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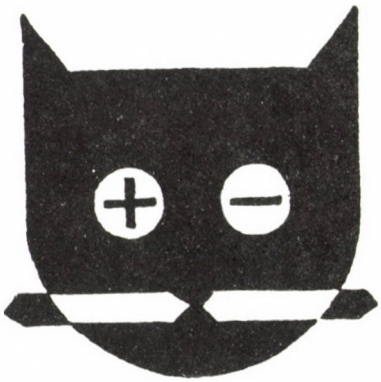

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ZN-DTC INTERFERENCE ON P.LIVIDUS ARS ACTIVITY .

E.E.Alia, M.A.Fighetti, F.A.P.Giannasi. Istituto di Istologia ed Embriologia Generale. Facoltà di Medicina e Chirurgia. Università di Sassari. Viale Mancini, 5. 07100 Sassari (Italy).

Key Words: Sea Urchin, Differentiation, Arylsulfatase.

Zinc-ethylenebisdithiocarbamate (Zn-DTC) strongly affects the development of the P.lividus sea urchin fertilized eggs. Abnormal plutea formation have been observed in fertilized eggs grown in the presence of 0.4 ug/ml Zn-DTC solution. To dissolve the salt in the sea water, DMSO is required. We preferred to try without the use of DMSO, to achieve more realistic conditions. And for these experiments we used Zn-DTC suspended in natural sea water containing fertilized eggs. The contamination was performed 15 mins after the fertilization. A 99% fertilization index was observed every time.

The contamination seems to inhibit the mitosis of the blastomeres: the 24 ug/ml concentration blocks the segmentation at 2-16 blastomeres; but the salt seems also to interfere with the migration of the cells: the 0.4 ug/ml concentration (solution in presence of DMSO) produces abnormal larvae. Different experimental conditions, most of them due to different Zn-DTC concentrations employed, induced the formation of blastomeres of various number and shape. It is not clear at which stage the inhibition takes place and which is the more affected event.

It has been suggested that extracellular proteoglycans play an important role in cell migration (Giudice G., 1986) and because of

the dramatic morphological modifications observed, we went on to study the eventual modification of the arylsulfatase (ARS) activity during the contamination. This enzyme is known to degrade glycosaminoglycans and sulfatides (Kolodni E.H. et al., 1983) and it has been shown to be synthesized in a large quantity in the sea urchin during development (Sasaki H. et al., 1988).

Our study was done with 21 hour treated (with 24 ug/ml Zn-DTC) fertilized eggs. In this case, everything is affected: cell division, cell morphology, zygote development and differentiation. Strong ARS activity inhibition was expected. The ARS activity was visualized using the method of Dubois (1973), over a non-denaturating polyacrylamide slab gel electrophoresis, APS-polymerized as a modification of the method of Davis (1964). The sea urchin ARS activity is non-inhibited by APS and shows a single band near the buffer front in control samples.

Treated samples showed a new strong reaction band above the first one, which is also present (at least partially). The Zn-DTC does not affect the ARS activity; it may be supposed it induces the formation of a higher molecular weight peptide, but it is not known whether this would be so in vivo.

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LAXATIVES AND THE PRODUCTION OF KININS BY RAT COLON

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Department of Experimental Pharmacology, via D. Montesano 49, 80131 Naples, Italy.
Keywords: laxatives, kinins, cellulose suplhate.

Some studies have suggested that prostaglandins (PGs), histamine and serotonin, are involved in the mechanism by which castor oil, senna, phenolphthalein and sulfosuccinate alter colonic fluid and electrolyte movement (Beubler and Juan, 1979; Capasso et al., 1986). The studies demonstrated that these laxatives, in addition to their ability to alter water and electrolyte absorption and/or secretion, also increased the release of PGs, histamine and serotonin into the colonic tissue and lumen. Recently it has been demonstrated that there is also an increase in kininogen content in the colonic mucosa of rats treated with phenolphthalein (Autore et al., in press). These results are consistent with the hypothesis that kinins contribute to the laxative effect of phenolphthalein. In the present study we have determined whether other laxatives lead to changes in the levels of kininogen in the rat colon and if a treatment which alters kinins production affect the response to laxative in a corresponding manner. Male Wistar rats (120-140 g) were deprived of food overnight but allowed free access to water. Bisacodyl (5 mg/kg), castor oil (2 ml/rat), mannitol (10 mg/kg), senna (30 mg/kg), sulfosuccinate (20 mg/kg) and water (control, 2 ml/rat) were administered by gavage. When diarrhoea was evident, the rats were killed and specimens of colon were immediately removed, homogenised in ethanol and processed for kininogen assay as previously described (Autore et al., 1989). Experiments were performed also in rats pretreated with cellulose sulphate (1 mg/kg injected i.v.) 1 h before administration of laxative. Control rats were pretreated with saline solution (NaCl 0.9 %, 3 ml/kg). Data obtained from laxative-treated rats are shown in Table 1. All extracts contained kininogen, a kinin precursor, which was activated by incubation with trypsin. However the mean amounts were significantly ($P < 0.01$) higher only in tissue extracts from cator oil-treated rats. Senna and sulfosuccinate had little effect while bisacodyl and mannitol had no effect. Pretreatment of the animals with cellulose sulphate reduced drastically kininogen content both in control and in laxative-treated rats.
All the drugs produced unformed faeces in 2-4 h, but pretreatment with cellulose sulphate reduced only the effect of castrol oil (-39%; $P < 0.05$). Cellulose sulphate given to control rats did not cause diarrhoea or modify the number of pellets excreted. The present results demonstrate that only castor oil stimulated colonic formation of kininogen in rats, and this was reduced by cellulose sulphate, a compound able to deplete kininogen. Cellulose sulphate also reduced the laxative effect of castor oil. These findings indicate that castor oil may act in part through kinins release.

Table 1. Kinin precursor levels in normal colon and in colon of rats treated with laxatives (bisacodyl, castor oil, mannitol, senna, sulfosuccinate) and pretreated or not with cellulose sulphate.

Treatment	Kininogen content (ug/g Bk)	
	None	Cellulose sulphate
Control	7.5±2.9	1.9±0.5
Bisacodyl	7.7±2.7	2.9±0.5
Castor oil	16.3±3.3a	5.4±0.9
Mannitol	7.1±3.0	1.7±0.4
Senna	11.7+3.3	2.9+0.6
Sulfosuccinate	10.4+3.0	2.0+0.5

Laxatives were given orally 2-4 h before the animals were killed. Cellulose sulphate 1 mg/kg was given intravenously on three occasions at 10 min intervals. Each result is the mean S.E. of 8 experiments. Kininogen content represents the amount of kininogen releasing measured as bradykinin (Bk) equivalents per g tissue wet weight.
a $P<0.01$ compared with control. Student's t-test.

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BIOCHEMICAL EFFECTS OF GYKI-12 743

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Keywords: Antihypertensive; ^{86}Rb efflux, α_2-selectivity;

GYKI-12 743 was demonstrated to display antihypertensive and vascular smooth muscle relaxing activity. Pharmacological tests suggested that it had antiα_2-adrenergic and potassium channel activiting effects. Receptor-binding studies were performed in order to corraborate the above findings with biochemical data. Human platelet preparation was used to test for α_2-receptor displacement with (^3H)-yohimbin as radioligand (Motulsky et al. 1983). GYKI-12 743 had high affinity for the yohimbin binding sites (IC_{50}=5,8 nM and 41 nM for yohimbin and 12 743, respectively). Rat myocardial membrane preparation was used, as a source ofα_1-adrenoreceptors (Glossmann et al., 1980). GYKI-12 743 inhibited (^3H)-prazosin binding in a concentration dependent manner with an IC_{50} of 530 nM, demonstrating the selectivity of GYKI-12 743 for α_2-receptors. Our compound had no appreciable activity for either verapamil or dihydropyridine binding sites.

Potassium channel activating capacity can be demonstrated by measuring enhancement of ^{86}Rb efflux, since membrane permeability for Rb^+ parallels that of K^+ (Hamilton et al., 1986).

We found that GYKI-12 743 opened K^+-channels of rabbit arteria pulmonaris more efficiently than pinacidil. Its efficacy was similar to that of cromakalim. Thus, biochemical and pharmacological data concerning the mechanism of action of GYKI-12 743 are in good agreement.

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CYTOCHEMICAL EFFECTS OF BIOLOGICALLY ACTIVE SUBSTANCES ON THE HEART MUSCLE

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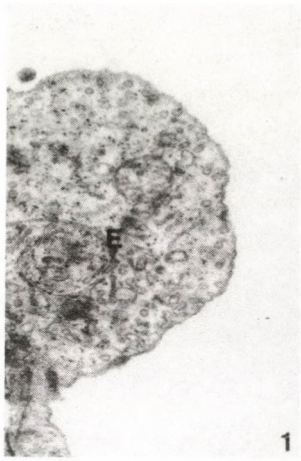
Semmelweis Medical School, Budapest, Hungary.

