. Endocrinol. 140, R5-R8.

Grossman, S. P. and Grossman, L. (1982) Iontophoretic injections of kainic acid into the rat lateral hypothalamus : effects on ingestive behavior. Physiol. Behav. 29, 553-559.

Kadekaro, M., Terrell, M. L., Harmann, P. and Summy-Long, J. Y. (1994) Central inhibition of nitric oxide synthase attenuates water intake but does not alter enhanced glucose utilization in the hypothalamo-neurohypophysial system of dehydrated rats. Neurosci. Lett. 173, 115-118.

Lahera, V., Salom, M. G., Miranda-Guardiola, F., Moncada, S. and Romero, J. C. (1991) Effects of N^G-nitro-L-arginine methyl ester on renal function and blood pressure. Am. J. Physiol. 261, F1033-F1037.

Pasqualotto, B. A., Hope, B. T. and Vincent, S. R. (1991) Citrulline in the rat brain: immunohistochemistry and coexistence with NADPH-diaphorase. Neurosci. Lett. 128, 155-160.

Pow, D. V. (1992) NADPH-diaphorase (nitric oxide synthase) staining in the rat supraoptic nucleus is activity-dependent: possible functional implications. J. Neuroendocrinol. 4, 377-380.

Rees, D. D., Palmer, R. M. J., Schulz, R., Hodson, H. F. and Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. Br. J. Pharmacol. 101, 746-752.

Robinson, M. M. and Evered, M. D. (1987) Pressor action of intravenous angiotensin II reduces drinking response in rats. Am. J. Physiol. 252, R 754-R759.

Sagar, S. M. and Ferriero, D. M. (1987) NADPH diaphorase activity in the posterior pituitary: relation to neuronal function. Brain Res. 400, 348-352.

Simpson, J. B., Epstein, A. N. and Camardo, Jr., J. S. (1978) Localization of receptors for the dipsogenic action of angiotensin II in the subfornical organ of rat. J. Comp. Physiol. Psychol. 92, 581-601.

Stuehr, D. J. and Griffith, O. W. (1992) Mammalian nitric oxide synthases. Adv. Enzymol. Relat. Areas Mol. Biol. 65, 287-346.

Summy-Long, J. Y., Salisbury, R., Marietta, M. P., Hartman, R. D. and Weisz, J. (1984)

Pathways of hydrogen utilization from NADPH generated by glucose-6-phosphate dehydrogenase in circumventricular organs and the hypothalamo-neurohypophysial system: a cytochemical study. Brain Res. 294, 23-35.

Thrasher, T. N., Keil, L. C. and Ramsay, D. J. (1982) Lesions of the organum vasculosum lamina terminalis (OVLT) attenuate osmotically-induced drinking and vasopressin secretion in the dog. Endocrinology 110, 1837-1839.

Vincent, S. R. and Kimura, H. (1992) Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46, 755-784.

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ACIDIC FIBROBLAST GROWTH FACTOR PROTECTS MEMORY AND IMMUNOREACTIVITY IMPAIRMENT IN SENESCENCE ACCELERATED MICE

Oomura, Y.[1], Sasaki, K.[2], Li, A.[3], Yoshii, H.[1], Fukata, Y.[1], Yago, H.[1], Kimura, H.[4], Tooyama, I.[4], Hanai, K.[4], Nomura, Y.[5], Kitamura, Y.[5] and Yanaihara, N.[6]

Inst. Bio-Active Sci, Nippon Zoki Pharm. Co., Hyogo[1]; Fac. Eng., Tooyama Univ., Toyama[2]; Fac. Med., Kyushu Univ., Fukuoka[3]; Shiga Med. Univ., Shiga[4]; Fac. Pharm., Hokkaido Univ., Sapporo[5]; Fac. Pharm., Shizuoka Pref. Univ., Shizuoka[6]

Subcutaneous injection of aFGF once per a week into senescence accelerated mice (SAM)P8 was begun at 3 weeks after birth and continued for 10 months. Saline was injected as a control. Learning and memory and cellular immunological functions in the aFGF (F) group were enhanced significantly and while those of the saline (S) group deteriorated.

The number of cholinergic neurons was decreased slightly and choline aceetyltransferase activity in individual neurons in the medial septum which send monosyonaptic terminals to the hippocampus was significantly decreased in the S group, but were more spared in the F group. The MAO-B activity was significantly lower in the F group than in the S group. The respective densities of muscarinic and NMDA receptors and the aFGF receptor, i.e. FGFR-1 in the hippocampus were also significantly higher in the F group than in the S group.

The delayed type hypersensitivity reactions (DTH) in the footpad caused by challenge with trinitrophenyl or sheep red blood cells as measured at the end of the 2nd and 7th months, indicated the T cell immune response. Both types of DTHs were reduced in the 7th month as compared with the 2nd month in the S group. However, aFGF administration protected against this reduction in response with age.

These results show that aFGF provides protection against impairment of not only learning and memory but also the DTH immunoreactivity in SAMP8, indicating thereby a close relationship between learning-memory and T cell immune function.

Key Words: aFGF, choline acetyltransferase, FGFR-1, hippocampus, immune function, medial septum, passive avoidance test, senescence-accelerated mice, subcutaneous injection water maze test

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INTRODUCTION

Feeding in rats increased the concentration of aFGF in CSF from a prefeeding level of 0.73 ± 0.2 pmol (mean ± S.E.M.) to 0.75 ± 0.2 nmol within 2 h and i.p. injection of glucose was shown to cause dose dependent release of aFGF (Hanai et al., 1989). aFGF is released from the ependymal cells responding to an increase in glucose in CSF (Oomura et al., 1992). Intracerebroventricular (i.c.v.) infusion of aFGF results in a dose dependent suppression of food intake, minimum effective dose being 3.3 pmol.

Food intake is increased significantly by anti-aFGF antibody administration in the lateral hypothalamic area (LHA), but not by preimmune IgG (Sasaki et al.,1994 b). Also, aFGF appears in the neurons of LHA, medial septum, hippocampus and some other areas 2 h after a meal or an i.p. injection of 300 mg/kg glucose (Oomura et al, 1992). The appearance of aFGF in the hippocampus led us to investigate the effect of endogenous aFGF on learning and memory and we have reported that 2 h after glucose i.p. injection affective and spatial memory as tested with a passive avoidance task and a Morris water maze task are significantly enhanced (Oomura et al, 1993). This memory facilitation is abolished by pretreatment with an anti-aFGF antibody administered i.c.v.. The facilitation of short-term and long-term potentiation (LTP) of the synaptic potentials evoked at the hippocampal CA1 layer by stimulation of Schaffer collateral/commissural afferents is obtained after aFGF application (Sasaki et al., 1994a). In view of these evidences, we studied if aFGF also provides any protection against learning and memory loss and immunological deterioration that occurs in senescence-accelerated mice (SAM)P8.

METHOD

The SAMP8 display aging-like physical symptoms and a progressive retardation of learning and memory after about 2 month of age (Yagi et al., 1988). Male and female SAMP8 were obtained from Takeda Pharmaceutical Co., Ltd. They were administered s.c. aFGF (bovine aFGF, carrier free, R & D systems Ins.,USA) at 0.7 μ *g/kg or 7 μ* g/kg body weight (0.05 or 0.5 nmol/kg) once a week from 3 weeks to I0 months of age. The learning and memory of these mice were assayed monthly using passive avoidance test (affective memory) and after the end of 10 months Morris water maze test was conducted in a 70 cm diameter water maze pool with 20 cm depth had a small platform fixed 1 cm below the water surface (Oomura et al., 1992). The mean latency to climb on the platform was measured for 4 trials with 10 min intertrial intervals. This was done every 2 days. The normal controls were senescence-resistant inbred strains, SAMRI. Control groups of SAMP8 received saline s.c. injections on the same schedule. The choline acetyltransferase (ChAT) concentration in the cholinergic neurons was immunohistochemically detected using anti ChAT antibody

(Chemicon, AB-143, USA). The aFGF receptor density was immunohistochemically detected using anti FGFR-1 antibody (Matsuo et al., 1994). The density of NMDA and muscarinic receptors was measured according to Zhao et al., (I990) and Kitamura et al., (1989). The concentration of MAO-B in the whole brain was measured as per Nomura et al. (1989). For the study of LTP, synaptic potentials in the CAl region in SAMP8 hippocampal slice preparations were recorded by stimulation of Schaffer collateral/commissural afferents at the end of 10 months. Tetanic stimulation was applied for one s at 100 Hz. Care was taken to increase the stimulus intensity gradually because it is known that strong and sudden stimulation can damage the tissue in which case LTP facilitation may never take place. Data were expressed as mean ± S.D. Statistical analysis was performed using Student's t test.

Measurement of tissue immunological function (T cell immune system). *Preparation of TNP (trinitrophenyl)-SRBC: 2,* 4, 6-trinitrobenzene sulfonic acid (TNBS)-sheep red blood cells (SRBC) were prepared as follows: TNBS was dissolved in phosphate buffered saline (PBS) at 20 mg/7.0 ml and was adjusted at pH 7.2 with NaOH solution.

The pellet (1.0 ml) of SRBC after washing with saline was dropped into the TNBS solution under dark condition. After gentle mixing for 20 min at room temperature, the TNP-SRBC formed were washed with saline three times. *TNP-specific DTH response:* Mice were sensitized i.p. with 10^9 TNP-SRBC and then challenged with 25 μ 1 of the TNBS solution (20 mg/7.0 ml in PBS at pH 7.2, as described above) into the footpad 14 days after sensitization. *SRBC specific DTH response:* Mice were sensitized with $1x10^7$ cells in the left footpad and were challenged with $1x1O^8$ cells 4 days after sensitization in the right footpad. The degrees of swellings of the right and left footpads were measured with a dial thickness gauge 24 and 48 h after challenge. The DTH response was expressed as the difference in thickness (x 0.01 mm) between the two footpads. Data were expressed as the mean ± S.E.M. Statistical analysis was performed using Student's t test.

RESULTS

Protection of learning and memory by aFGF

Administration of aFGF s.c. prevented the retardation of learning and memory in SAMP8.

Passive Avoidance Test: The affective memory retention was significantly higher in the aFGF treated group over a 9 month period than in the saline controls (Table 1).

The retained latencies in the 7 μ *g/kg* aFGF group were signiftcantly higher than those in the saline group. The retained latencies on 6th month were a bit low but still signiftcant. The experiment was repeated in another SAMP8 group and again it was found that retained latencies in the 7 μ *g/kg* aFGF group were signiftcantly higher than those in the saline group in 5 and 6 month olds shown in Table 2. This protection occurred both in males and females. At the end of 9th month, another experiment, retained latencies both in 0.7 *μ g/kg* and 7 μ *g/kg*

aFGF groups (tested only in male, n=10) were signiftcantly higher when compared with the saline group, e.g. 123.3±11.6 s and 129.9±4.9 s in the two groups vs. 54.9±6.61 s in the saline group.

TABLE 1

Effect of intermittent aFGF injections s.c. on passive avoidence task in SAMP8

			Latency in retention trial (sec)				
Treatment	Sex	n	2 months	3 months	5 months	6 months	9 months
saline	male	5	16.9±3.4	15.4±5.8	10.4±1.5	8.8±1.7	8.6±3.0
aFGF	male	7	41.0±9.2**	34.2±13.8*	34.6±9.5**	16.6±2.2*	30.6±12.6*
saline	female	8	11.9±1.4	15.2±6.7	7.0±0.6	7.1±1.1	20.8±8.3
aFGF	female	10	38.3±6.5**	58.5±11.6**	25.1±6.9*	16.9±3.2*	48.6±20.6

*: $p<0.05$; **: $p<0.01$

TABLE 2

Effect of intermittent aFGF injection s.c. on passive avoidance task in elder SAMP8

			Latency in retention trial (sec)	
Treatment	Sex	n	5 months	6 months
saline	male	9	23.0±4.7	17.60±2.8
aFGF	male	10	77.2±18.8*	75.8±23.2*
saline	female	8	18.2±5.4	13.2±1.7
aFGF	female	9	40.6±10.1**	51.4±17.3**

*: $p<0.05$; **: $P<0.01$

Water Maze Test: With regard to spatial memory of the SAMP8 at the end of the 10 months treatment period, as shown in table 3 the mean latency of 4 trials to climb on the platform on day 1 (1st block) both in males and females was significantly shorter in the 7 μ *g/kg* aFGF group than in the saline group

TABLE 3

Effect of intermittent aFGF injection s.c. on Morris water maze task in SAMP8

			Mean Latency to find platform (sec)			
Treatment	Sex	n	1 block	2 block	3 block	4 block
saline	male	9	50.7±7.4	25.0±3.7	18.9±2.4	14.4±1.7
aFGF	male	10	30.7±2.5*	16.3±1.3*	12.6±0.9*	12.2±1.0
saline	female	8	40.1±4.6	22.1±1.6	15.1±1.3	13.4±0.8
aFGF	female	9	25.9±1.9*	15.6±2.0*	15.1±1.5	12.5±1.0

*: $p<0.05$

On subsequent trials these differences though diminished remained significant in the males. The significance however was lost in the 4th block in the 3rd or 4th blocks in the female. In another group of SAMP8 (tested only in male n=10), experiments were repeated giving similar results. The mean latencies at the end of 10 months on day 1 in 0.7 *μ/kg* and 7 *μ /kg* aFGF groups were 27.0±3.2 s and 17.2±1.6 s respectively, which were significantly shorter than in saline group with a mean latency of 3 5.2±3.7 s.

Mechanism of Protection Provided by aFGF

Cholinergic Neurons in the Medial Septum: The distribution of ChAT in cholinergic neurons in the septum, the diagonal band of Broca and Meynert nucleus as measured immunohistochemically after s.c. aFGF and saline, SAMP8 saline SAMP8 and SAMR1 showed that cholinergic neurons were quite identical in diagonal band of Broca and Meynert nucleus.

Figure 1 give a relative idea of ChAT density aFGF and saline injected animals. Quantitative estimation showed that cholinergic neurons in the medial septum numbered 53± 5/mm^2 (n=10) in the aFGF group, and 50±7/mm^2 in the SAMR1, but significantly low i.e. 40±7/mm^2 in the saline group. The ChAT level in the medial septum neurons of the aFGF group averaged 1478.5 grey level (n=1179) which was significantly higher than the saline

group, 958.0 grey level (n=892). In the SAMR1 the average was 2206.1 grey level, which did not show significant statistical difference from the aFGF group.