Keywords: heart muscle, trace elements, cytochemistry, PAF

Using electronmicroscopic and cytochemical methods the effects of different biologically active substances (platelet activating factor: PAF, trace elements) were studied on Langendorff preparation of guinea pig and rat hearts. Hypoxic, ischemic and toxic alterations can be examined on the vessel wall, pericapillary space and surrounding myocardialcells. Damages of endothelial cells, hypoxia, ischemia, (cadmium, cobalt, vanadium, nickel, zinc, selenium, mercury) (Fig. 1.), intraluminal platelet adhesion and aggregation (PAF-platelet activating factor and its antagonist) (Fig. 8,9) the membrane bound adenylate cyclase enzyme localised in sarcolemma and tubuli of sarcoplasmic reticuli (Fig. 2), the intracellular calcium pools of myocardical cell (Fig. 5, 6, 7), some special mitochondrial enzymes (cytochromoxydase, Fig. 3.; succinic dehydrogenase Fig. 4.) can be tested. Cytochemical techniques for trace elements accumulation, the alteration of membrane permeability (lanthanium, ruthenium-red, dimethylglioxime, dithison, salicylicic aldoxime, rubeanic acid) have been used. These methods can be used for investigating the effect of some "cardioprotective" substances (prostacyclin) on isolated heart muscle cell.

Fig. 1. Cadmium effect on the endothelial cell, Fig. 2. Adenylate cyclase cytochemistry, Fig. 3. Cytochromic oxydase cytochemistry, Fig. 4. Succinic dehydrogenase, Fig. 5. Cacliumcytochemistry, Fig. 6, 7. Intramitochondrial calcium deposits, Fig. 8, 9. Effect of platelet activating factor, thrombocytic adhesion and aggregation in capillary.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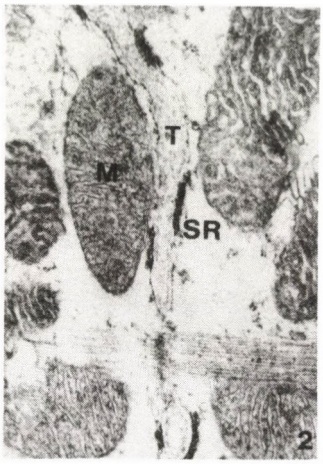
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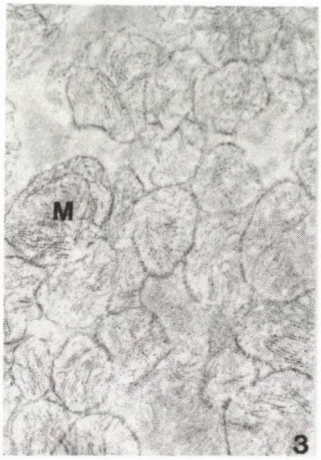
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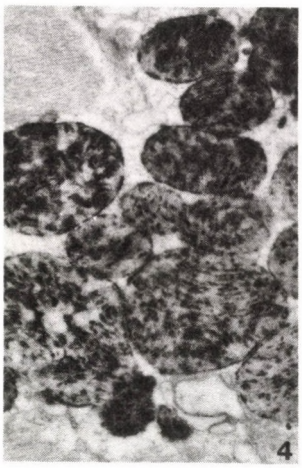
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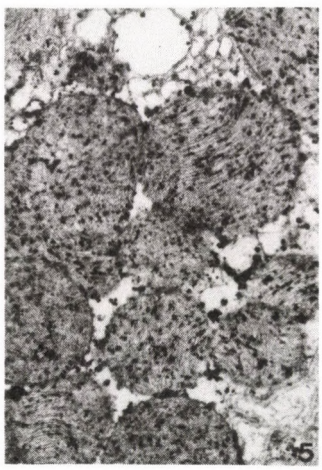
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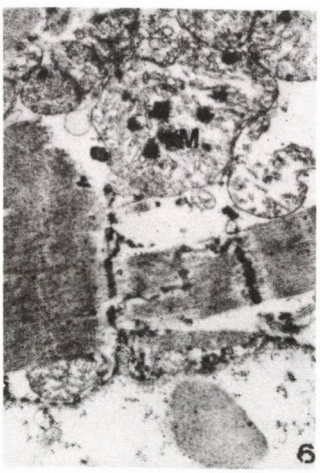
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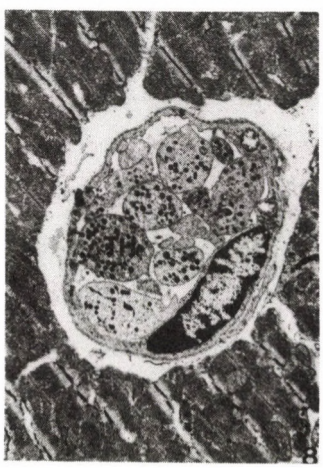
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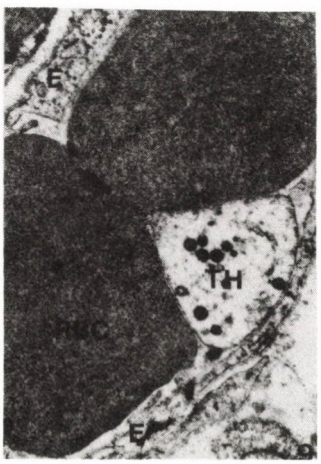
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URINE RETENTION INDUCED IN RATS BY INTRA-SPINAL CORD INJECTION OF COLCHICINE AS A TOOL TO FIND NEW PHARMACOLOGICAL TREATMENTS

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Keywores: micturition-colchicine-Bremazocine-GM_1-NGF

Available treatments for bladder disturbances seem to be unsatisfactory and the attempt to find new drugs require a better understanding of the machinery which regulates micturition and suitable animal models.
We have recently described (P. Zanoli and M. Baraldi, 1988) that the intra-spinal cord (i.s.c.) injection of low doses of colchicine (2-5 ug/rat) induced urine retention with a latency period of 24 hrs. Rats sacrified 72 hrs after the single injection of colchicine showed a bladder hypertrophy which is associated with a retention of more than 5 ml of urine and a minimum output of urine due to overflow (P. Zanoli and M. Baraldi, 1989). Substance P has been shown to play a key role in the regulation of the voiding reflex. Here we report that colchicine induces a dramatic decrease of the Substance P immunomaterial in the lumbar tract of the spinal cord. However, neither i.s.c. injection of Substance P nor systemic injection of eledoisine counteracted urine retention. Hence changes in Substance P alone cannot explain the observed phenomena. Indeed the i.s.c. administration of colchicine, probably by blocking axonal transport, induced also an increase of Met-Enkephalin associated with a reduced affinity constant of delta-receptors labelled by ^{3}H-DADLE. Though Met-Enkephalin exerts an inhibitory activity on the bladder function, we tested the ability of naloxone to counteract the colchicine-induced urine retention with negative results. Furthermore we tested whether or not Dynorphin and opioid K receptor subtypes were altered in colchicine treated rats with urine retention and we found an up-regulation of these receptors labelled by ^{3}H-Ethylketocyclazocine. The administration of the K_2 receptor agonist Bremazocine, but not that of the K_1 agonist U-69593 was able to prevent the colchicine induced urine retention and the bladder hypertrophy. Thus the colchicine-induced urine retention was attributed to an imbalance between the different neuropeptide systems in the spinal cord and the effect of Bremazocine to its agonist-antagonist property. Colchicine (2 μg/rat), when i.s.c. injected,

induces a mild urine retention lasting for about two-three weeks with a slow recovery. Herein we report (Table 1) that the administration of 30 mg/kg/day of monosialoganglioside (GM1) in combination with a single i.s.c. administration of NGF (0.8 μg/rat) improved the functional recovery of the micturition function judging from urine emission and content in the bladder at day 3 and 7.

Table 1: Influence of Nerve Growth Factor and Monosialoganglioside (GM_1) administration on colchicine-induced urine retention

Treatment	Drugs	Urine output (ml/8 hrs)			
		3	7	12	day
Saline	Saline	2.5±0.5	3.8±0.6	3.0±0.7	
Colchicine	Saline	0.8±0.4*	1.6±0.2*	2.5±0.3	
Colchicine	NGF+GM_1	2.5±0.3	4.1±0.4	2.5±0.4	

Mean values (±S.D.) of 6 rats/group.
NGF (0.8 μg/rat, i.s.c.) was injected once 24 h before colchicine, while GM_1 (30 mg/kg/day, i.p.) was dosed chronically starting 3 days before colchicine. Student's t-test: * $p < 0.01$ vs control.

These findings seem to demonstrate that our knowledge on the mechanisms regulating bladder function is still poor and that the use of suitable animal model can lead to find out new rational pharmacological approaches.

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ACTIVITY OF PGT/1A, A NEW IMMUNOSTIMULATING DRUG, ON MACROPHAGES AND LYMPHOCYTES IN IMMUNODEPRESSED MICE.

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Key words: PGT/1A; immunostimulating activity; macrophages; lymphocytes; bacterial infections.

In the last years many progress were obtained in the knowledge of microbial and mammalian peptides endowed with immunostimulating activities. Significant activities on the immune system were found on peptides derived by thymus gland, but immunoenhancing activity was also present in small peptides like TP-5 (Arg-Lys-Asp-Val-Tyr) and tuftsin (Thr-Lys-Pro-Arg).

Following our researches on new immunostimulating drugs, we found a new interesting peptide-like compound PGT/1A (3-L-pyroglutamyl-L-thiazolidine-4-carboxylic acid) (Poli, 1989).

In this study we report the activity of this new immunostimulating drug on cellular immune system and more precisely, on splenic lymphocytes and peritoneal macrophages in immunodepressed mice.

Mice CD-1 (C.River) were depressed by prednisolone (5 mg/kg/die s.c. for two days) and orally (200-400 mg/kg/die for 7 days) and intraperitoneally (100-200 mg/kg/die for 7 days) treated with PGT/1A. The treatment with the new compound stimulated the number of splenic lymphocytes producing rosette against sheep erythrocytes (Wilson, 1987l); the percent variations ranged from +25.8% to 44.6%.

In mice immunodepressed by cyclophosphamide (120 mg/kg i.p. for two days), PGT/1A administered orally (200 and 400 mg/kg die per 12 days) and intraperitoneally (100 and 200 mg/kg die per 12 days) increased clearance of colloidal charcoal (Biozzi et al., 1953) from blood restoring to levels of

no immunodepressed mice.

The new immunostimulating drug protected the mice against many bacterial infections. The compound was administered intraperitoneally (50 to 200 mg/kg) for five times (12 hours of interval) before infections; last drug administration was 8 hours before bacterial challenge.

Table

Treatment	Dose mg/kg/die x 5 times	Survival after infections (%)				
		E.coli P102	E.coli P103	P.mirabilis P203	Ps.aeruginosa P279	P.morganii P120
Control	-	0	25	30	0	10
PGT/1A	100	70	70	100	90	40

PGT/1A showed significant protections; the activity was evident only after 5 treatments and was dose-related; the activity was similar to those of TP-5 and thymostimulin (E.coli infections).

From these preliminary results we can conclude that PGT/1A is a new immunostimulating drug which increases the activity of lymphocytes and macrophages in immunodepressed mice and shows good protections against bacterial infections in mice.

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EFFECTS OF CENTRAL PUTATIVE NEUROTRANSMITTERS ON DIMETHYLPHENYL-PIPERAZINIUM-INDUCED EMESIS: PHARMACOLOGICAL AND PHYSIOLOGICAL IMPLICATIONS

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Key words: DMPP - Neurotransmitters - Emesis - Area postrema

Nicotinic receptors in the cat's area postrema mediating emesis have already been described (Beleslin and Krstić, 1986), but little is known on the neuronal circuitry or transmitters involved in this aspect of emesis response. However, there is a report that nicotinic receptors might be under central opioid control (Beleslin et al., 1981). Therefore, it was of interest to further investigate the effects of putative neurotransmitters present in the area postrema on the emesis induced by dimethylphenylpiperazinium (DMPP) injected intracerebroventricularly (i.c.v.) to unanaesthetized cats.

In an aseptic operation under pentobarbital sodium (35-40 mg/kg, i.p.) anaesthesia, an infusion cannula was implanted into the left lateral cerebral ventricle of cats of either sex (2-4 kg), so that i.c.v. injections of drugs could later be made without anaesthesia. In one group of the cats, the area postrema was destroyed electrolytically. The solutions of drugs were injected manually from a 1 ml syringe in a volume of 0.1 or 0.2 ml over a period of 15-20 seconds and washed with 0.1 ml of saline under the same conditions as for the drugs. Only expulsion of the gastric content was taken as a positive emetic response. Successive experiments, carried out on 6 cats were separated by an interval of at least 5 days. The regimen for the i.c.v. injections of DMPP and antagonists was randomized so that each animal was included in each of the experimental conditions. A coefficient of correlation (r) of linear regression was used to determine the existence of dose-response. An analysis of variance was used to test for differences between treatment and appropriate control groups.

DMPP (0.2-2.0 mg) given i.c.v. produced emesis, which appeared within 15 minutes after the i.c.v. injection and lasted from 3 to 50 minutes. The percentage of emetic responses induced by DMPP was dose-dependent ($r=0.94$; p 0.05). However, the percentage of cats showing emesis, even with the largest doses of DMPP (1.0 - 2.0 mg) amounted to 83 %, but never reached 100 %.

Of ganglionic blocking agents, hexamethonium (0.5 - 2.0 mg) was used to prevent the emesis produced by 1.0 mg of DMPP. The ganglionic blocking agent was injected i.c.v. 15-20 minutes before DMPP was similarly administered. Hexamethonium depressed or abolished ($p < 0.01$) the percentage of DMPP-induced emesis.

The catecholamines, dopamine (0.5 - 1.0 mg), noradrenaline (0.5 - 1.0 mg) and adrenaline (0.5 - 1.0 mg), as well as GABA (0.5 - 1.0 mg), glycine (0.5 - 1.0 mg), 5-hydroxytryptamine (0.5 - 1.0 mg) and histamine (0.5-

1.0 mg) were injected i.c.v. 15 - 20 minutes before the emetic challenge of 1.0 mg DMPP. By that time, the emesis induced by catecholamines and histamine subsided. Catecholamines, GABA, glycine, 5-hydroxytryptamine and histamine had virtually no effects on DMPP-induced emesis.

In cats with a lesion of the area postrema, 1.0 mg of DMPP injected i.c.v. did not evoke emesis.

In control experiments, two repeated i.c.v. injections of 0.9 % saline in volumes of 0.2 ml at intervals of 15-20 minutes did not produce any visible behavioural, autonomic or motor phenomena.

The area postrema of the cat contains catecholaminergic, 5-hydroxytryptaminergic and gabergic nervous elements (Fuxe and Owman, 1965; Armstrong et al., 1981; Newton and Maley, 1987). The present experiments revealed that catecholamines and histamine, but not 5-hydroxytryptamine, GABA and glycine induce emesis and that neither of them modify the emesis produced by DMPP. Since ablation of the area postrema abolished DMPP-induced emesis, it is apparent that catecholaminergic, 5-hydroxytryptaminergic, histaminergic and gabergic mechanisms do not modulate the emetic responses induced through nicotinic receptors in the area postrema of the cat. The present experiments further suggest that GABA, of neurotransmitters present in the largest amounts in the area postrema(Newton and Maley, 1987), cannot be an inhibitory neurotransmitter in the control of emesis evoked by an action on nicotinic receptors in this area of the brainstem.

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THE EFFECT OF CH-38083 ON THE MOTILITY AND PASSIVE AVOIDANCE BEHAVIOR OF MALE SPRAGUE-DAWLEY RATS

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(alpha-2 adrenoreceptor antagonist, CH-38083, clonidine, CNS)

CH-38083 (7,8-[methylenedioxy]-14-α-hydroxyalloberbane-HCl) is a selective and specific antagonist on alpha-2 adrenoreceptors, Vizi et al., (1986). However, the effect of CH-38083 on the central nervous system in mammals has only been partially characterized. In the present work we aimed to examine the CNS effect of CH-38083 given on itself and also its presumed alpha-2 adrenoreceptor antagonistic effect against the alpha-2 agonist, clonidine. A four-channel computerized Omnitech's Digiscan Animal Activity Monitor served to measure the motility of male Sprague-Dawly rats, weighing between 100-260 g. Clonidine was administered subcutaneously 20 min before, while CH-38083 was given orally 40 min before the begining of the measurements. Data were collected for 30 min and from the recorded 20 type of movements the mean ± SD of percentage difference in TOTAL ACTIVITY between untreated (control) and treated pair of rats was calculated. For statistical evaluations the unpaired t test (2 treatment groups consisting of different individuals) and analysis of variance (more treatment groups consisting of different individuals) were used. To assess the effects on learning and memory a six-channel shuttle box system was used. Drugs were applied after five days of shuttle box training. We observed the passive avoidance behavior of rats during the training and retesting (after treatment) periods, and changes in the acquired performance were expressed as mean ± SD percentage difference between periods. For statistical evaluation the unpaired t-test, analysis of variance, and the paired t test (before and after a single treatment in the same individuals) were used.