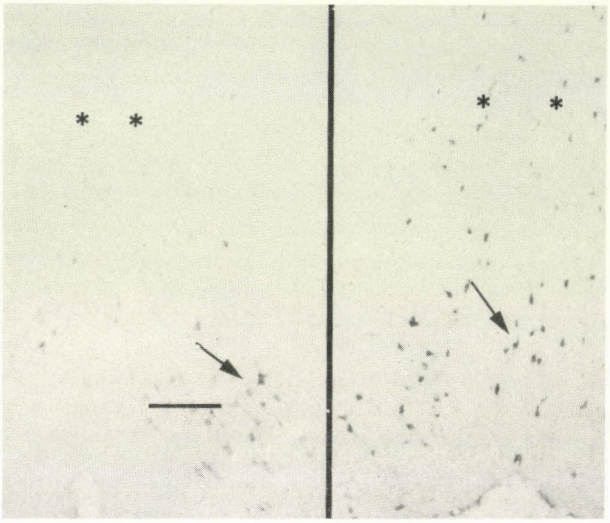

Fig. 1. Immnohistochemically stained choline acetyltransferase (ChAT) positive neurons in the septum and diagonal band on a frontal section. The medial septum is in continuum with the diagonal band. These sections are from the brains of 10th month old animals. Left, SAMP8, saline s.c. injection once a week. Right, SAMP8, aFGF s.c. injection, 7 μ *g/kg* once a week. Cholinergic neurons in the medial septum (upper, stars, bilateral) clearly seen in the right. Cholinergic neurons in the diagonal band of Broca (arrow), clearly seen in both left and right. It shows that in aFGF treated SAMP8, the ChAT concentration is higher than the saline group. Bar, 25 μ m.

LTP: The synaptic potentials at CA1 30 min after a tetanic stimulation increased by 34.9 ±8.3% in the saline group (n=8), by 90.2±11.5% in the 7 μ g/kg aFGF (n=8) and 69.9±8.5% in the 0.7 μ *g/kg* aFGF groups. The difference between the saline and aFGF groups were statistically significant. Thus, the LTP was significantly facilitated in the aFGF group. The increase in the normal SAMR1 was 114.8± 18.9%.

Receptor Density of Muscarinic, NMDA and aFGF Receptors: The density of muscarinic receptors in the hippocampus assayed by [^{3}H] - QNB binding was significantly higher (111.6±6.7 fmol/mg wet weight, n=6) in the male aFGF group than in the male saline group (54.7±17.9 fmol/mg, n=4). The same was true in the cortex (111.6 ± 5.6 fmol/mg wet in aFGF; 77.7±6.0 fmol/mg in saline).

The NMDA receptor density assayed by [^{3}H] MK 801 binding in the hippocampus tended to be higher in male aFGF group (42.7±4.5 fmol/mg, wet weight, n=6) than in the male saline group (26.5±11.6 fmol/mg, n=4), but did not reach a statistically significant level. However, that in the cortex was significantly higher in the male aFGF group (48.8±1.5, fmol/mg n=4) than in the saline group (24.8±6.8 fmol/mg, n=4). These receptor densities when tested in the females were not different between the aFGF and saline groups.

aFGF receptor density immunohistochemically stained by anti FGFR-1 antibody, on hippocampal neurons in the dentate nucleus, CA1, CA2 and CA3 regions was significantly higher in the aFGF and SAMR1 groups than in the saline group.

MAO-B concentration: The MAO-B activity in the whole brain which is known to increase in the aged, was 15.5±2.0 dpm/g protein (n=5) as assayed for male SAMP8 treated with aFGF. This was significantly lower than in the saline male group, 22.2 dpm/g (n=6). Again, the females did not show any significantl differences.

Immune response: The DTH in the end of the 2nd and 7th months caused by challenge with SRBC in the saline group (male, n=6) was 42.8±4.0 and 29.5±5.1 (10^{-2}mm) respectively, while in those treated with aFGF (male n=6) at the end of 7th month was 52.0±5.6 (10^{-2}mm). This difference was statistically significantl. The DTH in the same period caused by challenge with TNP in the saline group (male n=6) was 47.9±9.6 and 32.6±7.1 (10^{-2}mm) respectively, while it was 41.8±5.6 (10^{-2}mm) in the end of 7th month in the aFGF group (male n=6). Although the immune response increased by aFGF in 7th month, the difference was not statistically significant.

Transportation of aFGF in CSF: Studies in aFGF transportation, into rat CSF after aFGF s.c. injection showed that the entire aFGF molecule did not enter into CSF. Instead, immunochemical measurement with anti aFGF (1-15) antibody showed a significant increase in the aFGF(1-15) from 146.7±79.9 fmol/ml to 919.9±333.3 fmol/ml (n=8), 13 hr after treatment. Thus, an aFGF fragment could be elevated in the brain, at least the 1-15 and similar fragments.

DISCUSSION

The present data indicate that aFGF provides protection against impairment of not only learning and memory but also the T cell immunoreactivity in SAMP8.

The mutual connections between the medial septum and hippocampus, and the Meynert nucleus and the cortex are closely related to learning and memory. The distribution of ChAT in cholinergic neurons in the medial septum was severely decreased but it was not in the

Meynert nucleus in SAMP8 and aFGF restored it. The depleted densities of muscarinic and aFGF receptors in the hippocampal neurons in SAMP8 were also restored by aFGF. Such receptor functions of hippocampal neurons possibly constitute the basis of hippocampal role in learning and memory, and are compromised in the SAMP8. The present study also shows that LTP produced in the CA1 hippocampal neurons by tetanization of Schaffer collateral commissural afferents is much facilitated after aFGF administration in the SAMP8. This seems to bi in contrast to Katsuki et al. (1990) who could not observe any difference of LTP on CA1 synaptic potentials between SAMP8 and SAMR1. However, it is likely that Katsuki et al. could not observe the LTP difference in the SAMP8 because of technical differences. Axon terminals of the cholinergic neurons from the medial septum to CA1 neurons pass partly through the commissural afferents. In rats the disconnection between the cholinergic neurons in the medial septum and the hippocampus by severing the fimbria, deteriorated the learning and memory function but the implant of fetal septal neurons into the hippocampus and synaptic formation between them improved that (Dunnett et al., 1982). Severe damage on learning and memory function is known to take place by the lesions of the rat medial septum but not by those of lateral septum (M'Harzi et al., 1992). These all indicate the importance of the medial septo-hippocampal circuitry for learning and memory formation.

The aFGF mechanism for the restoration of the decrease in the tissue immune function with age in SAMP8 is not yet clear. Cultured T lymphocytes isolated from normal human peripheral blood synthesize and export bFGF (Blotmick et al., 1994). bFGF stimulates the release of interferon - γ produced from natural killer cells (Lewis et al., 1991). Our own preliminary experiment indicates an activation of phagocyte function of macrophages after their immersion in aFGF solution. Such information, even though limited, supports the hypothesis of aFGF induced activation of the tissue immune function.

In conclusion, aFGF prevents the impairment of learning and memory function and immune activity in SAMP8. These observations also indicate the close relationship between these the two functions.

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REFERENCES

Blotnick,S., Peoples,G.E., Freeman,M.R., Eberlein,T.J. and Klagsbrun, M.(1994) T lymphocytes synthesize and export heparin-binding epidermal growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD_4^+ and CD_8^+ T cells. Proc. Natl. Acad. Sci. USA 91 2890-2894.

Dunnett,S.B., Lon,W.C., Uverson,S.D., Stenevi,U. and Bjorklund,A. (1982) Septal transplants restore maze learning in rats with fornix-fimbria lesions. Brain Res. 251, 335-348.

Hanai,K., Oomura,Y., Kai,Y., Nishikawa,K., Morita,H. and Plata-Salaman,CR (1989): Central action of acidic fibroblast growth factor (aFGF) on feeding regulation Am. J.Physiol. 256, R217-R223

Katsuki,H., Ishihara,K., Shimada,A., Takeda,T. and Satoh,M. (1990) Age-related deterioration of longh-term potentiation in the CA3 and CA1 regions of hippocampal slices from the senescence-accelerated mouse. Arch. Gerontol. Geriatr. 11, 77-83.

Kitamura,Y., Zhao,X.-H., Ohnuki,T. and Nomura,Y. (1989) Legend-binding characteristics of [^{3}H]QNB, [^{3}H]prazosin, [^{3}H]rauwolscine, [^{3}H]TCP and [^{3}H]nitrendipine to cerebral cortical and hippocampal membranes of senescence accelerated mouse. Nuerosci. lett. 106, 334-338.

Lewis,C.E., Ramshaw,A.L., Lorenzen,J. and McGee,J.O'D. 1991 Basic fibroblast growth factor and interleukins 4 and 6 stimulate the release of IFN-γ by individual NK cells. Cell Immunol.132, 158-167.

Matsuo,A., Tooyama,I., Isobe,S., Oomura,Y., Akiguchi,I., Hanai,K., Kimura,J. and Kimura,H. (1994) Immunohistochemical localization of fibroblast growth factor receptor-1 in the rat brain. Neurosci. 60, 40-66.

M'Harzi,M. and Jarrard,L.E. (1992) Effect of medical and lateral septum lesions on acquisition of a place and cue radial maze task. Behav. Brain Res. 49, 159-165.

Nomura,Y., Wang,B., Xi,S., Namba,T. and Kaneko,S. (1989) Biochemical changes related to aging in the senescence-accelerated mouse. Exp. Geront. 24, 49- 55.

Oomura,Y., Sasaki,K. and Li,A. (1993): Memory facilitation educed by food intake. Physiol. Behav. 54, 493-498

Oomura,Y., Sasaki,K., Suzuki,K, Muto,T., Li,A., Ogita,Z., Hanai,K., Tooyama,I., Kimura,H. and Yanaihara,N.(1992): New brain glucosensor and its physiological significance. Am. J. Clin. Nutr. 55, 278S-282S

Sasaki,K., Oomura,Y., Figrov,A. and Yagi,H. (1994a): Acidic fibroblast growth factor facilitates generation of long-term potentiation in rat hippocampal slices. Brain Res. Bull. 33, 505-511

Sasaki,K., Oomura,Y., Muto,T., Hanai,K., Tooyama,I., Kimura,H., Yanaihara,N., Yagi,H. and Hori,T. (1994b): Effects of fibroblast growth factors and related peptides on food intake by rats. Physiol. Behav. 56, 211-218.

Yagi,H., Katoh,S., Akiguchi,I. and Takeda,T. (1988) Age related deterioration of ability of acquisition in memory and learning in senescence accelerated mouse: SAMP8 as an animal model of disturbance in recent memory. Brain Res. 474, 86-93.

Zhao,X.-H., Kitamura,Y. and Nomura,Y. (1990) Age-related changes of NMDA receptor and its agonist-induced acetylcholine release from brain slices in senescence-accelerated mouse. Japan J. Pharmacol. 52 Suppl.I:75p

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REFLECTIONS OF A "SENIOR PHYSIOLOGIST": BRIDGE OVER THE PACIFIC OCEAN

Koizumi, K.

Department of Physiology, State University of New York
Health Science Center at Brooklyn, New York 11203, USA

I am overwhelmed by the kindness and warmth of my colleagues and friends who gathered here today to honor Dr. Oomura and myself. Although I do not deserve such an honor as Dr. Oomura, I must say that my luck was to be born in the same year as Dr. Oomura.

When I reached a milestone of my life last September, I met with three surprises. Shortly after my birthday a letter from the US Government informed me that I would soon begin to receive Social Security payments. The second surprise was a letter from the American Physiological Society wishing me a happy birthday but at the same time asking me what I was doing with my life since I had joined a group of "senior physiologists". Of course, that was such a shock to me that I have not replied yet. If I were to do so, the Society would publish my letter in the Society's publication, *The Physiologist,* in a section called "Letters from senior physiologists" and then every physiologist in America will find out how old I am. Now this symposium is the third surprise and the most pleasant one. It is so wonderful and heart-warming to learn that many physiologists who worked with me in the past in the USA as well as in Japan are still offering me encouragement and help.

Rather than talking about my small research endeavor, I wish to reflect on the road I took in the past 45 years. One can say that this is a sign of aging. I also wish to reflect on how far

"our" physiology has come, and how we continue in the future in the difficult times we face.

As many "ordinary" people in the world have experienced, my life as a woman and a scientist has been largely determined by fate and luck. "By fate" I mean that, at every turn in my career, an opportinity was presented to me, and I just took that opportunity. It went like this. I went to a medical school, because that was the only choice for a woman in my days in Japan who desired to study any kind of science. The only other choice was to become a high school science teacher. After graduating from the women-only medical school, I decided to do research in physiology, because I was so excited and charmed by lectures in physiology given by a young and handsome professor, Dr. Isamu Suda. Once I was told by another cynical professor that I was following Lorelei's voice! I decided to go to the USA to study, because the laboratory where I was working for 8 months burned down in a fire and I could not continue to work. Luckily I passed an examination for a scholarship given by a group of American churches. Thus, in 1949 I went to Wayne State University in Detroit to become a graduate student in Physiological Chemistry, because that was the only place where I could receive a full scholarship. When I completed my master's degree after two years, there was no decent job for a woman physiologist in Japan, so I decided to stay in America for a little while. My vigorous job search brought me to Dr. Chandler McCuskey Brooks who was building and expanding the Department of Physiology at the newly created State University of New York, College of Medicine. I was hired as an instructor in 1951 and stayed there ever since.

It so happened that just before this period Dr. Brooks was in New Zealand working with Sir John C. Eccles for two years to learn neurophysiological techniques. The reason was that Dr. Brooks, who had been studying the hypothalamic control of the pituitary gland, felt that the new electrophysiological approach would be necessary

to further his work. This was before the discovery of the portal circulation by Geoffery W. Harris. Eccles told him that trying to record from hypothalamic neurosecretory cells was impossible and a nonsense, and advised him to work together on the spinal cord. They published a series of nice studies on Golgi inhibitory neurons. This was the period when the chemical transmission theory in the central nervous system was not yet accepted. I began to work with Dr. Brooks on the spinal cord, succeeding to a visiting professor from Italy who had just left for the National Institute of Health. Thus, I became a neurophysiologist by chance.

Nearly 10 years of my work on the neurophysiology of the spinal cord and the reticular formation in the midbrain was very rewarding. We were successful in finding mechanisms of temperature effects both at the reflex and cellular levels. We even found presynaptic inhibition at motoneurons, but never really pursued the "big question". Since our Department had a system of having a visiting professor every year, at the early stage of my career I had the priviledge of working with established investigators including Dr. J. Laurence Malcolm of Scotland, Dr. Isamu Suda of Japan and Dr. David R. Curtis of Australia (Brooks, Koizumi and Malcolm, 1955; Brooks and Koizumi, 1956; Suda, Koizumi and Brooks, 1958; Curtis and Koizumi, 1961). In the late 50's Dr. Junji Ushiyama from Japan joined us who was an expert in making capillary microelectrodes **by hand** for quite successful intracellular recordings (Koizumi, Ushiyama and Brooks, 1960; Ushiyama, Koizumi and Brooks, 1966).

Around 1960 I decided to change directions of my research, because I wished to explore a new field. By this period the field of neurophysiology of the spinal cord and brain was very active and "hot" after the discoveries of EPSPs and IPSPs in the spinal motoneurons by Eccles and his associates, who had established a chemical transmission theory in the central nervous system (Eccles, 1953). It was rather easy for me to choose the new research field,

since Dr. Chandler Brooks, my life long mentor and friend, was interested in both the autonomic nervous system and the hypothalamus. In his early career Dr. Brooks worked with Walter B. Cannon at Harvard Medical School. At Johns Hopkins Medical School he was associated with Phyllip Bard who made an "isolated hypothalamic island" in the chronic dog, and studied the hypothalamic control of the pituitary functions. As I mentioned before, in the back of his mind Dr. Brooks wished to record from the secretory neurons in the hypothalamus. Thus, we began our projects, applying electrophysiological techniques in studies of both the neuroendocrine and the autonomic nervous systems.