The results are summarized in the following tables:
THE EFFECT OF CLONIDINE, CH-38083 AND CLONIDINE + CH-38083 ON THE MOTILITY OF RATS

Compound	N	Dose (mg/kg)	% Difference in total activity	
Clonidine	22	0.1 (s.c.)	- 82 ± 6.	p<0.001*
	12	1.0	+ 8 ± 25.6	
CH-38083	10	5.0 ORAL	+ 12 ± 27.9	N.S.**
	19	10.0	+ 31 ± 42.4	
Clonidine		0.1 (s.c.)		
+	5	1.0	- 80 ± 7.3	
CH-38083	5	5.0 ORAL	- 79 ± 3.5	p<0.05**
	7	10.0 *(p<0.001)	- 22 ± 27.3	

Values are mean ± SD percentage difference in motility between control and treated group of rats; *unpaired t test; ** one-way ANOVA.
THE EFFECT OF CLONIDINE, CH-38083 AND CLONIDINE + CH-38083 ON THE PASSIVE AVOIDANCE BEHAVIOR OF RATS

Compound	N	Dose (mg/kg)	% Difference in total activity	
Clonidine	15	0.1 (s.c)	- 94 ± 5.20	p<0.001***
	11	1.0	- 0.5 ± 6.40	
CH-38083	6	5.0 ORAL	- 3.1 ± 1.90	N.S**
	21	10.0	- 4.7 ± 5.6	
Clonidine		0.1 (s.c.)		
+				
CH-38083	6	5.0	- 93 ± 6.60	
	14	10.0 *p<0.001	- 33 ± 30.0	p<0.05**

Values are mean ± SD percentage difference in the acquired performance before and after drug treatment; * = unpaired t test; **one way ANOVA; *** = paired t test.

Thus, 0.1 mg/kg clonidine (s.c.) was highly effective in decreasing the motility of rats (- 82 ± %). CH-38083 alone, has dose-dependent, moderate activity increasing effect. 1 mg/kg dose of CH-38083 did not influence the effect of clonidine. In 5 mg/kg concentration it had a moderate and in 10 mg/kg dose it showed submaximal antagonistic effect. CH-38083 alone was without effect on the passive avoidance behavior of rats, while clonidine significantly impaired their performance. In case of coadministration of a 10 mg/kg dose of CH-38083 greatly improved the clonidine-debased behavior.

IN SUMMARY, CH-38083 given orally, affects CNS function of rats and this effect has a special alpha-2 antagonistic component.

E.S. Vizi, L.G. Hársing Jr., J. Gaál, T. Kapocsi, S. Bernáth, G.T. Somogyi J. Pharmacol. Exp. Ther 238. 701-706 (1986)

EXCITATORY AMINO ACIDS AND CARDIOVASCULAR APPARATUS: EXPERIMENTAL STUDIES ON CONSCIOUS RATS WITH L-GLUTAMATE, N-METHYL-D-ASPARTATE, KAINATE AND QUISQUALATE

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Key words: EAA, arterial blood pressure, AVP, catecholamines, rat

It is currently known that L-glutamate can act as an excitatory synaptic transmitter in the CNS. Moreover, there is little information about the involvement of excitatory amino acids (EAA) in the manteinance of the cardiovascular tone (Marmo, 1988). We evaluated the effects of icv (3rd and right lateral ventricles, posterior hypothalamus and striatum) EAA (L-glutamate, N-methyl-D-aspartate, kainate and quisqualate) on arterial blood pressure, on heart rate, on catecholamines and arginine vasopressin plasma levels, and on the behaviour of conscious normotensive rats.

All these drugs significantly increased arterial blood pressure, heart rate and catecholamines and arginine vasopressin plasma levels. Regarding behaviour, all animals presented psychomotor agitation, stereotiped movements and hyper-excitability.

A systemic (intravenously or subcutaneously) pretreatment with reserpine, propranolol, prazosin, phentolamine, clonidine, hexamethonium, diltiazem, and CGP 25838 (a selective antagonist of V_1 receptor of arginine vasopressin) reduced cardiovascular, behavioural and biochemical responses of icv EAA. In addition, the bisurrenectomy and the spinal transectomy (at

C7) considerably reduced responses caused by EAA.

On the contrary, naloxone, atropine and bivagotomy significantly enhanced EAA-induced responses.

Icv (3rd and right lateral ventricles, posterior hypothalamus and striatum) pretreatment with 2-APV, a selective antagonist for NMDA receptors, induced the strongest inhibition of the EAA effects.

These data show that the glutamergic transmission partecipates, through a central mechanism mainly mediated by specific receptors (e.g. NMDA, KA subtypes) in the regulation of cardiovascular function in conscious rats. In addition, this research point out an increase in the central sympathetic efferent activity and in arginine vasopressin release and an involvement of parasympathetic and opiod systems.

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ALCOHOL WITHDRAWAL AND PROLACTIN

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Keywords: etanol withdarawl, prolactin

It is known that prolactin (PRL) plays important role(s) in various functions of central nervous system, e.g. the hormone results in alterations in the paternal and maternal behavior; migration; feeding behavior; learning and memory; sexual behavior, etc. The role of PRL in drug-induced behavioral responses has also been suggested (Drago, 1982). There is a large body of evidence indicating that alterations of PRL secretion are common in chronic alcoholic patients, (Noth and Walters, 1984) but it has not been analyzed systematically, whether altered PRL secretion might have a role in clinical symptoms of ethanol withdrawal or in endocrine and clinico-chemical disturbances observed during withdrawal period. In the present investigations 90 male chronic alcoholics attending the Alcohology Unit of the Markusovszky Teaching Hospital, were investigated concerning changes in clinico-chemical and endocrinological parameters during a 40 days period of alcohol withdrawal. The serum levels of PRL, LH, testosteron (TEST), ACTH and cortisol (CORT) were determined by radioimmunoassay. The results were analyzed with SAS Manova test (SAS Institutes Inc., USA). One group of group of alcoholic patients exhibited normal- or slightly low PRL levels at the first day of withdrawal. Patients in the second group exhibited hyperprolactinemia. Hypogonadism in chronic alcoholics is induced either by a direct peripheral effect of alcohol (Legros et al., 1980), or in a secondary manner, by hyperprolactinemia (Thorner et al., 1980). There were no PRL-related differences in the activity of pituitary-adrenal axis, or pituitary-gonadal axis. There was, on the other hand, a very marked PRL-related difference in the incidene

of delirium tremens (7% in normoprolacinemic patients and 39% in hyperprolactinemic patients (p 0.05)). It is concluded that PRL might have an important role in ethanol withdrawal, but changes in the activity of pituitary-adrenal and pituitary-gonadal axis seem to be independent of altered PRL secretion pattern.

		day a #	day 2 #	day 10 #	day 40 #
PRL	N	144.6±12.0 ○	217.5±22.4×○	240.6±23.4×○	252.1±51.5×
	H	583.6±43.1	495.1±98.8×	375.0±61.4×	314.1±58.9×
LH	N	16.0± 4.0	12.4± 2.0	11.8± 1.2	9.8± 0.7
	H	19.9± 4.7	16.7± 2.1	16.5± 2.2	11.1± 1.2
TEST	N	17.5± 2.9	14.1± 1.5	16.0± 2.2	22.8± 3.0
	H	14.1± 3.2	14.8± 1.8	18.3± 2.5	26.1± 4.0
ACTH	N	25.3±10.9	no data	26.1± 7.2	no data
	H	30.6±16.2	no data	45.8±31.8	no data
CORT	N	784.1±59.8	630.0±55.0	602.9±33.6	554.1±69.3×
	H	673.7±74.2	639.2±77.7	532.2±44.1	557.6±60.1

Table 1. The serum hormone levels in chronic male alcoholics during the withdrawal period. **N** indicates the normoprolactinemic groups and **H** indicates the hyperprolactinemic group. #: mean ± S.E.; ×: significantly different form the first day; ○: significantly different from the hyperprolactinemic group on the same day.

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THE EFFECTS OF RECEPTOR BLOCKERS ON ATRIAL NATRIURETIC PEPTIDE-INDUCED ACTION ON PASSIVE AVOIDANCE LEARNING IN RATS

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Key words: Atrial natriuretic factor, receptor blockers

Introduction

In our previous experiments we have demonstrated that rat atrial natriuretic peptide (ANP) is able to lengthen the passive avoidance response in a dose-dependent manner. This indicates that ANP is able to facilitate learning in this paradigm.

In the present experiments we tried to reply the question that whether neurotransmitters are involved in the behavioural actions of ANP. In the present report an account is given on the effects of different receptor blockers on ANP-induced action of one-trial learning of the passive avoidance behaviour.

Methods

The passive avoidance behaviour was performed by the method of Ader et al. (1972). The action of the peptide and the interactions with the receptor blocker were studied on consolidation of the memory when the peptide was given immediatelly after the learning trial. The receptor blockers were given 20-30 min before the peptide administration. The dose of the peptide was selected according to our earlier experience in this paradigm, in which the peptide per se would not interfere with the behaviour. The

dose of the ANF was 200 ng and it was given icv. following the learning trial. The animals were tested 24 hr later.

Results and Discussion

The results are presented on Table 1.

Table 1. The effects of receptor blockers on ANP-induced action of consolidation of the passive avoidance learning.

Treatment	Passive avoidance response (sec)	Significance versus ANP
Untreated	100 ± 20	-
ANP (200 ng icv.)	200 ± 20	-
ANP+Haloperidol (10 ug/kg i.p)	90 ± 15	p 0,05
ANP+Phenoxybenzamide (2 mg/kg i.p.)	189 ± 34	NS
ANP+Propranolol (10 mg/kg i.p.)	156 ± 31	NS
ANP+Methysergide (5 mg/kg i.p.)	171,6± 26	NS
ANP+Bicuculline (2 mg/kg sc.)	229,2± 35	NS
ANP+Naloxone (0.3 mg/kg sc.)	195 ± 38	NS
ANP+Atropine (2 mg/kg i.p.)	85,9± 21	p 0,05

The receptor blockers alone had no influence on the consolidation in the doses used. The minimal number of animals used in a group was lo.

The data presented here suggest that in the action of ANP on facilitating the consolidation of the passive avoidance learning, dopaminergic and cholinergic mediations are involved. Blocking the receptors, the ANP action will be diminished. Other receptors such as alpha-, or beta-receptors, GABA receptors, endogenous opiates, or serotonergic receptors are not involved in this mediation.

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INHIBITORY AND STIMULANT EFFECTS OF BACLOFEN ON GASTRIC ACID SECRETION

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Key words: Baclofen, phaclofen, GABA-B receptors, gastric acid secretion

Introduction - The parenteral administration of the GABA-B receptor agonist baclofen in anaesthetized rats increased gastric acid secretion (a.s.) with a parallel increase in atropine-sensitive vagal efferent activity, suggesting that baclofen activates parasympathetic outflow to the stomach centrally (Goto et al., 1985). However the putative central GABA receptors which might mediate the effects of baclofen on a.s. remain to be characterized. The present study investigates the nature of central GABA receptors involved in the secretory effects of baclofen.

Materials and methods - Experiments were carried out in conscious pylorus-ligated rats (200-220 g, 24 h fasting). In a group of animals a chronic cannula was implanted into the lateral ventricle of the brain.

Results - Baclofen 1-9 μg/rat i.c.v. induced a phaclofen-sensitive inhibition of a.s.. Similar results were obtained with 1 mg/kg i.v., whereas baclofen 9 and 27 mg/kg i.v. induced a non phaclofen-sensitive increase in a.s.. Muscimol was without effect (Fig. 1)

Discussion - The present inhibitory effects of i.c.v. or i.v. baclofen on hypersecretion evoked by pylorus ligation indicate the involvement of GABA-B receptors. On the basis of these results, the decrease in a.s. following i.c.v. GABA in conscious rats (Bhargava et al., 1985) may be explained by central GABA-B receptor activation. Since acid hypersecretion by pylorus ligation is mediated by central cholinergic pathways (Sharma et

al., 1963), the present antisecretory effects of baclofen may be mediated by central phaclofen-sensitive GABA-B receptors inhibiting vagal activity. Furthermore, i.v. baclofen at high doses increased a.s., as observed by Goto et al. (1985) in anaesthetized rats. However, although baclofen may behave as a partial GABA-A agonist (Del Tacca et al., 1989), the lack of secretory effects of the GABA-A agonist muscimol suggests that neither central GABA-A nor GABA-B receptors participate in the present stimulant effect of i.v. baclofen.

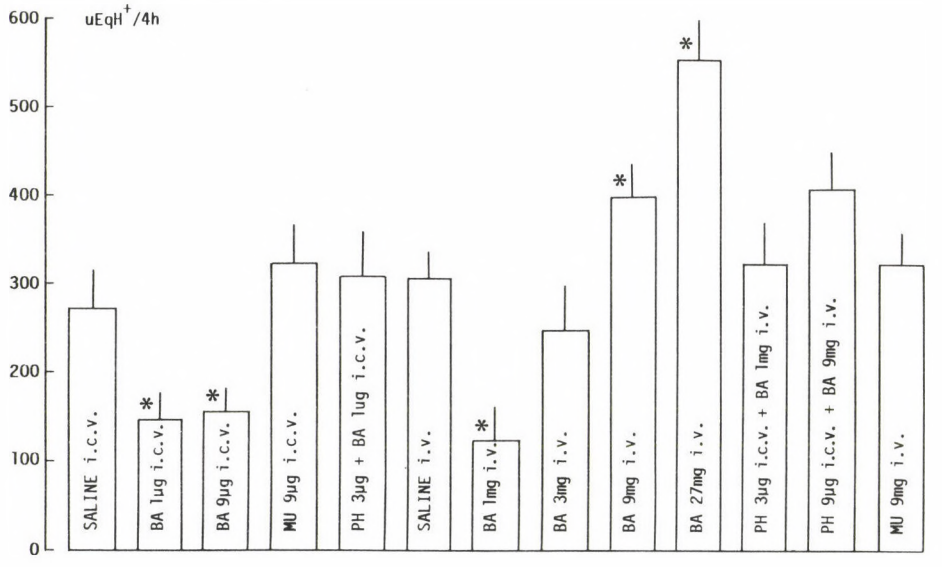

Figure 1 - Effects of baclofen (BA), muscimol (MU), and phaclofen (PF) on gastric acid output ($\mu EqH^+/4h$). Values represent the mean of 8 experiments±S.E. Student's t-test for unpaired data, *$p < 0.05$.

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THE EFFECT OF CIMETIDINE ON THE RAT SCIATIC NERVE - GASTROCNEMIUS MUSCLE PREPARATION I N V I V O

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Keywords: Cimetidine, Neuromuscular transmission, Interactions, I n v i v o .

We have reported that cimetidine and ranitidine produced neuromuscular blockade in the isolated phrenic nerve - diaphragm preparation of the rat and increased the neuromuscular blockade induced by non depolarizing relaxants and aminoglycoside antibiotics. Neuromuscular blockade produced by the two H2 antagonists was reversed by $CaCl_2$ and 4-aminopyridine and not by neostigmine or dimparit; on directly stimulated preparations the two drugs did not produce any block. (Bossa et al. 1981, 1982, 1988).

These results are obtained i n v i t r o at high concentrations, far above plasmatic levels required for antisecretory activity. For this reason we investigated the effects of cimetidine on neuromuscular transmission in vivo. Male rats of 250-350 g were anesthetized with urethane (1.5 g/kg i.p.) and kept under artificial respiration; indirectly elicited muscle contractions were obtained by using the sciatic nerve - gastrocnemius muscle preparation; the parameters of stimulation of the nerve were: frequency 0.2 Hz, duration 1 msec, at supramaximal voltage. As the tetanic stimulation, a frequency of 50 Hz was applied for 20 sec. Drugs were administrated in the jugular vein.

Single intravenous injection of cimetidine (20 mg/kg) did not alter twitch tension; single intravenous injection of d-tubocurarine (45 µg/kg) produced a reduction of twitch tension that was potentiated by successive treatment with cimetidine (Table I). We obtained similar results when we substituted d-tubocurarine with pancuronium (150 µg/kg), sisomicin

(20 mg/kg) and netilmicin (20 mg/kg).

Tetanic contraction and post-tetanic potentiation were reduced after cimetidine administration (60 mg/kg). Continuous infusion of cimetidine (30 mg/kg/min) provoked a reduction of twitch tension (50% of basal value after 12 min): there is a complete recovery after treatment with 4-aminopiridine (2 mg/kg i.v.) (Lund, H., 1978).

Our *in vitro* and *in vivo* results suggest that neuromuscular effects of cimetidine are not related to its specific activity on histamine H2 receptors, but to an inhibition of transmitter release at the nerve ending through a reduction of calcium influx at presynaptic level.