In those "ancient" days, though it was merely 30 to 40 years ago, neurophysiology was almost restricted to studying the somatic system. Except for a few pioneering physiologists here and there, most physiologists did not attempt to record from cells other than neurons, probably because they were not interested in them. By the same token, most physiologists thought then that the cerebral cortex and the cerebellum had little to do with the autonomic nervous system.

We wanted to find out if changes in electrical activities of the neurosecretory cells in the hypothalamus were the cause of hormone release from the pituitary. If so, we could record an increase or a decrease in the electrical activity of these neurosecretory neurons in response to physiological stimuli and this would affect the amount of hormone released at the pituitary gland. These are all well known facts today but not so in those days. In the autonomic research we tried to find out if nerve impulses in the autonomic nerves were altered by cortical or cerebellar stimulation. It was known then that a few brain regions, when electrically stimulated, caused changes in gut movement or blood pressure, but we had little knowledge on what was happening in the autonomic nerves. In both situations the question was how to identify the cells or nerves from which we recorded their electrical activities.

Here again, my fate took me to Japan, because I had to leave the USA for at least two years due to my overdue visitor's visa. While I was working with Dr. Isamu Suda at University of Kobe Medical College, he helped me establish the techniques of recording from "single" sympathetic postganglionic fibers to the gut by a floating microelectrode. He also developed a very unique method of recording from "visually and chemically identified" neurosecretory neurons in the exposed hypothalamus of hemispherectomized cats (Koizumi and Suda, 1963; Suda, Koizumi and Brooks, 1963). As any researcher discovers all the time, there are always some pioneers in any field. We knew that D. W. Bronk and his associates had already in the 30's recorded action potentials from "single" cardiac sympathetic fibers (Bronk, 1935), and B. A. Cross and J. D. Green recorded unitary activity from hypothalamic neurosecretory neurons in the 50's (Cross and Green, 1959). However, their work somehow ended there.

Thus, in my mid-career I began to work on the "new" field, utilizing the method I learned from working on the spinal cord. It was the "good old days" and I did not even think of the money needed to start the new projects. Somehow there was always money available for me to use for our research on the subject we thought was interesting.

After two years' stay in Japan and Scotland, I was allowed to return to the USA in 1962. Since that time I have been blessed with having a continuous flow of Japanese and some European scientists as post-doctoral fellows in our laboratory in the USA. Every year or two a young, energetic and bright fellow came to Brooklyn on a fellowship to contribute to our research projects. Since every post-doctoral fellow had a somewhat unique idea and interest, our work gradually expanded to cover a more wider horizon.

With the help of Dr. Tomoe Ishikawa from Kobe, Japan and Dr. H. H. Lu of Taiwan, we discovered that neurosecretory neurons in

the hypothalamus were activated by physiological stimulation of the uterus and the vagina in post-partum and lactating cats, as well as by osmotic stimuli. I reported that work at the International Congress of Physiological Sciences (now IUPS) in Tokyo in 1965, half believing that our findings were true, and half being afraid that something might be wrong, because our results fitted our idea too beautifully. Sir Barry Cross of England was very excited about our results and later he and his co-workers demonstrated similar phenomena and expanded our work using rats and rabbits. When I learned that the same finding was made in other species with much more refined technique, I was very pleased and breathed a sigh of relief. As a neurophysiologist I never dreamed of using rabbits which are mostly used by endocrinologists for studying reproductive behavior, even though lactating cats were very difficult to work on (Koizumi, Ishikawa and Brooks, 1964, Brooks et al, 1966). Dr. Ishikawa returned to Brooklyn again and we found the facilitatory recurrent system studying bullfrog neurosecretory system in vitro (Ishikawa and Koizumi, 1975).

In 1967 Dr. Richard Dyball of Cambridge, then from Bristol, came to my laboratory to learn electrophysiological technique and introduced me to use rats in neuroendocrine work. He also made me keenly aware of the need of hormone assay in our study of hypothalamic neurons. Together, we began to use antidromic stimulation through the pituitary stalk to identify neurosecretory neurons (Dyball and Koizumi, 1969). Dr. Hiroshi Yamashita who first came to Brooklyn in 1968 from Kobe launched on "in vivo" intracellular recordings from hypothalamic neurosecretory neurons in dogs and cats (Koizumi and Yamashita, 1972). I believe we were the first and probably the last to do so in these species, because it is no longer easy to use large animals for this type of work. Since that time our collaboration has continued to this day, experiments being carried out in the laboratories in Kitakyushu, Japan and Brooklyn, USA.

I had my first taste of working on the lateral hypothalamus and the circadian rhythm around the early 70's, because one of my pre-doctoral students, Mary Schmitt, had a very severe trigeminal neuralgia and preferred to work during the night when she did not have to speak to anyone in the laboratory. Thus, she discovered diurnal changes in the activity of hypothalamic neurons. She also studied liver inputs to the lateral hypothalamic neurons. This was my first experience working on feeding. Dr. Hitoo Nishino then came to our laboratory in 1973 from Kanazawa and worked on the ventromedial and lateral hypothalamic neurons as well as on the suprachiasmatic nuclei neurons. Dr. Nishino also began pharmacological studies on hypothalamic neurons, using iontophoretic method with five barrel electrodes, the technique he mastered working with Dr. Oomura in Kanazawa (Koizumi and Nishino, 1976; Nishino and Koizumi, 1977). We also studied the pineal gland and its control through the autonomic nerve (Nishino, Koizumi and Brooks, 1977). I regret that we did not pursue these studies further, because the topic became very "hot" in later years.

The autonomic research we began in Japan was continued with the help of Dr. Akio Sato who came to us in 1966. In 1969 Dr. Horst Seller of Heidelberg, then of München, came and we continued the work also with my collaborator in Brooklyn, Dr. Albert Kaufman (Koizumi, Sato et al., 1968; Koiumi, Seller et al., 1971). Later Dr. Naohito Terui of Tsukuba joined us in 1981. We investigated relationships between the autonomic and somatic systems, recording from both sympathetic and vagal nerve fibers (Terui and Koizumi, 1984).

In 1976 I was fortunate to be joined by Dr. Mark Kollai of Budapest, who was in Philadelphia at that time. After talking to me at one of the Physiological Society Meetings in New York, he decided to come to my laboratory to do the autonomic research and literally moved into my laboratory on a very short notice. Thereafter, we worked together on and off for 15 years, exploring

interrelationships between the sympathetic and parasympathetic control of the heart. This collaboration also gave me a chance to spend pleasant six months in Budapest in 1979-80 (Kollai and Koizumi, 1979; Koizumi and Kollai, 1992).

Eventually our studies on the neuroendocrine and the autonomic nervous systems were joined together by investigating how vasopressin neurons participate in baroreceptor reflex, how changes in the atrial volume affect vasopressin neurons and hence release of vasopressin. Dr. Hiroshi Kannan of Miyazaki spent a year in my laboratory in 1978-79 (Kannan and Koizumi, 1981). Drs. Yamashita and Kollai also worked on these problems (Koizumi and Yamashita, 1978; Kollai et al., 1978; Yamashita et al, 1983). Our interest in the neuroendocrine physiology also extended to the studies of the subfornical organ, carried out with collaboration of Dr. Yamashita's Department in Japan. Dr. Toshimasa Osaka came to my laboratory in Brooklyn in 1986 for these studies (Osaka, Yamashita and Koizumi, 1988).

In the last several years my work turned around a third time. Again this occurred by chance, not because I was restless in studying the similar subjects for 25 years, but also I am always attracted by "something new". A fellow in the Department of Medicine in our Medical Center had strange inbred mice that were extremely polydipsic. He asked Dr. Yamashita and me if we could find why these mice drank so much. We both thought that the problem was intriguing but could be solved in one or two years. I had then the luck of having three Japanese energetic post-doctoral fellows from 1987 to 89 who worked on the polydipsic mice. Dr. Yukio Hattori from Okayama, Dr. Toshihiko Katafuchi from Fukuoka, and Dr. Itsugi Nagatomo from Kagoshima joined me and studied the contribution of the brain opiates on the polydipsia (Hattori, Katafuchi and Koizumi, 1991, Katafuchi et al. 1991, a, b). For a while it looked as though the mystery was solved. However, the more we studied, the more the problem of the polydipsia became

mysterious, and at present we are still in the dark. I transferred a part of my mice colony to Dr. Yamashita's laboratory and we are working together on this problem. I often joked that I had to write in my will that someone should eventually solve the mystery, then letting me know wherever I would be. A friend of mine quickly reminded me to leave some money for this purpose in my will.

During all these years my foreign collaborators not only helped me to continue my research in depth and width, but also shared their knowledge, enthusiasm and friendship. Looking back now on 45 years of my life as a scientist working in Brooklyn as well as in Japan, Hungary, Germany and Scotland, I am overwhelmed by the kindness and good will of my colleagues. Without my co-workers and my mentor, Dr. Brooks, who also helped me to have many associates from abroad, I would never have reached where I am, and I could never have had such a rich and enjoyable life. Most of all, a strong bond of friendship which ties us around the world is the most precious gift given to me in my life.

At present we all realize that physiology is at a crossroad. All of us who are interested in any aspect of the hypothalamus and its vast functions wonder how our future research will go and how much of the mysteries we will be able to unravel. When I began my career as a physiologist in 1950, we could pursue what we were interested in and the society allowed us to do so. Can we expect to continue to do so? Already in the USA one tends to select or alter one's research direction in order to obtain grant money to continue one's work.

I used to say that the requirements for a good physiologist are a bit of brain and some dexterity, but what is needed more is curiousity, enthusiasm and perseverance. If one is very bright, one has no patience to sit for hours in order to see one beautiful action potential to appear. One successful experiment will put us in heaven, while the next 5 miserable experiments almost drag us down. And yet we continue to try again and again. Does Science

attract us because it is so interesting? In our daily experiments, that is not what pushes us ahead. It is perhaps by nature that we wish to continue until we succeed. To borrow from Dr. Setsuro Ebashi, an outstanding muscle physiologist, a researcher must find a pleasure and joy in a very small discovery or a small success in daily work. If that cannot be done, experiments become a burden to an investigator. An experimentation itself is often a hard and almost "stupid" work. To find a great joy in such a small thing may be called talent (Ebashi, 1991). I have no doubt that there always will be young "talented" scientists who will continue exploring the mystery of life, brain and the hypothalamus, because physiology is too attractive and rewarding to be abandoned.

Working with my "talented" colleagues gave me such a pleasure to do research, regardless of what the outcome was. I always enjoyed doing research, i.e., doing work by myself when I was young, and watching others work when I became older. I am grateful to many colleagues of mine who allowed me to do many experiments with them and who permitted me just to watch them do their work.

And now it is beyond my comprehension that my former colleagues and friends so generously give me this honor.

REFERENCES

Bronk, D. W. (1935) The nervous mechanism of cardiovascular control. Harvey Lect. 1933-34, 245-262.

Brooks, C. McC., Ishikawa, T., Koizumi, K. and Lu, H. H. (1966) Activity of neurones in the paraventricular nucleus of the hypothalamus and its control. J. Physiol. 182, 217-231.

Brooks, C. McC. and Koizumi, K. (1956) Origin of the dorsal root reflex. J. Neurophysiol. 19, 61-74.

Brooks, C. McC., Koizumi, K. and Malcolm, J. L. (1955) Effects of changes in temperature on reactions of the spinal cord. J. Neurophysiol. 18, 205-216.

Cross, B. A. and Green, D. J. (1959) Activity of single neurones in the hypothalamus. Effect of osmotic and other stimuli. J. Physiol. 148, 554-569.

Curtis, D. R. and Koizumi, K. (1961) Chemical transmitter substances in brain stem of cat. J. Neurophysiol. 24, 80-90.

Dyball, R. E. J. and Koizumi, K. (1969) Electrical activity in the supraoptic and paraventricular nuclei associated with neurohypophysial hormone release. J. Physiol. 201, 511-722.

Ebashi, S. (1991) Kenkyu suru tanoshimi (Joy of doing research). Jikken Igaku 9 (no. 14), 66-74.

Eccles, J. C. (1953) Neurophysiological basis of Mind. Clarendon Press, Oxford.

Hattori, Y., Katafuchi, T. and Koizumi, K. (1991) Characterization of opioid-sensitive neurons in the anteroventral third ventricle region of polydipsic inbred mice in vitro. Brain Res. 538, 283-288.

Ishikawa, T. and Koizumi, K. (1975) A study of hypothalamic neurosecretory cells of bull frogs in vitro. J. Physiol. 253, 1-19.

Kannan, H. and Koizumi, K. (1981) Pathways between the nucleus tractus solitarius and neurosecretory neurons of the supraoptic nucleus: Electrophysiological studies. Brain Res. 213, 17-28.

Katafuchi, T., Hattori, Y., Nagatomo, I. and Koizumi, K. (1991a) Kappa opioid antagonist strongly attenuates drinking of genetically polydipsic mice. Brain Res. 546, 1-7.

Katafuchi, T., Hattori, Y., Nagatomo, I., Koizumi, K. and Silverstein, E. (1991b) Involvement of angiotensin II in water intake of genetically polydipsic mice. Am. J. Physiol. 260, R1152-R1158.

Koizumi, K., Ishikawa, T. and Brooks, C. McC. (1964) Control of activity of neurons in the supraoptic nucleus. J. Neurophysiol. 27, 878-892.

Koizumi, K. and Kollai, M. (1992) Multiple modes of operation of cardic autonomic control: Development of the ideas from Cannon and Brooks to the present. J. Auton. Nerv. Syst. 41, 19-29.

Koizumi, K. and Nishino, H. (1976) Circadian and other rhythmic activity of neurones in the ventromedial nuclei and lateral hypothalamic area. J. Physiol. 263, 331-356.

Koizumi, K., Sato, A., Kaufman, A. and Brooks, C. McC. (1968) Studies of sympathetic neuron discharges modified by central and peripheral excitation. Brain Res. 11, 212-224.

Koizumi, K., Seller, H., Kaufman, A. and Brooks, C. McC. (1971) Pattern of sympathetic discharges and their relation to

baroreceptor and respiratory activities. Brain Res. 17, 281-294.

Koizumi, K. and Suda, I. (1963) Induced modulations in autonomic efferent neuron activity. Am. J. Physiol. 205, 738-744

Koizumi, K., Ushiyama, J. and Brooks, C. McC. (1960) Effect of hypothermia on excitability of spinal neurons. J. Neurophysiol. 23, 421-431.

Koizumi, K. and Yamashita, H. (1972) Studies of antidromically identified neurosecretory cells of the hypothalamus by intracellular and extracellular recordings. J. Physiol. 221, 683-705.

Koizumi, K. and Yamashita, H. (1978) Influence of atrial stretch receptors on hypothalamic neurosecretory neurones. J. Physiol. 285, 341-358.

Kollai, M. and Koizumi, K. (1979) Reciprocal and non-reciprocal action of the vagal and sympathetic nerves innervating heart. J. Auton. Nerv. Syst. 1, 33-52.

Kollai, M., Koizumi, K., Yamashita, H. and Brooks, C. McC. (1978) Study of cardiac sympathetic and vagal activity during reflex responses produced by stretch of the atria. Brain Res. 150, 519-532.

Nishino, H. and Koizumi, K. (1977) Responses of neurons in the suprachiasmatic nuclei of the hypothalamus to putative transmitters. Brain Res. 120, 167-172.

Nishino, H., Koizumi, K. and Brooks, C. McC. (1976) The role of suprachiasmatic nuclei of the hypothalamus in the production of circadian rhythm. Brain Res. 112, 45-59.

Osaka, T., Yamashita, H. and Koizumi, K. (1988) Inhibition of paraventricular neurons by subfornical organ and AV3V in cats. Am. J. Physiol. 255, R961-R967.

Suda, I., Koizumi, K. and Brooks, C. McC. (1958) Reticular formation influences on neurons of spinal reflex pathway. J. Neurophysiol. 21, 113-123.

Suda, I., Koizumi, K. and Brooks, C. McC. (1963) Study of unitary activity in the supraoptic nucleus of the hypothalamus. Jap. J. Physiol. 13, 374-385.

Terui, N. and Koizumi, K. (1984) Responses of cardic vagus and sympathetic nerves to excitation of somatic and visceral nerves. J. Auton. Nerv. Syst. 10, 73-91

Ushiyama, J., Koizumi, K. and Brooks, C. McC. (1966) Accommodative reactions of neuronal elements in the spinal cord. J. Neurophysiol. 29, 1028-1045.

Yamashita, H., Kannan, H., Inenaga, K. and Koizumi, K. (1983) Role of neurons in the supraoptic and paraventricular nuclei in cardiovascular control. Progr. Brain Res. 80, 459-468.

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EFFECTS OF HYPOTHALAMIC PARAVENTRICULAR NUCLEUS: ELECTRICAL STIMULATION PRODUCE MARKED ANALGESIA IN RATS

Shiraishi, T., Onoe, M., Kojima, T.*, Sameshima, Y.* and Kageyama, T.*

Department of Neurophysiology, Division of Human Structure and Function, The Tokai University School of Medicine, Isehara, Kanagawa 259-11 and *Research Institute of Oriental Medicine, Shibuya, Tokyo 150 Japan

Summary: The effect of the electrical stimulation induced analgesia (ESIA) on the hypothalamic paraventricular nucleus (PVN) was investigated by the paw pressure test, which was used to avoid any tissue damage to the paw of Wistar-SPF/VAF male rats. A stimulating electrode was chronically implanted in the parvocellular (PVN-prv) or magnocellular (PVN-mgn) divisions of the PVN. The ESIA was examined at least 10 days after surgery. The elctrical stimulation of the PVN markedly showed analgesia (ESIA), but stimulation of most locations outside the PVN did not produce ESIA. Stimulation threshold for the ESIA was lower from PVN-prv than from PVN-mgn, but neither region was affected by naloxone administration (10 mg/kg, i.p.). These results indicate that the PVN is a part of the pain inhibitory system in the CNS, and show that PVN-ESIA might not be mediated either by opioids or by neuropeptides such as vasopressin.

Key Words: hypothalamic paraventricular nucleus (PVN), parvocellular division of the PVN, analgesia, pain threshold, paw pressure test, Wistar male rat

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INTRODUCTION

In the experiments conducted, interesting and important results were found and reported. Mainly, relations between the hypothalamic neuronal activity and its related functions, such as regulation of feeding, control of gastric acid secretion were studied, (Shiraishi, 1980c; Shiraishi et al., 1982; Shiraishi and Simpson, 1982, 1987; Shiraishi, 1990; Shiraishi, 1991a, etc.).

In our researches, recently, it was found that the hypothalamic paraventricular nucleus (PVN) plays an important role in the cerebral pain inhibitory system/mechanisms. We have studied electrical stimulation induced feeding by stimulation of the lateral hypothalamus (LHA) and PVN. In these experiments, occasionally, some of the animals, in this case rats, showed 'analgesic' behavior similarly to sedation drowsiness depending on stimulating current intensities.

Up to the present, we have known that stimulations (Oliveras and Besson, 1988) of lateral (Cox and Valenstein, 1965), medial (Vidal and Jacob, 1980), and posterior (Rhods and Liebeskind, 1978) hypothalamic area have been reported to elicit analgesia. It is presumed that PVN participates in the pain inhibitory system for following reasons. The PVN neurons project to various parts of the CNS which are involved in nociception, such as periaqueductal gray, nucleus raphe magnus, dorsal vagal complex of the medulla and the spinal cord (Cochetto and Saper, 1988; Hosoya, 1980; Sawchenko and Swanson, 1980). Neurons in the PVN synthesize and secrete numerous neuropeptides, some of which have been shown to produce analgesia on exogenous administration, for example, vasopressin (Bodnar et al., 1986), Met-and Leu-enkephalin (Belluzzi et al., 1976), dynorphin (Han and Xie, 1982), somatostatin (Chrubasik et al., 1984), neurotensin and Fallon, 1986; Nemeroff et al., 1979), TRH (Boschi et al., 1983), and CRF (Hargreaves et al., 1987).

The purpose of the present study was to reveal the effects of the PVN electrical stimulation induced analgesia (PVN-ESIA), through the chronically stimulating electrodes implanted into either magnocellular division of PVN (PVN-mgn) and/or parvocellular division of PVN PVN-prv) in Wistar-SPF/VAF male rats, by the paw pressure test. Some of the results described in this paper have been previously presented in preliminary form (Shiraishi et al., 1993).

MATERIALS AND METHODS

Animals

Wistar SPF/VAF male rats, 4 weeks old (80-90 g), were caged individually under standarized conditions with 12 h dark-light cycles (light on, 7:00-19:00) and free access to rat chow pellets (Japan Clea Co., Tokyo) and tap water until surgical preparation for the chronic stimulating electrodes implantation. Each animal received a handling every day 15-30 min by the examiner.

Surgery

When 9-10 weeks old (200-230 g), the animals were fixed in a stereotaxic apparatus (Narishige, Tokyo, SN-) under pentobarbital sodium (Nembutal, 45 mg/kg, i.p.) anesthesia. An 80-100 μm diameter stainless steel wire, single electrical stimulating electrode, with cashew varnish coating, except 0.3 mm of the tip, was stereotaxically implanted bilaterally into the PVN-prv or PVN-mgn, and fixed to bone with anchor screws and dental cement. Loci of the PVN were: anterior, 1.0 mm to posterior, 0.5 mm; lateral, 0.2 mm (PVN-prv) or 0.8 mm (PVN-mgn); and vertical 8.0 mm from the surface of the parietal bone at the Bregma (Köning and Klippel, 1967). After surgery, each animal was housed in a separate cage. The experiment was started at least 10 days after surgery. During recovering time from surgery, even on the day of experiments, the animals still received 15-30 min handling every day. In accordance with the preliminary baseline 'pain' threshold (Shiraishi, 1991b; also see RESULTS), in morning (09:00-12:00) it was significantly higher than in afternoon (14:00-18:00) testing. All experiments were thus carried out in the afternoon at daylight.

Procedure

Analgesia was tested by the rat paw pressure test. This device was a recently developed apparatus for measuring the 'pain' threshold (Shiraishi, 1991b). Briefly, the left hind paw of the well trained rat was gently restrained on a plinth, while pressure was exerted by a weight, traveling at a uniform rate along a scale on the home-made analgesic scale meter. The position of the weight on the scale meter indicated the 'pain' threshold of the subjects. Determination of threshold was: when the subject struggled or vocalized, the paw was withdrawn and the position of the pressure maker was recorded in units of 20 g. The maximum scale was set to 25 units (500 g). This was to avoid any tissue damage to the paw in the course of analgesia testing.

Three baseline withdrawal thresholds (BT) were recorded at intervals of 20 min prior to administration of drugs or next procedure. Test thresholds (TT) were measured at 20 min intervals for 2 h, beginning 20 min after the PVN stimulations or drug

administrations. The BT was generally in the range between 140 and 180 g in this study. The TT was expressed as percentage of Maximal Possible Effect (%MPE) using the equation:

$$\%MPE = \{(TT-BT)/(500-BT)\} \times 100$$

PVN-Electrical Stimulation Induced Analgesia (PVN-ESIA)

The analgesic effect on the PVN-prv (n=6) or PVN-mgn (n=7) electrical stimulation was examined using the above described method. i) Twenty min after determined BT, the TT was assessed by determined the mean response for the last 3 of 5 trials. Stimulation was delivered at frequency of 30-50 Hz, and pulse duration of 0.1-0.5 ms. To determine PVN-ESIA current threshold, stimulation began at an intensity of 10μA and was increased in 20-μA steps to 100-400μA until a current level was reached that almost completely inhibited the paw pressure 'pain' test. ii) Twenty min after the determined BT, the animals were administrated naloxone (NLX, n=6, 10 mg/kg, i.p.) or physiological saline (SLN, 0.1 ml/100 g body weight; n=5). Twenty min later, PVN-ESIA threshold (TT) was re-examined by stimulating the rat at the previously determined threshold (BT). All produces were conducted in accordance with the Japan Physiological Society Guide for the Care and Use of Laboratory Animals, as approved by the Tokai University School Medicine Committee on Animal Care and Use.

Drugs

Morphine Hydrochrolide (Sankyo Pharmaceut., Tokyo), Naloxone Hydrochrolide (Sigma, St. Louis), and vasopressin ([Arg^8]-vasopressin, Sigma and Protein Research Foundation, Osaka) were dissolved in 0.85 % physiological saline and administrated in a volume of 0.1 ml/100 g body weight intraperitoneally.

Histology and Analysis

Postexperimental histological examinations were routinely done to check PVN-prv or PVN-mgn electrical stimulating sites in the hypothalamic periventricular region. After each experiment, the animal was injected an overdose of Nembutal (60 mg/kg, i.p.) and then perfused with physiological saline and 10 % formalin. The brain was removed and fixed, and 40-60 μm coronal sections were cut, mounted, and stained according to methods previously described (Shiraishi and Mager, 1980a, 1980b).

All results are expressed as mean ±SE. Statistical analyses were made by χ^2-test for the occurrence percentage, Bartlett's Test for variance, ANOVA (*F*-test) for means, and Duncan's Multi Test for comparing between groups with a saline control, except analysis of diurnal variations was done using the paired *t*-test (Shiraishi, 1990).

RESULTS AND DISCUSSION

Baseline 'pain' threshold was compared on the same rats AM (09:00-12:00) and PM (14:00-18:00). Paw Pressure Testing AM (8-9 units, i.e. 160-180 g, mean 170.0±12.6 g, n=6) was significantly higher than PM (7-8 units, 140-160 g; 146.7±8.2 g, t=3.80, df=6, $p<0.01$). Diurnal variations in the analgesic efficacy of opioids has been reported (Fredrickson et al., 1977; Rosenfield and Rice, 1979). These suggest that the lower baseline in the afternoon group might be due to the higher concentration of endogenous morphine-like substance in the afternoon. All tests of 'pain' threshold were thus performed PM (14:00-18:00, see METHODS).

Effects of morphine on rat paw pressure test

The aim was to examine whether and how the rat paw pressure test is useful for the 'pain' threshold measurements. So, the initial study was performed to determine the effect of morphine in the rat paw pressure test. Fig. 1 shows the morphine induced analgesia in the paw pressure test in rats (n=6). Morphine, at 1.5, 3, 6, 12 mg/kg and physiological saline as control, was randomly and repeatedly administrated (5 doses x 6 rats = 30 times, i.p., 2-3 doses/day) and 'pain' thresholds were determined at intervals of 20 min for 2 h after injection. Morphine produced marked rise in 'pain' thresholds (analgesia) during the 2 h examination, in a dose-dependent ($y=3.98x + 22.67$, $r=0.827$, $p<0.01$) manner. The rat paw pressure test thus is a useful, quantitative, highly efficient and outstanding method for the measurement of 'pain' thresholds. The following experiments were carried out by this method.

PVN-Electrical Stimulation Induced Analgesia (PVN-ESIA)

The PVN stimulations were observed to induce marked analgesia (PVN-ESIA). From the post-experimental histological examination, the location on the brain at which electrical stimulation induced analgesic responses were in the periventricular region (6.5 mm to 7.5 mm from parietal bone surface, bilaterally within 0.5 mm, and anterior 1.2 mm to posterior 0.2 mm from the Bregma) in the hypothalamus (histological microphotograph

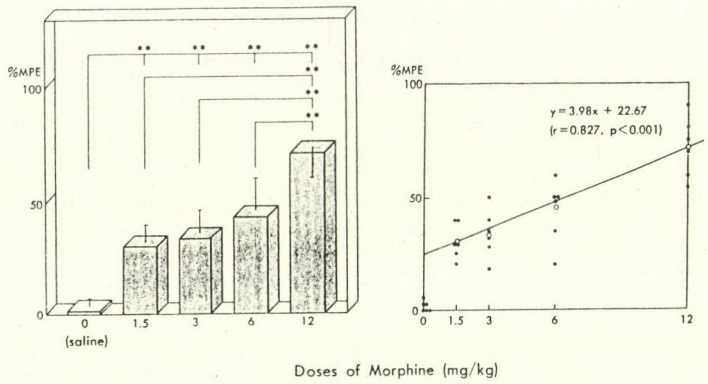

Fig.1: Effects of morphine on 'pain' threshold in the paw pressure test in the rat (n=6). Abscissa; doses of morphine (mg/kg, i.p.) with a physiological saline control, indicate 0. Ordinates; the percentage maximum possible effect (%MPE) calculated as described in the text. Right; Dose response curve for morphine. Closed circles indicate individual value, and open circles indicate their mean value at the each dose. Note significant ($p<0.001$) dose-dependent manner was observed

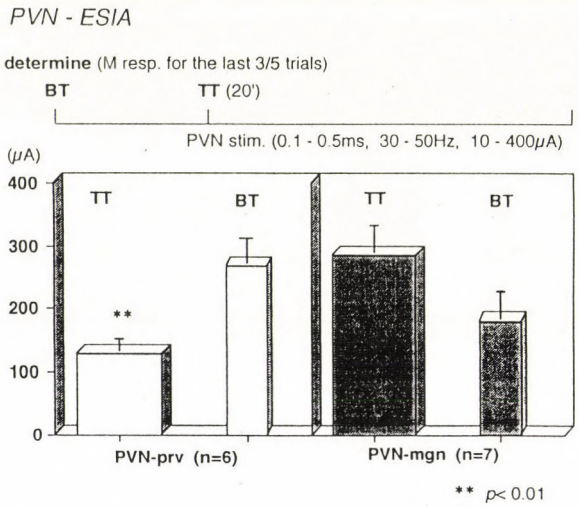

Fig.2: Effects of electrical stimulation of the parvocellular (-prv, n=6) and magnocellular (-mgn, n=7) divisions of paraventricular nucleus (PVN) induced analgesia (ESIA) on the determination of baseline threshold (BT) and 20 min after of BT, test threshold (TT). Determination of both BT and TT was assessed by determining the mean response for the last 3 of 5 trials (express; M resp. for the last 3/5 trials). Mean (± S.E.M.) threshold currents (ordinate, μA) is expressed. Note that stimulation threshold for the ESIA was significantly lower ($p<0.01$) from the PVN-prv (left) than from the PVN-mgn (right) on the TT

not shown here). The ESIA responding sites were strictly identified within the PVN. Stimulations of most locations outside the PVN did not induce analgesia at the highest ($\geq$ 0.3 mA) stimulation level tested.