The importance of this action is the possibility of respiratory depression or prolonged apnoea induced by interaction with curare or other muscle relaxants (Argov Z. et al., 1979).

Table I: Effect of d-tubocurarine and cimetidine on the neuromuscular transmission (sciatic nerve - gastrocnemius preparation of rat)

	CONTROLS	d-TUBOCURARINE	CIMETIDINE *
DOSE		45 μg/kg	20 mg/kg
TWITCH TENSION	100 (56.3±18.7 g)	61.45±23.70	18.11±14.56**

* = Cimetidine was administrated 60 sec after treatment with d-tubocurarine;
** = $p < 0.01$ versus d-tubocurarine value

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CHARACTERIZATION OF THE ALPHA-2-ADRENOCEPTOR LABELLING SPECIFICITY OF [^{3}H]CH-38083

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(alpha-2-adrenergic receptors, [^{3}H]CH-38083, rat brain)

We previously reported the alpha-2-adrenoreceptor selectivity and specificity of CH-38083, a berbane derivative (7,8-methylene-dioxy-14 α -hydroxy alloberbane-HCl) in binding studies, carried out in rat brain crude membrane preparations, using [^{3}H]Prazosin and [^{3}H]Idazoxan as reference substances. The selectivity ratio of CH-38083 (K_d alpha$_1$/K_d alpha$_2$) was found to be 1368, Vizi et al., (1986).

The tritiated derivative of CH-38083 was synthetized by catalytic isotope change reaction using tritium gas (specific activity: 67.6 Ci/mmol). The characteristics of [^{3}H]CH-38083 were determined in association, dissociation and saturation studies, including pH, temperature, incubation time, protein, mono- and divalent cation contents optimization, Gaál et al., (1988).

In the present displacement studies we aimed to establish the degree of specificity of [^{3}H]CH-38083 as labelling ligand for alpha-2-adrenergic receptors. The binding of [^{3}H]CH-38083 was established in the mixture of 500 µl crude rat brain membrane preparation (0.7-0.9 mg/ml protein), 100 µl buffer and drugs, respectively and 400 µl buffer (50 mM Tris/HCl, pH 7.4, 25 ^{o}C) in a total volume of 1.0 ml.

The concentration of [^{3}H]CH-38083 was 2 nM for competition assays. The tubes were incubated at 25 ^{o}C for 45 min and incubation was terminated by rapid filtration under vacuum through GF/C glass fibre filters. The radioactivity was measured by liquid scintillation spectrometry. The specific binding was 60 % of the total activity determined by the

application of 10 µM unlabelled CH-38083. The experiments were evaluated by a non-linear curve fitting program, Batke and Gaál (1986).

The results are summarized in the following table:

Compound	K_d (nM)	Compound	K_d (nM)
alpha-adrenergic		**cholinergic**	
CH-38083	2,7	Atropine	> 10000
Idazoxan	8,5	Carbamylcholine	3900
Yohimbine	30	QNB	> 10000
Clonidine	59	**serotoninergic**	
Phentolamine	30	Methysergide	1800
Prazosin	280	Spiperone	> 10000
beta-adrenergic		**antidepressants**	
Isoprenaline	3000	Imipramine	1200
Propranolol	2800	Desipramine	3000
Dihydroalprenolol	> 10000	**MAO inhibitors**	
Terbutaline	> 10000	(-)-Deprenyl	1800
histaminergic		Pargyline	> 10000
Histamine	3800		
Cimetidine	2400		

Thus, only the alpha-adrenergic substances showed significant activity on the [^{3}H]CH-38083 labelled alpha-2-adrenoceptors. The order of their relative potencies is: CH-38083 > Idazoxan > Yohimbine = Phentolamine > Clonidine >> Prazosin >>> compounds with different receptor specifities. Within the group of compounds acting on alpha-adrenergic receptors CH-38083, Idazoxan and Yohimbine can be regarded as specific, i.e. the receptors labelled by [^{3}H]CH-38083 were the alpha-2 type.

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CALCIUM-ANTAGONIST EFFECT ON PURINE RELEASE FROM DISSOCIATED PRIMARY GLIAL CULTURES OF RAT STRIATUM.

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Key words: purine release, glial cultures, Ca^{2+}-channels.

An increasing number of experimental evidences seems to give glial cells new functional roles in the central nervous system. As a matter of fact, besides exerting a trophic activity, astrocytes would be excitable and able to modulate the neuronal transmission through specific mechanisms of uptake and release. Our previous studies pointed out that dissociated primary cultures of glial cells are able to release labelled purines (^{3}H-P), traced by ^{3}H-adenosine used for the incubation, at rest. The ^{3}H-P outflow rised, under electrical stimulation, in a frequency-dependent and Na^+-independent manner (Caciagli, 1988). The presence of voltage-sensitive Ca^{2+} channels (VSCC) in cultured glial cells have been shown by electrophysiological studies (Mac Vicar, 1984). This finding has been successively confirmed by the individuation of high affinity binding sites for nitrendipine, a potent and specific L type of VSCC antagonist, on glial cell membrane (Litzinger, 1986). Thus, it is possible to infer that astrocytes represent a potential target for Ca^{2+} channel blockers.

The aim of the present study was to evaluate the eventual Ca^{2+}-dependence of both basal and electrically evoked ^{3}H-P release from dissociated primary cultures of rat striatum glial cells, focusing on the role played by VSCC in such a mechanism. 10-90nM Nitrendipine, chosen as representative drug of dihydropyridines, and 100nM w-conotoxin (w-CgTx), a toxin able to block both N and L types of VSCC, alone or in combination, were added to the superfusion medium 60 min before the electrical stimulation.

30-120μM Verapamil was used too, even if, besides blocking VSCC, it is known to affect other cellular mechanisms (Miller and Freedman, 1984). All the assayed Ca^{2+}-antagonists significantly reduced, in a dose-dependent manner, the evoked ^{3}H-P release; while the basal outflow was incresed, even if this latter effect was variable and not dose-dependent. Consequently, the ratio between evoked and basal ^{3}H-P fractional release (rFR=S1/Sp1) resulted significantly reduced. The rank order was the following: w-CgTx ≥ nitrendipine > verapamil. When 100nM w-CgTx was added to the cultures already submitted to 90nM nitrendipine treatment, no further significant effect on ^{3}H-P release, compared to that of nitrendipine or w-CgTx alone, was observed.

These findings, though preliminary, suggest that at glial level the L type of VSCC play a relevant role in the regulation of P release, whereas the N channels seem not to be involved in such a mechanism.

Since astrocytes have been found to be provided with Ca^{2+}-activated K^{+} channels and K^{+} currents represent important events for the physiological glial activity, the effect of 10mM tetraethylammonium (TEA), a Ca^{2+}-activated K^{+} channel blocker, was assayed. The drug, added to the culture superfusion medium 60 min before the stimulation, induced a significant reduction of fractional ^{3}H-P release, increasing the basal outflow and decreasing the evoked one. The simultaneous culture treatment with TEA and nitrendipine confirmed that, at glial level, K^{+} and Ca^{2+}-channels are functionally connected and suggested that they together participate in controlling ^{3}H-P release.

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ß- CYCLODEXTRIN COMPLEXATION IMPROVES ABSORPTION AND GASTRIC TOLERABILITY OF PIROXICAM

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KEY WORDS: piroxicam-ß-cyclodextrin; gastric tolerability

ß-cyclodextrin is an oligosaccaride with a ring structure of 7 glucose monomers, which can form inclusion complexes with molecules that are pharmacologically active. These complexes offer considerable improvements in water solubility, rate of absorption after oral administration and a reduction in possible irritant effects on the mucosa of the gastrointestinal tract (Nambu et al., 1988).

Piroxicam, a modern and particularly effective non-steroidal anti-inflammatory and analgesic drug (NSAID), is characterized by a long elimination half-life (38-45 hours) but slow and gradual absorption: peak plasma levels are achieved 3 to 4 hours after oral administration.

In order to obtain a more rapid onset of peak plasma concentration and a better tolerability, piroxicam has been complexed with ß-cyclodextrin in the molar ratio of 1:2.5. The product obtained (piroxicam-ß-cyclodextrin; P-ß-CD) proved to have the same pattern of anti-inflammatory and analgesic activity, but a faster appearance of effects. In rats the inhibition of paw oedema was infact 60 min. after administration considerably greater for P-ß-CD in respect to plain piroxicam (P-ß-CD 58% inhib.; piroxicam 39% inhibit.: $P<0.05$; dose 3 mg.kg p.o. of active principle). No statistical difference was observed in the overall anti-inflammatory activity (reduction of AUC "oedema against time": P-ß-CD: 50%; piroxicam 46%).