The finding that the differences in ESIA threshold within subdivisions of the PVN, suggest that the stimulation of the PVN itself, rather than current spread to adjacent areas, induces analgesia. PVN-ESIA was always observed at stimulation onset and terminated immediately after stimulation offset. This indicates that PVN-ESIA is not mediated humorally but is direct neurally mediated analgesia.

Effects of PVN-prv or PVN-mgn stimulation on the ESIA

Fig. 2 shows that significantly ($p < 0.01$) higher intensities were required to induce analgesia by stimulation of the PVN-mgn than of the PVN-prv at the determination of TT. At stimulation of the PVN-prv, the TT is also significantly ($p < 0.01$) lower than the BT. But at stimulation of the PVN-mgn, the TT did not show clear ESIA compared to the measurement of the BT. It appears to be a current of ESIA for the TT rather than BT's.

The finding that lower currents were needed to induce ESIA from the PVN-prv than from the PVN-mgn might support that PVN-ESIA is a neural mediation to the ESIA.

Neurons of the PVN-mgn primarily project to the pituitary, while PVN-mgn neurons project to 'pain' related regions in the brainstem and spinal cord (Sawchenko and Swanson, 1982; Cechetto and Saper, 1989). Hosoya (1980) reported as many as 1/3 of the total number of hypothalamic neurons directly projecting to the spinal cord originate in the PVN. Many of these fibers terminate in the substantia gelatinosa of the dorsal horn (Holstege, 1987), i.e. near the first central synapse of nociceptive afferents (Dubner and Bennet, 1983).

Effects of naloxone on the PVN-ESIA

Fig. 3 shows the effects of naloxone (NLX) on the ESIA at the determination of the Test Threshold (TT), compared to physiological saline (SLN) control. TTs of both NLX and SLN, 20 min after administrations, were significantly ($p < 0.05$) higher than the Baseline Threshold (BT) at either PVN-prv or PVN-mgn. Explanation of the effect of SLN on the PVN-ESIA is not discussed here. The BT and TT current for ESIA was once again

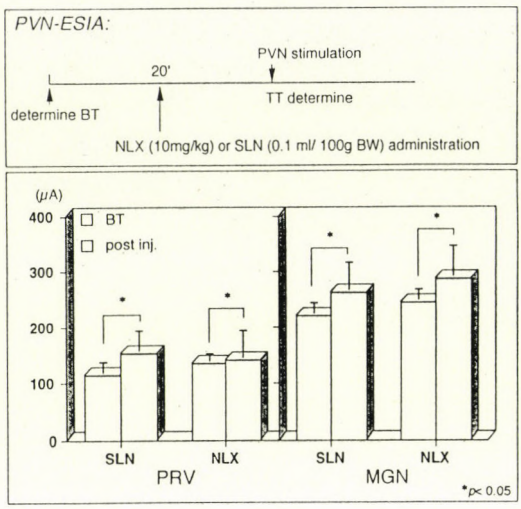

Fig.3: Effects of naloxone (NLX) and saline (SLN) on the PVN–prv (left) and PVN–mgn (right) in the ESIA;compared between BT and post–administration (post inj., shadows) stimulation currents (ordinate). PVN–ESIA was not affected by NLX, indicating opioids are not involved in mediating this analgesia.

significantly ($p < 0.05$, but an asterisk mark not seen in the figure) lower in PVN-prv than in PVN-mgn as described above.

The finding that PVN-ESIA was not mediated by NLX (doses of 3 to 15 mg/kg injected i.p.), but only results of 10 mg/kg are illustrated in the figures, indicates that opioids (at least μ-receptor) are not involved in estimating this electrically induced-analgesia.

The PVN neurons have been known to synthesize endogenous opioid peptides, i.e. enkephalin (Rossier et al., 1979) and dynorphin (Watson et al., 1982) and to regulate β-endorphin release from the pituitary (Meyerhoff et al., 1987). Probably we need more selective and other opioid subtypes (e.g., δ- and/or κ-receptor) antagonists. It is reported that an analgesic response to exogenously administrated vasopressin (Berntson and Berson, 1980; Kordower and Bodnar, 1984), an altered pain responsiveness in animals with altered vasopressinergic systems (Bodnar et al., 1986), and projections of PVN vasopressinergic neurons to brain and spinal cord areas involved in nociception (Sawchenko and Swanson, 1982; Cechetto and Saper, 1988) suggesting the existence of the PVN vasopressinergic pain-modulating system. In the present study, however, we have verified the facts that

various doses of [Arg^8]-vasopressin (0, 0.1, 1, 10 and 100 μg/kg, i.p.) could not reveal the difference between SLN and vasopressin on the PVN-ESIA (data and figures not represented here). The present study suggest that vasopressin is not necessarily involved in the pain-modulating function of the PVN, at least concerning ESIA. The roles of these pain-related endogenous neuropeptides are still not clear in terms of the PVN-ESIA and should be further investigated.

SUMMARY AND CONCLUSION

1) PVN electrical stimulation produced marked analgesia (PVN-ESIA).

2) PVN-ESIA was observed at stimulation onset, and terminated immediately after stimulation offset.

3) Stimulation at location outside the PVN did not produce analgesia.

4) Stimulation threshold for the PVN-ESIA was lower ($p < 0.05$) from PVN-prv that from PVN-mgn. This indicates that PVN-mgn neurons project to the pituitary (neurohypophysis), PVN-prv project to the brainstem and spinal cord ('pain') pathway.

5) Various doses of vasopressin did not have any influence on the PVN-ESIA.

6) Threshold (TT) 20 min after administration of both saline (SLN) and naloxone (NLX) was higher ($p < 0.05$) than before injection (BT). But there was no difference between the threshold elevation after SLN or NLX in the PVN-prv and PVN-mgn; this indicates that opioids are not involved in mediating PVN-ESIA.

In conclusion, the present study indicates that the PVN is a part of the 'pain' inhibitory system in the CNS, and shows that the PVN-ESIA may not be mediated either by opioids or by neuropeptides, such as vasopressin.

REFERENCES

Belluzzi,J.D., Grant,N., Garsky,V., Srantakis,C.D., Wise,C.D. and Stein,L.(1976) Analgesia induced in vivo by central administration of enkephalin in rat. Nature, 260, 625–627.

Berntson,G.G. and Berson,B.S. (1980) Antinociceptive effects of intraventricular or systemic administration of vasopressin in the rat. Life Sci. 26, 455–459.

Bodnar,R.J., Truesdell,L.S., Haldar,J., Aral,I.A., Kordower,J.H. and Nilaver,G. (1986) Elimination of vasopressin analgesia following lesion placed in the rat hypothalamic paraventricular nucleus. Peptides 7, 111–117.

Boschi,G., Desiles,M., Reny,V., Rips,R. and Wigglesworth,S. (1983) Antinociceptive properties of thyrotropin releasing hormone in mice: comparison with morphine. Br.J.Pharmacol. 79, 85–92.

Cechetto,D.F. and Saper,C.B. (1988) Neurochemical organization of the hypothalamic projection to the spinal cord in the rat. J.Com.Neurol. 272, 579–604.

Chrubasik,J., Meynadier,J., Blond,S., Scherpereel,P., Ackerman,E., Weistock,M., Bonath,K.,Cramer,H. and Wunsch,E. Somatostatin, a potent analgesic. Lancet,ii, 1208–1209.

Code,R.A, and Fallon,J.H. (1986) Some projections of dynorphin–immunoreactive neurons in the rat central nervous system. Neuropeptides 8, 165–172.

Cox,V.C. and Valenstein,E.S. (1965) Attenuation of aversive properties of peripheral shock by hypothalamic stimulation. Science 149, 323–325.

Dubner,R. and Bennet,G.J. (1983) Spinal and trigeminal mechanisms of nociception. Ann.Rev.Neurosci. 6, 381–418.

Fredrickson,R.C.A., Burgis,V. and Edward,S.D. (1977) Hyperalgesia induced by naloxone follows diurnal rhythm in responsitivity to painful stimuli. Science 198, 756–758.

Han,J.S. and Xie,C.W. (1982) Dynorphin:potent analgesic effect in spinal cord of the rat. Life Sci. 31, 1781–1784.

Hargreaves,K.G., Dubner,R., Goldstein,D. and Dionne,R. CRF produces analgesia in humans and rats. Brain Res. 422, 154–157.

Hosoya,Y. (1980) The distribution of spinal projection neurons in the hypothalamus of the rat, study with the HRP method. Exp.Brain.Res. 40, 79–87.

Köning,J.F.R. and Klippel,R.A. (1967) The rat atlas: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore.

Kordower,J.H. and Bodnar,R.J. (1984) Vasopressin analgesia: specificity of action and non–opioid effects. Peptides 5, 747–756.

Meyerhoff,J.L., Mougey,E.H. and Kant,G.J. (1987) Paraventricular lesions abolish the stress–induced rise in pituitary cyclic adenosine monophosphate and attenuate the increase in plasma levels of proopiomelanocortin–derived peptides and prolactin. Neuroendocrinology. 46, 222–230.

Oliveras,J.L. and Besson,J.M. (1988) Stimulation–produced analgesia in animals: behavioral investigations. In: Progress in Brain Research, vol.77. pp 141–157 (Fields,H.L. and Besson,J,M. eds.), Elsevier, Amsterdam.

Rhods,D.L. and Liebeskind,J.C. (1978). Analgesia from rostral brainstem stimulation in the rat. Brain Res. 143, 521–532.

Rosenfield,J.P. and Rice,P.E. (1979) Diurnal rhythms in nociceptive threshold of rats. Physiol. Behav. 23, 419–420.

Rossier,J.E., Battenberg,E., Pittman,O., Bayon,A., Koda,L., Miller,R., Guillemin,R. and Bloom,F.E. (1979) Hypothalamic enkephalin neurons may regulate the neurohypophysis. Nature, 227, 653–655.

Sawchenco, P.E. and Swanson,L.W. (1982) Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J.Com.Neurol. 205, 260–272.

Shiraishi,T. and Mager,M. (1980a) Hypothermia following injection of 2–deoxy–D–glucose in to selected hypothalamic sites. Am.J.Physiol. 239, R265–R269.

Shiraishi,T. and Mager,M. (1980b) 2–Deoxy–D–glucose induced hypothermia: thermoregulatory pathways in rat. Am.J.Physiol. 239, R270–R276.

Shiraishi,T. (1980c) Effects of lateral hypothalamic stimulation on the medulla oblongata and gastric vagal responses. Brain Res.Bull. 8, 645–651.

Shiraishi,T. and Simpson,A.D. (1982) Lateral hypothalamus neuron responses to electroosmotic 2–deoxy–D–glucose. Brain Res.Bull. 8, 645–651.

Shiraishi,T.,Tsutsui,T.,Sakata,T. and Simpson,A.D. (1982) 2-Deoxy-D-glucose effects on hypothalamic neurons, and on long term feeding. In: The Neural Basis of Feeding and Reward (eds. Hoebal,B.G. and Novin, D.) 373-390, Hear Institute for Electrophysiological Research, ME.

Shiraishi,T. and Simpson, A.D. (1987) Central control of gastric acid secretion by extralateral hypothalamic nuclei. Brain.Res.Bull. 18, 309-314.

Shiraishi,T. (1987) Gastric related properties of rat paraventricular neurons. Brain Res. Bull.18, 315-323.

Shiraishi,T. (1990) CCK as a central satiety factor: Behavioral and electrophysiological evidence. Physiol. Behav. 48, 879-886.

Shiraishi,T. (1991a) NE neurons modulate lateral hypothalamic chemical and electrical stimulation-induced feeding by sated rats. Brain Res. Bull. 27, 347-351.

Shiraishi,T. (1991b) CCK modulate morphine induced analgesia via the central CCK-B-receptors on the rat paw pressure test. Neurosci.Res. suppl.16, s39.

Shiraishi,T., Kawashima,M., Kojima,T., Sameshima,Y. and Kageyama,T. (1993) Electrical stimulation of the hypothalamic paraventricular nucleus produces analgesia on the rat paw pressure test. XXXIInd Congress of the IUPS Abstracts(Thursday), p72, 332.7/o

Vidal,C. and Jacob,J. (1980) The effect of medial hypothalamic lesions on pain control. Brain Res. 199, 89-100.

Watson,S.J., Fischli,W. Doldstein,A., Zimmerman,E., Nilaver,G. and Van Wimersma Greidanus,T.V. (1982) Dynorphin and vasopressin: common localization in magnocellular neurons. Science 216, 85-87.

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PREDATORY AGGRESSION INDUCED BY HYPOTHALAMIC STIMULATION: MODULATION BY MIDBRAIN PERIAQUEDUCTAL GRAY (PAG)

Manchanda, S. K., Poddar, A., Saha, S., Bhatia, S. C., Kumar, V. M. and Nayar, U.

Department of Physiology, All India Institute of Medical Sciences, New Delhi - 110029 and Centre for EEG and Clinical Neurophysiology, Vikas Puri, New Delhi - 110018, India

Summary: Adequate electrical stimulation of extreme lateral hypothalamic regions of healthy, non-aggressive male cats was employed to produce aggression on live but anaesthetized rats. Stimulus response (S-R) curves based on scoring systems for both somatic and affective display components of behaviour were used to assess how manipulation of midbrain PAG by electrocoagulative lesions or drug microinjections affected the sensitivity of attack producing hypothalamic loci. Anodal lesions of dorsal PAG and adjoining tectum increased the excitability of hypothalamic loci producing predatory attack. Microinjection of 250 ng of delta-alanine-methionine enkephalin (DAME) in dPAG completely suppressed the somatomotor components of attack behaviour and markedly inhibited the affective display components. Administration of naloxone, an opioid antagonist (1 μg) at the same sites facilitated the hypothalamically induced attack behaviour and annulled the inhibitory effect of DAME. These findings indicate the involvement of midbrain enkephalinergic mechanisms in the modulation of predatory attack behaviour elaborated by hypothalamic stimulation.

Key Words: Predatory attack, Aggressive behaviour, Periaqueductal gray, Lateral hypothalamic area, Delta-alanine-methionine enkephalin, Naloxone, Enkephalinergic modulation

Correspondence should be addressed to: Dr. S. K. Manchanda
Centre for EEG and Clinical Neurophysiology
66 Charak Sadan, Vikas Puri, New Delhi - 110018, India

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INTRODUCTION

It is well established that predatory aggression or quiet biting attack can be elicited by adequate electrical stimulation of loci in the extreme lateral regions of hypothalamus in cats. Stimulation of more medial regions, e.g. periformical areas produces affective attack (Wasman and Flynn, 1962; Bandler, 1977). The projections related to these two types of attack behaviour elicited from hypothalamus to midbrain have been traced (Chi and Flynn, 1971; Chi et al, 1976; Fuchs et al, 1981). Midbrain is considered an important region for the integration of aggressive responses. Stimulation of dorsal PAG and adjoining midbrain tectum has been reported to produce affective attack and that of ventral PAG and tegmentum, predatory attack (Shaikh et al, 1987). It is also reported that lesions of dorsal tegmentum and PAG lead to blocking of both hypothalamically induced and naturally elicited aggressive behaviour (Fernandez de Molina and Hunsperger, 1962; Skultety, 1963; Bergquist, 1970; Berntson, 1972). In the present investigation we have shown that fairly large lesions of dorsal PAG and adjoining tectum did not abolish the hypothalamically induced predatory attack behaviour. Instead, this attack behaviour was now elicited at much lower strengths of hypothalamic stimulation. In view of the analgesic effect consequent to opioid release reportedly obtained on PAG stimulation (Hosubuchi et al, 1977; Fardin et al, 1984) we also investigated the role of PAG enkephalinergic mechanism in the modulation of hypothalamically induced predatory aggression and report the existence of such a mechanism.