In mice the almost maximal analgesic activity was reached after 5 min. from treatment. A quantitative assessment of gastroirritancy of P-ß-CD in comparison to piroxicam (4.5 mg/kg p.o. as active principle) was performed in Crl:CD (SD) male rats using two experimental models, namely:

a) gastrointestinal bleeding by means of measure of eme in faeces spectrophotometrically after conversion to pyridine-hemochromogen (Ghanayum et

Ahmld, 1982). Fasted male Crl:CD (SD) rats (g. 150-175) were treated by oral route and faeces were collected over the successive 24 hours.

b) Morphological assessment of hemorrhagic lesions in the gastric mucosa by evaluation of areas of extravasation of Monastral blue, a suspended blue tracer that normally crosses injured blood vessels only.

Fasted male Crl: CD (SD) rats (g. 200-250) were used. 5 hrs after treatment the stomach was removed, opened along the greater curvature, pinned flat on cork board and fixed in 10% buffered formaldehyde for 24 hrs and examined with reflected light using a stereomicroscope (Szabo et al.,1985)

Results obtained in both tests (Table I) show that P-ß-CD has a significant better gastric tolerability in respect to plain piroxicam.

Table I: Gastric tolerability of P-ß-CD in comparison to plain piroxicam in the rat. Mean value ± S.E.

Treatment	Doses* mg/kg	Fecal blood loss µl/24 hrs	Total area of extravasation
Vehicle	-	3.0 ± 0.7	0 ± 0
P-ß-CD	4.5	18.2 ± 5.4	0.37 ± 0.15
Piroxicam	4.5	53.2 ± 15.6	1.77 ± 0.70

* dose was given as active principle; statistical difference between P-ß-CD and piroxicam was in both tests $P < 0.05$ (Mann-Whitney "U" test).

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AMILORIDE-OUABAIN INTERACTION IN CARDIAC CONTRACTION: THE ROLE OF Na^+/Ca^{2+} EXCHANGE.

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Key words: Na^+/Ca^{2+}exchange, ouabain, amiloride, heart.

Most authors agree that the positive inotropic effect of ouabain is the result of an increase of intracellular Na^+ due to inhibition of Na^+/K^+ATPase (Repke, 1964). The reduction of Na^+ gradient through the sarcolemmal membrane, caused by the increase of $[Na^+]_i$, may induce a decrease of Ca^{2+} exit or an increase of Ca^{2+} entrance via Na^+/Ca^{2+} exchange. These two events may be not mutually exclusive, since in each cardiac cycle the Na^+/Ca^{2+} exchange system operates in either direction across the sarcolemma, promoting Ca^{2+} influx upon depolarization and Ca^{2+} efflux upon repolarization (Sheu and Blaustein, 1986). The positive inotropic effect of amiloride, known inhibitor of Na^+/Ca^{2+} exchange, has been ascribed to a reduction of Ca^{2+} efflux through Na^+/Ca^{2+} exchange (Floreani and Luciani, 1984). Therefore, ouabain and amiloride eventually share a common mechanism to induce positive inotropic effect, i.e. an increase of intracellular Ca^{2+} through a modification of Na^+/Ca^{2+} exchange activity.

Since it has been reported that amiloride increases the inotropic effect and counteracts the toxic responses of cardiac muscle to digitalis glycosides (Seller et al., 1975; Floreani and Luciani, 1984), the pattern of amiloride action on the ouabain-induced positive inotropic effect has been studied in isolated guinea-pig left atria to clarify the features of ouabain-amiloride interaction.

The addition of 0.5 mM amiloride to left atria previously exposed to 0.5 μM ouabain either increased, reversed or unaffected the fully developed positive inotropic effect of the cardiac glycoside, depending on the experimental conditions. When atria driven at 1 Hz were bathed in 1.8 mM Ca^{2+}, addition of amiloride induced a further increase of the force of contraction, whereas amiloride was uneffective at

0.45 mM external Ca^{2+}. However, the positive inotropic effect of ouabain in atria driven at 0.1 Hz was reversed by amiloride at 1.8 mM Ca^{2+}, while was enhanced at 3.6 mM Ca^{2+}.

These results suggest that the relevance of Na^{+}/Ca^{2+} exchange in promoting Ca^{2+} entry and efflux is different under different experimental conditions (Cargnelli et al., 1989). Therefore, the action of drugs affecting Na^{+}/Ca^{2+} exchange activity may have different consequences on cardiac contractility.

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RUFLOXACIN, A NEW FLUOROQUINOLONE IN BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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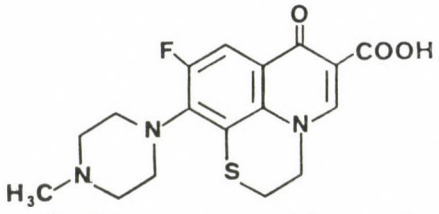
Key words: rufloxacin, biological fluids, HPLC.

Rufloxacin (I), 9-fluoro-10-(N-methylpiperazinyl)-7-oxo-2,3 dihydro-7H-pyrido (1,2,3-de)(1,4)benzothiazine-6-carboxylic acid is a new pyridone carboxylic acid derivative.



(I)

The last quinolone generation includes norfloxacin, pefloxacin, ciprofloxacin, difloxacin, fleroxacin, temafloxacin and many others in phase I and phase II clinical trials. The 4-pyridone-3-carboxylated moiety linked with an additional aromatic or heteroaromatic ring in position 5 and 6 represents the fundamental structure for the hypothesized quinolone mechanism of action (Crumplin et al., 1980). Experimental studies performed on animals demonstrated that the distribution of the drug in both tissues and organic fluids is rapid, and the reached concentration are higher than corresponding serum levels (Segre et al., 1988). In the rat, the urinary recovery determined by microbiological assay was higher than that of the unmodified drug, thus indicating the

presence of an active indeterminate metabolite (Cecchetti et al., 1984). In the present communication, we describe an extraction and quantitation procedure for measuring rufloxacin concentrations in human serum, and urine after oral administration of the drug. We used a Waters liquid chromatograph equipped with a model M6000A pump, a U6K injector, a model 740 Data module integrator and a Luminescence-LS-30 Perkin-Elmer detector. The chromatographic separation was achieved in a cyano column 5μm (25cm x 4.6mm, I.D.) connected by a 2cm disposable Pelliguard column LC-CN (40μm). The mobile phase used was a mixture of acetonitrile-phosphate buffer (pH=7.0). The excitation wavelength was set at 338nm and the emission wavelength at 526nm. The calibration curve for the determination of rufloxacin in human serum was linear over the range 0.1-5.0μg/ml and the corresponding equation was Y=0.7096X+0.034 (r=0.998); for urine the linear range 0.05-20μg/ml was described by the equation Y=0.80X+0.0145 (r=0.999) where Y is the peak area ratio of drug to internal standard and X is the rufloxacin concentration (μg/ml) in serum and urine. The intra-assay CV (n=5) was 5.6% and 6.3% for serum concentration of 0.1 and 5μg/ml and 4.6% and 1.6% for urine concentration, of 0.05-20μg/ml respectively. The extraction recovery of rufloxacin from human serum was 93% while for urine the recovery was 96,7%.

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QUANTITATION OF THE ENANTIOMERS OF FURPROFEN IN BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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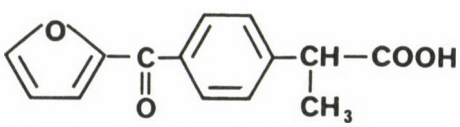
Key words: furprofen, HPLC.

Furprofen, 2-[4-phenyl-(2'-furoyl)] propionic acid (I), is an analgesic, antipyretic and antiinflammatory drug.



Arylpropionic acids are an important class of non steroidal anti-inflammatory agents displaying stereoselective activity inhibiting of prostaglandin synthesis. The (S)-enantiomers being consistently more active than the (R)-enantiomers (Gund et al. 1977). A previously reported method for assaying plasma levels of furprofen has quantified the total parent drug (Palumbo et al.,1988). The carboxylic group that partecipates in amide formation is retained by the principle oxidative metabolites of furprofen, therefore, we extended the above cited technique to allow quantitation of enantiomers in biological fluids. In this context it is of considerable significance the fact that the pharmacological activity of

these compounds is markedly influenced by their matabolic fate, in particular by their stereoselective biotrasformation (Hutt et al.,1983). The fate of furprofen enantiomers was studied in humans dosed with the racemate;for this purpose, the drug was derivatized and the diastereoisomeric amides determined by high performance liquid chhromatogrphy (HPLC). The HPLC system consisted of a Waters model M6000A pump, a U6K injector, a Lambda Max model 481 LC-spectrophotometer connected to a 740 Data Module integrator. The separation was performed at ambient temperature and the detector set at 0.1 a.u.f.s.. A 25cm x 4.6mmI.D. column paked with LiChrosorb Si 60 (10μm) was used. The mobile phase was isopropanol-cyclohexane (7:93, v/v). The wavelength of the detector was set at 245nm. Plasma samples were treated with 1-1'carbonyldiimidazole, then acidified and derivatized with S-(α)-methylbenzylamine and the final extraction was performed by hexane. Hexane was evaporated to dryness and the residue was taken up with mobile phase and used for chemical analysis. The method presented here is suitable for the simultaneous determination of enantiomers of furprofen.