MATERIALS AND METHODS

Well-tamed and healthy male cats weighing between 2.5 and 3.5 kg housed in separate individual cages placed in an air-conditioned room were used for the present study. All cats were tested for spontaneous aggression towards rats by leaving a live anaesthetized rat of about 300 gm in each cat's house cage for 24 hours. The cats which attacked the rat were not selected for experiments.

Each experimental cat had two bilaterally symmetrical permanently implanted bipolar concentric electrodes for hypothalamic stimulation to produce aggression and two permanently implanted symmetrical monopolar electrodes (uninsulated tip diameter 0.25 mm) in the dPAG for making anodal lesions. The stereotaxic coordinates for hypothalamic stimulation were : A 11.5-12.0, L 3.5-4.0 and V -3.5 to -4.0 mm and those for making lesions in the dPAG were : A 3.5-4.0, L 0.0-0.5 and V +1.0 to + 1.5 mm. The permanently implanted chemitrodes for microinjection of delta-alanine-methionine enkephalin (DAME) into the dPAG had similar coordinates as used for making lesions. Stereotaxic Atlas by Jasper and Ajmone-Marsan (1960) was consulted for reference while implanting electrodes and chemitrodes and later for anatomical reconstructions after the experiments were accomplished and the brains of experimental animals were histologically processed to identify the hypothalamic loci which were stimulated, PAG loci in which microinjections were done and for determining the dimensions of lesioned areas. The construction of electrodes for hypothalamic stimulation and chemitrodes for microinjections in the dPAG and the procedures of aseptic surgery for their permanent implantation are classical neuropysiological techniques and were recently described by us (Dawra et al, 1988; Saha et al,1993). The animals were allowed a post-operative recovery period of 7 to 8 days. Behavioural observations were made only after the animals fully recovered, started moving around freely, remained well-tamed, friendly and non-aggressive.

Behavioural Recording

The hypothalamic sites were tested with trains of square-ware electrical pulses (0.25 msec., 300-1000μA at 50-60 /sec) obtained from a Grass (Model S4E) stimulator and the responses were recorded on an already prepared protocol characterizing various somatomotor and affective display components and their respective weightages as described earlier (Dawra et al,1988). All behavioural recordings were done while observing the animal in a behaviour box (1m x 1m x 1m) with a sliding door for the entrance and exit of the animal. The box was so constructed that one side had a smoked glass while the other side had a clear glass for one way viewing, video recording and camera photography. Electrical stimulations at graded current strengths were repeated on successive days with ten ascending and descending trials with a gap of at least half an hour between the trials and at least five minutes between each successive graded stimulation. Stimulation trials repeated on successive days allowed the checking of reproducibility of observations.

After making control observations by stimulating 8 hypothalamic loci in 5 cats bilateral lesions were made in the dPAG of these cats by passing anodal constant current of 3 mA for a period of 30-40 sec. Twenty four hours after the lesions, same hypothalamic sites were stimulated again to observe differences if any from the control records. In another 7 cats DAME (250 ng in 0.5μl saline) was bilaterally microinjected in the dPAG and graded stimulations were given to the hypothalamic loci to make behavioural observations. Naloxone (1μg) was bilaterally administered while behavioural effects of DAME were at the peak and tests continued. After 3 days rest all these 7 cats were retested for control responses. Naloxone alone was then bilaterally administered in the dPAG loci and behavioural observations were made again. In these animals behavioural observations made after microinjections of saline (0.5μl) in the dPAG served as controls.

Histological Processing

After the completion of experiments all cats were sacrificed by over dosage of the anesthetic nembutal. Before sacrificing anodal lesions of minute dimensions were made at the sites of dPAG microinjections (1.5 mA for 5-6 sec constant current). The brains were fixed by perfusing trans-cardially with 10% formol saline dissolved in 2% potassium ferrocyanide solution and frozen. The cut coronal sections were stained with haematoxylin and eosin.

Statistical Analysis

Correlation coefficient between various strengths of stimulation and respective percentage responses were done in each series of experiments before and after midbrain PAG lesions by employing standard Pearson's method. To obtain a better fit the percentage responses were averaged out of three readings at the same stimulation strength.

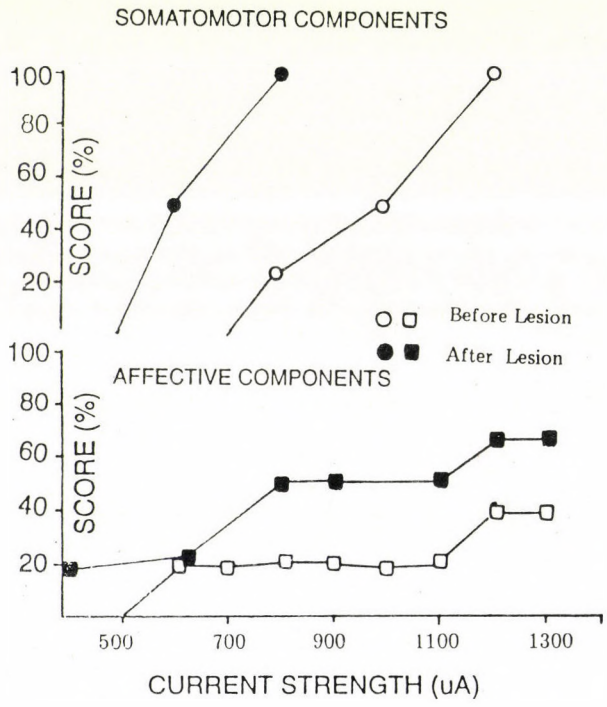

Figure 1: Stimulus response relationship of the somatomotor and affective components of predatory aggression obtained on stimulation of a locus in the extreme lateral part of middle hypothalamus before and after lesions in the dPAG. The curves shift to the left after the lesion indicating greater sensitivity of hypothalamic locus. Also note the low level of affective display before the lesions and its increase after lesions

RESULTS

Hypothalamically Induced Predatory Aggression

On stimulation of a locus in the extreme lateral part of the middle hypothalamus at low current strengths of 300-450 μA, the response began with alertness and pupillary dilatation. The cat looked from side to side and started moving around inside the behaviour box. With increase in stimulation strength i.e. 500-700μA salivation, respiratory acceleration and piloerection on the midline of the back and or tail were added to the response. At times the cat adopted a crouching posture. A further increase in stimulation strength led to movements which were faster, often encircling type with part unsheathing of claws. Soon it noted the anaesthetized rat and with only a mild growl or grunt approached the rat, struck it with a forepaw and bit it on the neck to kill. If the stimulation was stopped at this stage, the cat would drop the rat from its mouth and walk away. But if it was continued it kept on biting and ate up the whole rat. The predatory attack was never accompanied by baring of teeth, hissing or loud growls which often accompany the affective attack. Ear flattening occured only occasionally and there never was any urination or defaecation. The affective components never exceeded a score of 50%. Mostly it was less than 40%.

Effect of Midbrain Periaqueductal Gray Lesions

Figure 1 depicts the stimulus-response (S-R) relationships obtained on stimulation of a locus in the extreme lateral part of hypothalamus before and after dPAG lesions in cat no. 28. A typical predatory attack with an affective display amounting to a maximum of 40% was built up as the stimulus strength was progressively increased to about 1200 μA when the cat struck the rat and started biting it. After the lesions both affective display and somatomotor components started appearing at much less current strength i.e. 400 and 500 μA respectively and a full blown attack could be obtained just with 800 μA. Although no hissing was obtained, in any of the five cats employed in the lesion experiments loud growls did appear in all cats

after the lesions and the affective display score varied between 60 and 75%. Similar S-R relationships were obtained from all the 8 hypothalamic loci.

Pearson's correlation coefficients between the stimulation strengths and respective percentage responses were determined before and after lesions and are given in Table 1 below:-

Table 1: Correlation coefficients of stimulus - response data before and after dPAG lesions

Somatomotor Components		Affective Display Components	
Before Lesions	After Lesions	Before Lesions	After Lesions
0.919	0.831	0.954	0.955

All values were highly significant at $P < 0.001$

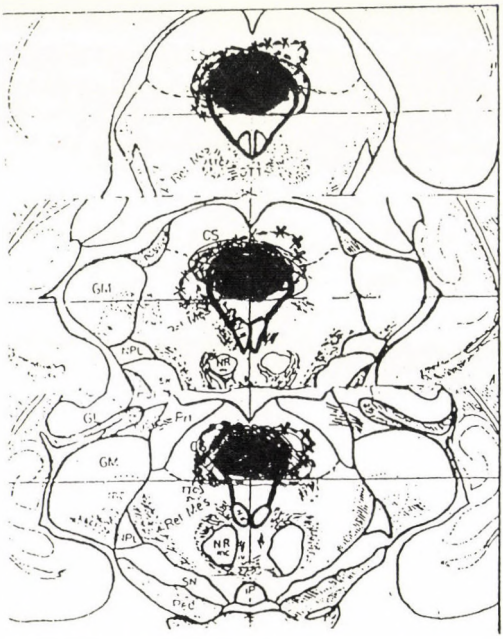

Figure 2: Reconstruction of the PAG lesions on coronal sections A2-A5 as per Jasper and Ajmone-Marsan (1960). The lesioned area common to all the five cats is black. Note that some parts of superior colliculi and adjacent area were also destroyed

Figure 2 gives the anatomical reconstruction of the midbrain region showing lesions to be in the dorsal PAG region and the adjacent tectum including parts of superior colliculi. The lesions were bilateral and extended over the coronal sections A1 to A5 as per the stereotaxic atlas of Jasper and Ajmone-Marsan (1960). The lesioned areas in A1, and A5 were very small i.e. about a mm or less in all cats. Therefore these are not included in the figure. These lesions had facilitatory effect on aggressive responses from all the hypothalamic loci in 5 cats.

Effect of Microinjection of DAME in dPAG

Tests were performed in seven cats in which hypothalamic stimulation produced typical predatory aggression, the affective display remaining at less than 50% on stimulation at maximal strength at which full-blown aggression culminating into the killing of rat was achieved. Within an hour after bilateral microinjection of 250ng of DAME in 0.5 µl saline in the dPAG no somatomotor components except extended neck was elicited even when the stimulation strength was raised to 1000 µA.

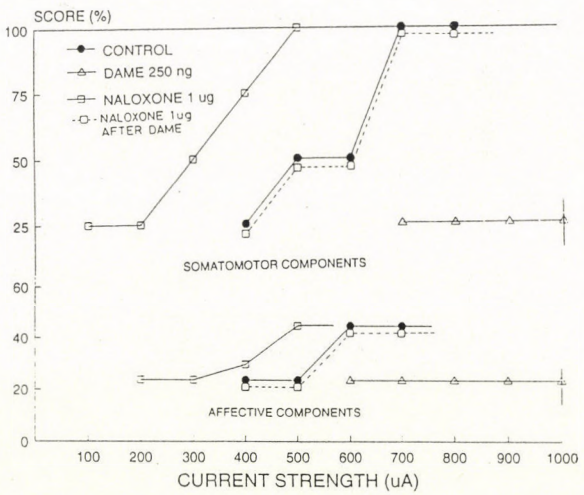

Figure 3: Stimulus Response relationship of somatomotor and affective components of predatory aggression obtained by stimulation of a hypothalamic locus. Microinjection of DAME in the dPAG inhibited the response shifting the S-R curve to the right. Naloxone had a facilitatory effect shifting the S-R curve to the left. Naloxone when given at the peak of DAME effect reversed the DAME effect bringing the S-R curve back to control level

The affective display components like alertness, pupillary dilatation and respiratory acceleration however continued to be elicited even at low levels of stimulation, but salivation, piloerection and growling were completely abolished. The blocking effect of DAME lasted for about 30-36 hours. After a further rest for 3 days 1 μg of naloxone in 0.5μl saline was bilaterally microinjected at the same dPAG sites. It was observed that the aggressive response and its build-up was now achieved at much lesser stimulation strength. In another series of experiments when microinjection of DAME had already shown its peak effect i.e. at about 6-8 hours, naloxone (1μg in 0.5μl saline) was bilaterally microinjected at the same sites. It was observed that within one hour the entire aggressive response as obtained in the control records had returned. These observations are charted in figure 3. The location sites of chemitrodes through which microinjections were given as confirmed histologically are depicted in figure 4. At all these sites bilateral microinjections of 0.5μl of normal saline did not change stimulus-response relationship from the control level.

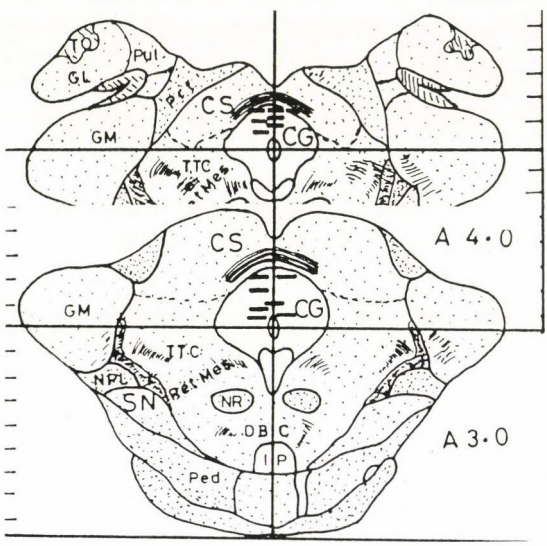

Figure 4: Localization of all the 14 dPAG sites where injections were made on coronal sections of midbrain as per Jasper and Ajmone-Marsan (1960)

DISCUSSION

Electrical stimulation of PAG, both in rostral and caudal midbrain has been reported to produce aggressive behaviour (Skultety, 1963; Sheard and Flynn, 1967). Bandler (1977) has shown that predatory aggression in the cat could be elicited by stimulation of ventromedial PAG and ventral midbrain tegmentum. Abolishment of naturally occuring as well as hypothalamically induced attack behaviour by PAG lesions has also been documented to fit in with the stimulation studies (Hunsperger, 1956; Fernandez de Molina and Hunsperger, 1962; Sprague et al, 1961; Skultety, 1963). In the present study it was demonstrated that the lesions restricted to the dPAG, adjoining tectum including parts of superior colliculi were not sufficient to abolish the hypothalamically elicited predatory attack. Descending pathway from hypothlamus mediating predatory attack, particularly the somatomotor components thus do not seem to pass through the dorsal PAG. Shaikh et al (1984, 1985, 1987) suggested a functional differentiation between the ventral and dorsal PAG, the ventral being responsible for predatory attack and the dorsal for affective attack. Our results support the view that predaatory attack is not mediated by dPAG and ventral PAG may be involved in its elaboration. However, our observations that after dPAG lesions, not only the stimulation threshold for eliciting affective components was lowered, but also more components of affective display were added at the same stimulation strength, do not support the concept of dPAG being the integrating centre for affective attack. Rather, these observations indicate that the eleboration of affective components accompanying aggression may be a more generalized affair and not restricted to dPAG. It seems that the dPAG possesses inhilbitory mechanisms which do not allow the elicitation of affective display and also raise the thresold of eliciting goal directed somatomotor components of attack behaviour. The fact that microinjections of DAME in the dPAG produced a profound inhibition of predatory attack and that such an inhibition was counteracted by naloxone microinjections proved the involvement of enkephalinergic mechnisms at the dPAG level for inhibiting the hypothalamically induced aggression. The literature on density of opioid receptor populations in various brain regions has been reviewed by Akil et al (1984) and dPAG is known to possess a rather high density of

enkephalin and opioid receptors. The PAG is not only rich in enkaphalins but also abounds in enkephalinergic neurones. Functionally stimulation of dPAG in also known to produce anlgesia (Fardin etal,1984). Somatotopic mapping of the sites in the dorsal central gray linked to analgesia limits it to the forepaw and head and neck regions (Soper and Melzak,1982) which of course will include the organs of prehension and biting, the two crucial omponents of attack in the cat.

Pott et al (1987) reported that infusion of DAME in the PAG produced both inhibition and facilitation of aggression. Weiner et al (1991) further elaborated this differentiation and mentioned the involvement of both u and delta receptors in the PAG. In the present study we restricted the enkephalinergic intervention only to the rostral aspects of dPAG and found only inhibitory effect of DAME on the hypothalamically induced predatory aggression and that in all tests this inhibition was completely reversed by naloxone. But the naloxone effects obtained by Pott et al (1987), Weiner et al (1991) and Shaikh and Siegal (1989) were not consistent for there were sites which did not produce reversal by naloxone indicating that non-opioid mechanism may be involved in producing dPAG induced inhibition of predatory aggression. It is likely that other transmitter mechanism may also be operating at the PAG level. Cholinergic (Dawra et al 1988 a and b) and adrenergic (Saha et al 1993) mechanisms of the PAG affecting hypothalamically induced aggressive responses have been already demonstrated. With regard to opioidergic aspects, Lipp (1991) has suggested that the activation of PAG analgesic areas may result from an interaction between more than one specific neurotransmitters such as serotonin, norepinepherine and enkephalins on receptors located on the neurons that transmit and inhibit pain. With regard to the modulation of aggressive behaviour by midbrain PAG, the facilitatory role of dopamine (Piazza et al 1986), inhibitory role of GABA (Shaikh and Siegal, 1990) and excitatory role of glutamic acid (Bandler and Carrive, 1988) have been reported. The emerging picture of neuronal interplay indeed may be as complex as the behaviour of an attacking individual.

In conclusion the present study emphasises the role of midbrain periaqueductal gray in modulating the predatory aggressive behaviour elaborated by the hypothalamus and that the dorsal and rostral aspects of periaqueductal gray employ opioidergic inhibitory mechanisams to

suppress the predatory attack. The study does not favour the view that the dorsal PAG integrates the affective attack behaviour.

REFERENCES

Akil, H., Watson, S.J., Young, E., Michael, E.L., Khachautrian, H. and Walker, M.(1984) Endogenous Opioids: Biology and Function. Ann. Rev. Neurosc. 7, 223-255.

Bandler, R.J. (1977) Predatory behaviour in the cat elicited by lower brainstem stimulation and hypothalamic stimulation. Brain. Behav. Evol. 14, 440-460.

Bandler, R.J. and Carrive, P. (1988). Integrated defense reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal gray regions of the unrestrained cat. Brain Res. 439, 95-106.

Bergquist, E.H. (1970) Output pathways of hypothalamic mechanisms for sexual, aggressive and other motivated behaviours in opossum. J. Comp. Physiol. Psychol. 70, 380-398.

Berntson, C. C. (1972) Blockade and release of hypothalamically and naturally elicited aggressive behaviour in cats following midbrain lesions. J. Comp. Physiol. Psychol. 81, 541-554.

Chi, C.C. and Flynn, J.P. (1971) Neural pathways associated with hypothalamically elicited attack behaviour in cats. Science. 171, 703-705.

Chi, C.C., Bandler, R.J. and Flynn, J.P. (1976) Neuroanatomic projections related to biting attack elicited from ventral midbrain in cats. Brain. Behav.Evol. 13,91-110.

Dawra, P.S., Aneja, I.S., Manchanda, S.K. Bhatia, S.C. and Tandon O.P. (1988). Midbrain cholinergic mechanisms in the elicitation of hypothalamic aggressive responses in cats. Prog. Neuropsychopharmacol. Bio.Psych. 12, 445-453 (a).

Dawra, P.S., Rao, P.S., Manchanda, S.K. and Tandon O.P. (1988) Midbrain cholinergic mechanisms regulating cardiovascular responses during hypothalamic defense reaction. Prog. Neuropsychopharmacol. Biol.Psych. 12, 617-627 (b).

Fardin, V., Oliveras, J.L. and Besson, J.M. (1984) A reinvestigation of the analgesic effects induced by stimulation of PAG matter in the rat. II. Differential characteristics of analgesia induced by ventral and dorsal PAG stimulation. Brain. Res. 306, 125-139.

Fernandez de Molina and Hunsperger R.W. (1962) Organization of the subcortical systems governing defense and fight reactions in the cat. J. Physiol. 160, 200-213.

Fuchs, S.A.G., Dalsass, M., Siegel, H. E. And Siegel, A. (1981) The neural pathways mediating quiet biting attack behaviour from hypothalamus in the cat; a functional autoradiographic study. Agg. Behav. 7, 51-68.

Hosobuchi, Y., Adams, J.C. and Linchitz, G. (1977) Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science. 197, 183-186.

Hunsperger, R. W. (1956) Affektreaktionen auf elektrische Reizung via Hirnstamm der Katz. Acta Helv. Physiol. Pharmacol. 14, 70-92.

Jasper, H.H. and Ajmone-Marsan, C. (1960) A Stereotaxic Atlas of the Diencephalon of the Cat. National Research Council of Canada, Ottawa.

Lipp, J. (1991) Possible mechanism of morphine analgesia. Clin. Neuropharmacol. 14, 131-147.

Piazza, P., Crescimanno, G., Benigno, A. and Amato, G. (1986) Stimulation of ventral tegmental area A 10 region enhances the hypothalamic attack behaviour in the cat. Behav. Brain. Res. 19, 179-182.

Pott, C. B., Kramer, S. Z. and Siegel, A. (1987) Central gray modulation of affective defense is differentially sensitive to naloxone. Physiol. Behav. 40, 207-213.

Saha, S., Manchanda, S.K., Bhatia, S.C. and Nayar, U. (1993) Midbrain adrenergic mechanism modulating predatory attack behaviour induced by hypothalamic stimulation. Indian J. Physiol. Pharmacol. 37, 121-126.

Shaikh, M. B., Brutus, M., Siegel, H. and Siegel, A. (1984) Differential control of aggression by the midbrain. Exp. Neurol. 83, 436-442

Shaikh, M.B. Brutus, M., Siegel, H. and Siegel A. (1985) Topographically organized midbrain modulation of predatory and defensive aggression in the cat. Brain. Res. 336, 308-312.

Shaikh, M.B., Barrett, J.A. and Siegel, A. (1987) The pathways mediating affective defense and quiet biting attack behaviour from the midbrain central gray of the cat - an autoradiographic study. Brain Res. 437, 9-25.

Shaikh, M. B. and Siegel, A. (1989) Naloxone induced modulation of feline aggression elicited from midbrain periaqueductal gray. Pharmacol. Biochem. Behav. 31, 791-796.

Shaikh, M.B. and Siegel, A. (1990) GABA mediated regulation of feline aggression elicited from midbrain periaqueductal gray. Brain Res. 507, 51-56.

Sheard, M.H. and Flynn, J.P. (1967) Facilitation of attack behaviour by stimulation of the midbrain of cats. Brain Res. 4, 324-333.

Skultety, F.M. (1963) Stimulation of periaqueductal gray and hypothalmus. A.M.A. Arch. Neurol. 8, 38-50.

Soper, W. and Melzak, R. (1982) Stimulation produced analgesia: Evidence for somatotopic organization in the midbrain. Brain Res. 251, 301-311

Sprague, J.M., Chambers, W.W. and Stellar, E. (1961) Attentive, affective and adaptive behaviour of savage behaviour in cats. Fed. Proceed. 20, Suppl. 1, 327.

Wasman, M., and Flynn, J.P. (1962) Directed attack elicited from hypothalmus. A.M.A. Arch. Neurol. 6, 220-227.

Weiner, S., Shaikh, M.B., Shaikh, A.B. and Siegel, A. (1991) Enkephalinergic involvement in periqueductal gray control of hypothalamically elicited attack in the cat. Physiol. Behav. 49, 1099-1105.

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CARDIOVASCULAR SYSTEM RELATED PEPTIDES AND HYPOTHALAMIC NEURONS

Yamashita, H., Ueta, Y., Inenaga, K.*, Nagatomo, T., Shibuya, I., Kabashima, N., Cui, L.-N., Li, Z. and Yamamoto, S.

Department of Physiology, University of Occupational and Environmental Health and Kyushu Dental College*, Japan

Summary: The hypothalamus is known to be an integrative site of cardiovascular, endocrine and autonomic functions. Our previous studies, using extracellular, intracellular and/or whole cell patch-clamp recordings in rat hypothalamic slice preparations, revealed that cardiovascular related peptides such as atrial natriuretic polypeptides (ANP), B-type polypeptides (BNP), endothelin (ET), angiotensin II (AII) and interleukin-1β (IL-1β) influence the hypothalamic neurons. ANP modulated the firing rates in the supraoptic nucleus (SON). BNP inhibited the SON neurons and these effects were mediated through cGMP and cGMP-dependent protein kinase. ET also inhibited approximately 60% of SON neurons. By using slice patch-clamp techniques, AII inhibited the transient outward potassium current in the SON neurons. IL-1β increased the firing rate and depolarized the membrane of the most SON neurons. A new type of transmitter, nitric oxide (NO), identified as an endothelial-derived relaxing factor (EDRF), modulated the glutaminergic inputs of the SON neurons. The results suggest that cardiovascular related peptides and NO modulate the neuronal activity of neurosecretory cells in the SON.

Key words: natriuretic polypeptides, endothelin, angiotensin II, interleukin 1β, supraoptic nucleus, nitric oxide

INTRODUCTION

The hypothalamus is an integrative site of cardiovascular, endocrine and autonomic function (Swanson and Sawchenko, 1983). Recently a variety of cardiovascular related peptides were discovered and investigated since they have a wide range of distribution and physiological functions in the whole body. We have studied how cardiovascular related peptides such as atrial natriuretic polypeptides (ANP), B-type polypeptides (BNP), endothelin (ET), angiotensin II (AII) and interleukin-1β (IL-1β) influence the hypothalamic neurons, using extracellular, intracellular and/or whole cell patch-clamp recordings in the rat hypothalamic slice preparation. In the present paper, we reviewed the findings published on the sensitivity of hypothalamic neurons to ANP (Okuya and Yamashita), 1987; Okuya et al., 1987), BNP (Yamamoto et al., 1991; Akamatsu et al., 1993), ET (Yamamoto et al., 1993), AII (Okuya et al., 1987; Nagamoto et al., 1995) and IL-1β (Li et al., 1992; Li et al., 1993). As there is accumulating evidence that nitric oxide (NO) influences neuroendocrine function (Grossman, 1994), we also studied the effects of NO on the glutaminergic inputs to the SON neurons, using intracellular recording (Cui et al., 1994).

MATERIALS AND METHODS

Male Wistar rats weighing 120-300 g were decapitated and the brains were quickly removed and placed in artificial cerebrospinal fluid (ACSF) of 4°C. After 400 μm-thick coronal sections were cut with a vibrotome, the trimmed slices were pre-incubated at 30°C. Prior to recording, a slice was transferred into a recording chamber, submerged and perfused with ASCF warmed to 36°C at a rate of 2 ml/min. There was a lag time of 0.5 min during which the perfusion medium reached the recording chamber from a reservoir. The ACSF contained (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1.24; $CaCl_2$, 2.1; $MgSO_4$, 1.3; $NaHCO_3$, 20 and glucose, 10. The ACSF was kept at pH 7.4 and was saturated with 95% O_2 and 5% CO_2. Extracellular recordings (Okuya and Yamashita, 1987), intracellular recording (Akamatsu et al., 1993) and whole cell patch-clamp recordings (Nagamoto et al., 1995) were performed as described elsewhere.

RESULTS AND DISCUSSION

ANP, BNP and AII

The ANP and BNP sensitivities of neurons in the SFO and anteroventral third ventricle (AV3V), of magnocellular neurons in the SON, and of parvocellular neurons in the PVN were compared and the effects of ANP on AII-induced neuronal activity in the subfornical organ (SFO) and the SON. The neurons in the SON, AV3V, SFO and PVN were inhibited by ANP and BNP. The numbers of responsive neurons varied from clusters to nucleus, the range being 14-75%, However, there were no excitatory responses except in the PVN. ANP and BNP caused inhibition in a dose dependent manner, with the same threshold concentration of 10^{-11} M. The action of BNP seems to be much more potent than that of ANP. The dose-response curves of BNP showed a rapid decrease in neural activity with increasing concentrations, and the number of putative vasopressin neurons that showed phasic firing pattern in the SON inhibited by BNP was twice as large (75%) as of those seen with ANP (29%). The inhibitory effect of ANP persisted even after synaptic blockade (low $[Ca^{2+}]$ and high $[Mg^{2+}]$). AV3V neurons were most sensitive to ANP and BNP.

We investigated the combined effect of ANP and AII on the activity of SFO and SON neurons. Although ANP had little effect on the spontaneous firing rate, ANP inhibited AII-induced excitation in most of the SFO neurons (87%). Since ANP failed to suppress the excitation induced by raising the potassium concentration in the perfusing medium, the depression by ANP was not due merely to its possible stabilizing effect on the cell membrane. As the topical application of ANP did not suppress the AII-induced excitation of SON neurons, ANP selectively depressed the excitatory actions of AII in the SFO by mechanisms that may include presynaptic inhibition or an antagonistic effect through the receptors on their receptor site. In the SON, AII and ANP did not interfere with each other. It is probably less likely that ANP acts as a neurotransmitter in the SFO, since there is very little ANP immunoreactivity in the SFO.

When BNP and ANP were applied to the AV3V neurons, the effects of both peptides were almost the same on the individual neurons in the AV3V. The possible presumption that both peptides may activate a similar subtype (ANP-A receptor) of receptor on the AV3V neurons. On the other hand, when we compared effects of BNP with those of AII on AV3V neurons, the number of neurons that responded to either peptide was significantly larger than that of neurons. This result implies that most neurons in the AV3V have mainly one type of receptor acting as a sensor for either peptide.

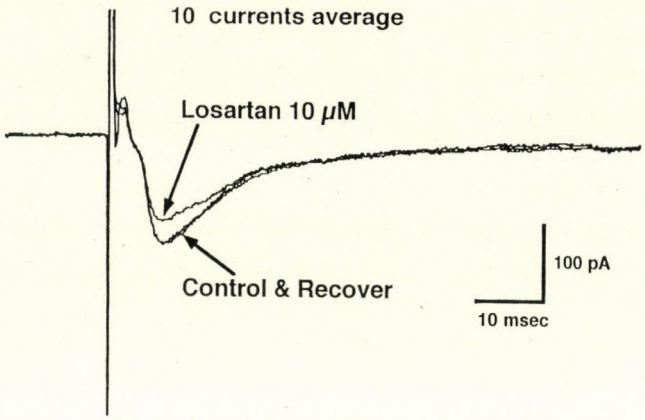

Fig. 1: Effects of losartan potassium on evoked excitatory postsynaptic current (EPSC) in SON neurons. Losartan potassium partially suppressed the evoked EPSC.

By using slice patch-clamp techniques in the rat slice preparation, we examined the effects of AII on transient outward current (I_A) and delayed rectifier current (I_K) of the SON neurons. To separate I_A and I_K, Na^+ free and CO^{2+} (2mM) containing perfusion medium were used to block Na^+ and Ca^{2+} related currents. According to their responses to AII, cells were classified into sensitive and insensitive groups. Bolus injection of AII (10 μ M, 100 μl) decreased the I_A amplitude by 25.1±2.4% in 7 of 13 neurons tested (sensitive group). Bolus injection of AII also decreased the I_K amplitude by 9.6±1.6% mV in 5 of the 11 neurons tested (sensitive group). Application of saralasin at 1μM, an AII antagonist, blocked the effects of AII on I_A. Perfusion of AII (1μM) increased the number of the spontaneous excitatory postsynaptic currents (EPSCs) without changing the mean amplitude of EPSCs. Losartan potassium (10 μM) partially suppressed the evoked EPSC (Fig. 1). These results suggest that AII excites SON neurons through suppression of I_A, acting postsynaptically and increasing the synaptic transmission.

Endothelin

Endothelin (ET) is a potent and long-lasting vasoconstrictor peptide discovered in 1988 (Yanagisawa et al., 1988). Although ET activated the release of AVP from rat explants, we demonstrated that ET inhibited the AVP release-dependently *in vitro* (Yamamoto et al., 1992). Using extracellular recordings from SON neurons in the rat slice preparation, 61% of recorded phasic cells (putative AVP-secreting neurons) were inhibited by ET at 10^{-7} M, 39% were non-responsive and none was excited. 17.5% of the non-phasic cells (putative oxytocin-secreting neurons) tested were inhibited by ET at 10^{-7} M. Ca-free medium and the Ca antagonist, nicardipine at 10^{-5} M did not have any effect on the inhibitory responses of SON neurons to ET. These results suggest that ET has a direct action as a neuropeptide on hypothalamic neurons.

Interleukin 1β

Interleukin 1β (IL-1β), a cytokine that is involved in immune responses, inflammation, fever and synthesis of acute phase proteins, has been shown to increase the secretion of CRF and ACTH after systemic or intracerebroventricular administration (Sapolsky et al., 1987). Significant increase in plasma levels of AVP and OXT has been shown to follow intravenous administration of IL-1β in conscious, freely moving rats (Naito et al., 1991).

Using intracellular recordings from the SON neurons in slice preparation, 42 SON neurons were recorded. Application of 10^{-8} to 10^{-9} M IL-1β caused membrane depolarization, or depolarization followed by a hyperpolarization, or a membrane hyperpolarization. Most (60%) neurons were depolarized and their discharges increased. In 11 other neurons, depolarization was followed by hyperpolarization. The membrane potentials were at first depolarized and then hyperpolarized. The amplitude of membrane depolarization was dose-related and response could still be seen at 10^{-12} M. Only 4 neurons were hyperpolarized, and the remaining 2 neurons were unresponsive to IL-1β. The hyperpolarizing effect masked a direct depolarizing effect of IL-1β since many SON neurons showed a slight depolarization and enhanced excitatory responses to IL-1β in the presence of bicuculline, a specific antagonist of GABA receptors. These results suggest that GABAergic inputs are responsible for IL-induced membrane hyperpolarization and tonically control SON neural activity. After perfusion of salicylate. the depolarizing responses to IL-1β were abolished in all neurons tested in our experiment. This suggests that neurosecretory

cells in the SON receive direct excitatory action of IL-1β as well as that an indirect action through the AV3V and prostaglandin metabolism also involved in the action of IL-1β in the SON. Glial cells in the brain can synthesize and secrete IL-1β. As the SON has an imperfect blood-brain barrier, IL-1β produced in the peripheral organs or in the other regions of the brain may enter the SON through blood vessels (Fig. 3).

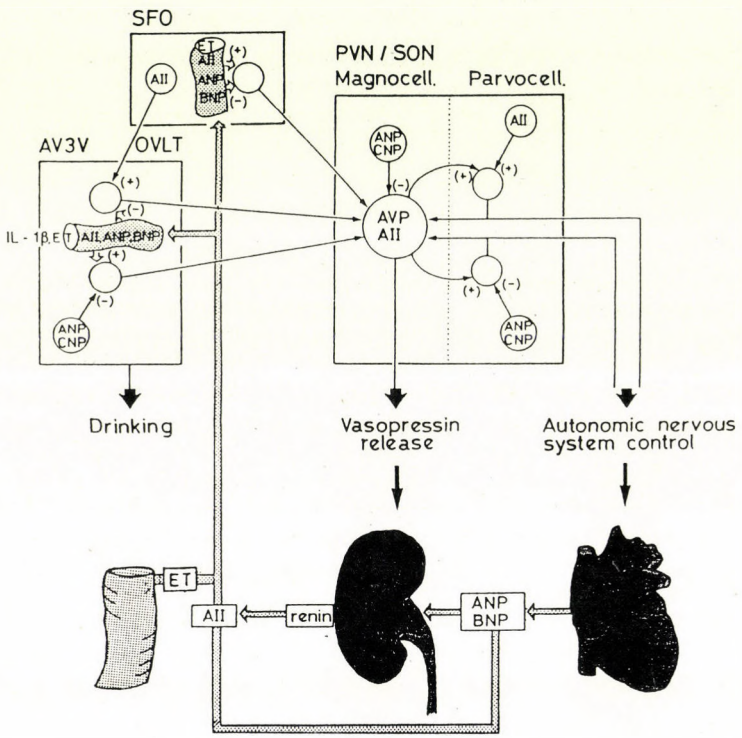

Fig. 2: The roles of cardiovascular related peptides in the anteroventral third ventricular region (AV3V), paraventricular nucleus (PVN), subfornical organ (SFO) and supraoptic nucleus (SON). They are involved in the control of drinking, vasopressin release and autonomic nervous system.

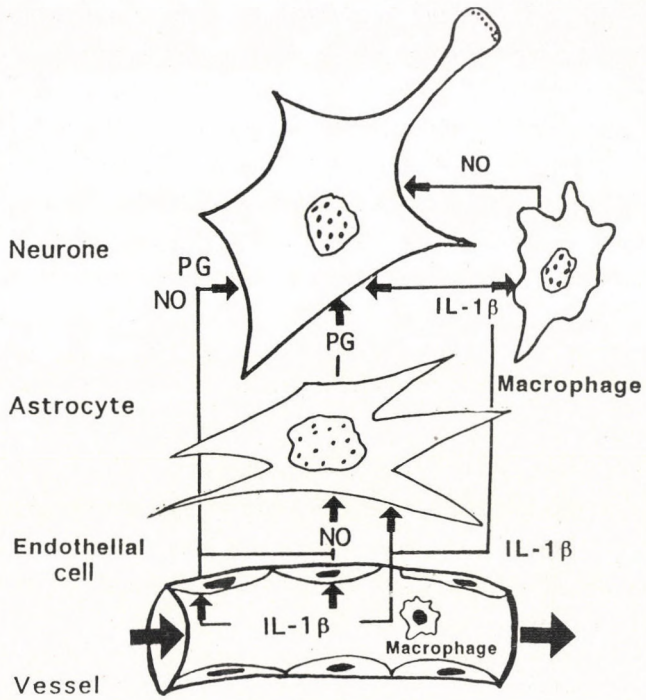

Fig. 3: Possible interactive effects of nitric oxide (NO) and Interleukin-1β (IL-1β) on the hypothalamic neurons. PG; prostaglandins

Nitric oxide

Recent evidence suggests that nitric oxide (NO) plays an important role in a number of central nervous system functions. NO synthase immunoreactivity and mRNA have been found in various parts of the CNS, in particular SON and PVN (Bredt et al., 1991). Osmotic stimulation induced an increase of NOS immuno-reactivity and NOS gene expression in the SON and PVN (Kadowaki et al., 1994). It has been demonstrated that NO and NO-releases substances modulate N-methyl-D-aspartate (NMDA)-induced inward currents in neurons (Hoyt et al., 1992). A previous study has indicated that NMDA receptor as well as non-NMDA receptors are involved in excitatory responses of magnocellular neurosecretory cells in the SON (Hu and Bourque, 1992). These studies suggest that NO may be involved in regulating hypothalamo-neurohypophysial functions.

The modulatory effects of NO on NMDA-induced response in neurons of the SON were studied by intracellular recording in the rat brain slice preparation. Depolarization induced by 100 μM NMDA was reduced by application of 1 to 3 mM of the NO-donors, sodium nitroprusside, and isosorbide dinitrate in all 8 neurons and in 6 of 10 neurons, respectively. The scavenger for NO, hemoglobin, and the inhibitor of NO synthase, N^G-nitro-L-arginine (LNNA) enhanced the NMDA-induced depolarization in four neurons and two of three neurons, respectively. Our results suggest that endogenous NO in the SON could be a physiological inhibitory factor that modulates NMDA-induced responses.

CONCLUSIONS

Our data show that cardiovascular-related peptides, cytokines and NO modulate the neuronal activity of the hypothalamic neurons, in particular neurosecretory cells in the SON. Although the present study may provide new insight into understanding of the physiological implication of the substances, many questions remain unanswered. For more complete understanding, further studies are necessary.

REFERENCES

Akamatsu, N., Inenaga, K. and Yamashita, H. (1993) Inhibitory effects of natriuretic peptides on vasopressin neurons mediated through cGMP and cGMP-dependent protein kinase *in vitro*. J. Neuroendocrinol. 5, 517-522.

Bredt, D. S., Glatt, C. E., Hwang, P. M., Fotuhi, M., Dawson, T.M. and Snyder, S. H. (1991) Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 7, 615-624.

Cui, L.N., Inenaga, K., Nagatomo, T. and Yamashita, H. (1994) Sodium nitroprusside modulates NMDA response in the rat supraoptic neurons invitro. Brain Res. Bull. 35, 253-260.

Grossman, A. (1994) NO news is good news. Endocrinology 134, 1003-1004.

Hoyt, N. R., Tang, L. H., Aizenman, H. and Reynolds, I. J. (1992) Nitric oxide modulates NMDA-induced increases in intracellular Ca2+ in cultured rat forebrain neurons. Brain Res. 592, 310-316.

Hu, B. and Bourque, C. W. (1991) N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors are expressed by rat supraoptic neurosecretory cells in vitro. J. Neuroendocrinol. 3, 509-514.

Kadowaki, K., Kishimoto, J., Leng, G. and Emson, P.C. (1994) Up-regulation of nitric oxide synthase (NOS) gene expression together with NOS activity in the rat hypothalamo-hypophysial ayatem after chronic salt loading: evidence of a neuromodulatory role of nitric oxide in arginine vasopressin and oxytocin secretion. Endocrinology 134, 1011-1017.

Li, Z., Inenaga, K., Kawano, S., Kannan, H. and Yamashita, H. (1992) Interleukin-1ß directly excites hypothalamic neurons in rats *in vitro*. NeuroReport 3, 91-93.

Li, Z., Inenaga, K. and Yamashita, H. (1993) GABAergic inputs modulate effects of interleukin-1ß on supraoptic neurones *in vitro*. NeuroReport 5, 181-183.

Nagatomo, T., Inenaga, K. and Yamashita, H. (1995) Transient outward current in adult rat supraoptic neurones with slice patch-clamp technique: inhibition by angiotensin II. J. Physiol., *in press*.

Naito, Y., Fukata, J., Shindo, K., Ebisui, O., Murakami, N., Tominaga, T., Nakai, Y., Mori, K., Kasting, N.W. and Imura, H. (1991) Effects of Interleukins on plasma arginin vasopressin and oxytocin levels in conscious, freely moving rats. Biochem. Biophys. Res. Commun. 174, 1189-1195.

Okuya , S. and Yamamoto, S. (1987) Effects of atrial natriuretic polypeptide on rat hypothalamic neurones *in vitro*. J. Physiol. 389, 717-728.

Okuya, S., Inenaga, K., Kaneko, T. and Yamashita, H. (1987) Angiotensin II sensitive neurons in the supraoptic nucleus, subfornical organ and anteroventral third ventricule of rats in vitro. Brain Res. 402, 58-67.

Okuya, S., Inenaga, K., Kaneko, T. and Yamashita, H. (1987) Angiotensin II sensitive neurons in the supraoptic nucleus, subfornical organ and anteroventral third ventricule of rats in vitro. Brain Res. 402, 58-67.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P. and Vale, W. (1987) Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238, 522-524.

Swanson, L.W. and Sawchenko, P.E. (1983) Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci. 6, 269-324.

Yamamoto, S., Inenaga, K. and Yamashita, H. (1991) Inhbition by brain natriuretic peptide of vasopressin neurons in the supraoptic nucleus and neurons in the region of the anteroventral third ventricule in rat hypothalamic slice preparations. J. Neuroendocrinol. 3, 45-49.

Yamamoto, S., Inenaga, K., Kannan, H., Eto, S. and Yamashita, H. (1993) The actions of endothelin on single cells in the anteroventral third ventricular region and supraoptic nucleus in rat hypothalamic slices. J. Neuroendocrinol. 5, 427-434.

Yamamoto, S., Morimoto, I., Yamashita, H. and Eto, S. (1992) Inhibitory effects of endothelin-3 on vasopressin release from rat supraoptic nucleus in vitro. Neurosci. Lett. 141, 147-150.

Yanagisawa, M., Kurihara, H., Kimura, S., Tômobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y. and Goto, K. A (1988) novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature, 332, 411-415.

EDITORIAL

I greatly appreciate and express my sincere thanks for the conscientious and professional work of all the Referees of Neurobiology. They were often asked to perform their reviewing work at great speed and frequently at the expense of their own important daily duties or necessary recreation time. Without their expert assistance, the regular editing of this Volume would not have been possible.

List of Referees of the manuscripts submitted for publication in Neurobiology, Vol. 3, Numbers 1 - 4

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Norbert Halász
Editor-in-Chief

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Balázs, R. and Richter, D. (1973) Effects of hormones on the biochemical maturation of the brain. In: Biochemistry of the Developing Brain (ed. Himwich, W.) Vol. 1, pp. 254-304. M. Dekker, New York.

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