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EFFECTS OF FREE RADICALS-GENERATING SYSTEM ON VOLTAGE-GATED Na CHANNELS OF SINGLE MYELINATED NERVE FIBRES

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Key words: node of Ranvier, sodium channels, free radicals

Active oxygen intermediates, such as superoxide and hydroxyl free radicals, which are probably formed in all living organisms, could be responsible for lipid peroxidation, leading to a sequence of reactions when not termined (Kappus, 1985). Therefore lipid peroxidation is a very attractive hypothesis for explaining many deseases and drug-induced toxicity. If oxygen-derived free radicals are involved in membrane injury by direct attack on lipids, it is important to question whether electrophysiological abnormalities of excitable cells ensue. In view of this possibility, the coventional voltage clamp analysis (Nonner, 1969) on single myelinated nerve fibres, dissected from the sciatic nerve of the frog, was used to study the effects of conditions promoting lipid peroxidation. Since this issue was heavily biased towards voltage-gated Na channels, K current was suppressed by replacing the end pool solution with 110 mM CsCl + 10 mM NaCl and adding tetraethylammonium (10 mM) to the external solution. At the beginning of an experiment the voltage-clamp was balanced in Ringer's solution to give a steady-state Na inactivation h of approximately 0.7. All the measurements were made before and after exposure to the "in vitro" free radicals generating system, $FeCl_3$· ADP·H_2O_2. Three to five minutes after exposure to 1 μM $FeCl_3$·10 μM ADP·0.1 μM H_2O_2 spontaneous action potentials appeared at a frequency of about 100 Hz. Neither the amplitude nor the duration of action potentials were significantly modified. Under voltage clamp-conditions

the main effect was the appearance of a maintained (late) inward current (I_L) during long lasting depolarizations; the inactivation time course of Na current could still be separated into two exponential phases with almost the same time constants and amplitude as those under control conditions. The peak Na current was not significantly modified. The late current activated and reversed at more negative voltages than the peak current. The steady-state inactivation-voltage relationship showed that a fraction of the sodium current, corresponding to the late current described above, did not inactivate and the curve, corresponding to the inactivable fraction of Na current, was shifted towards negative voltage by about 3 mV. When the contact between the nodal membrane and the free radicals generating system containing solution exceeded a critical period of 15 minutes, spontaneous action potentials were separated by silent periods of several tens of milliseconds; this effect ran parallel with the progressive disappearance of I_L, while the peak Na current was reduced to about 40 % of its control value. These in vitro experiments on hydroxyl radical formation by the classical Fenton reaction, show a two-phase effect: an early excitatory phase (due to modification of a fraction of Na channels) and a late depressant phase (due to blockade of membrane currents).

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DIHYDROPYRIDINO-SENSITIVE CALCIUM CHANNELS IN OPIOID-INDUCED THERMIC AND BEHAVIORAL EFFECTS

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Dynorphin - Met-enkephalin - Verapamil - Body temperature.

Intracerebroventricular (ICV) administration of opioids interacting with μ or k receptors in the rat elicits different pharmacological effects. For instance μ agonists induce, at lower doses, an increase in body temperature and a clear hypothermia at higher doses and a dose-related antinociception and catalepsy. On the contrary, dynorphin A (Dyn A), a purported k endogenous agonist elicits a significant hypothermia together with barrel rolling and bizarre postures without any change on nociceptive threshold (Cavicchini et al., 1988). It has been suggested a functional coupling of opioid receptors to voltage-dependent Ca++ channels and, in part, calcium-antagonists potentiate some effects induced by opioids. The aim of our research was to investigate possible involvement of dihydropyridine Ca++ antagonists on thermic and behavioral effects induced by ICV administration of dyn A and FK 33-824 a synthetic stable analogue of met-enkefalin, binding preferentially to μ receptors.

Male Sprague-Dawley rats (180-200 g) were prepared with a permanent ICV cannula and used 7 days after surgery. Rectal temperature was measured before and after ICV injection and, at 15- or 30- min intervals. Nociceptive threshold was monitored by tail-flick test and evaluation of catalepsy was timed placing the rat across twin plexiglass platforms (cut-off time 2 min). Dyn A (10 μg) provoked a clear hypothermia (Tab. 1). The calcium antagonist verapamil (10 μg ICV, 15 min before opioids) did not, by itself, affect basal body temperature, but significantly reduced the effects of Dyn A. Barrel rolling and bizarre postures were observed during 15 min after Dyn A and were not altered in verapamil pretreated rats. No significant change on nociceptive threshold was observed. Intracerebroventricular FK 33-824 elevated body temperature at the dose of 0.15 μg/rat and induced a long-lasting hypothermia at the dose of 0.25

µg/rat. Verapamil pretreatment changed in hypothermia the hyperthermic response induced by the lower dose of FK 33-824 and significantly enhanced the hypothermic effect of the higher dose. Antinociception and catalepsy induced by FK 33-824 (0.15 µg/rat) were potentiated by verapamil pretreatment.

Table 1.- Body temperature changes (°C) in rats treated with verapamil (VER) and opioid peptides (Dyn A and FK 33-824) at different time (min).

Treatment	15'	30'	45'	60'	90'	120'
Saline	+0.3	+0.2	+0.1	+0.1	0	+0.1
VER (10 µg) + Saline	0	+0.1	0	-0.1	0	+0.1
Dyn A (10 µg)	-1.2	-1.4	-1.3	-0.8	-0.6	-0.5
VER (10 µg) + Dyn A (10 µg)	-0.5	-0.2	-0.3	-0.4	+0.2	+0.3
FK 33-824 (0.15 µg)	+0.2	+0.2	+0.6	+1	+1.2	+1.2
VER (10 µg) + FK 33-824 (0.15 µ)	-1.5	-2	-2.3	-2.8	-3.2	-3.0
FK 33-824 (0.25 µg)	-0.1	-0.7	-1.3	-1.9	-2.4	-2.7
VER (10 µg) + FK 33-824 (0.25 µg)	-0.6	-1.2	-1.9	-2.5	-3.5	-4.2

It is widely accepted that molecular events subsequent to opioid receptor activation involve a change in intracellular Ca distribution. It was also found that k agonists, including dynorphin, are coupled to voltage dependent calcium channels. Analogously the calcium antagonist potentiates the antinociceptive effect induced by µ receptor agonists.

Our findings can be explained in the line of these results. As concerns behavioral effects, elicited by the k agonist Dyn A,seems that they are not related to dihydropyridine-sensitive calcium channels. On the contrary this channel seems to mediate antinociception and catalepsy induced by the µ agonist.

EFFECT OF D-PENICILLAMINE ON RAT BRAIN CORTEX LIPID PEROXIDATION INDUCED "IN VITRO" AND "IN VIVO" BY IRON-COMPOUNDS

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Key words: iron, lipid peroxidation, TBARS, lipid-soluble fluorescence, D-penicillamine.

Penicillamine is an agent used in the treatment of arthritis and as copper chelator in the treatment of copper over dose and Wilson's disease. Moreover this drug is reported to be an effective "in vitro" hydroxyl scavenger (Aruoma et al., 1988), although a possible generation of thiyl and peroxy radicals by attack of OH on penicillamine, as well as a possible hydrogen peroxide and hydroxyl radical formation enhancement caused by reducing and iron-binding properties of penicillamine have been pointed out (Gutteridge et al., 1979).

In order to study the scavenging effect of D-penicillamine on CNS peroxidative damage, experiments were performed both "in vitro" and "in vivo" on the brain cortex of Wistar rats.

Lipid peroxidation "in vitro" was stimulated in brain cortex homogenates (10% in phosphate buffer pH 7.4) by adding iron-chloride (20 μM) and ascorbic acid (100 μM). D-penicillamine HCl (50 to 1000 μM) added and the reaction mixtures were incubated at 37°C in b.m. for 30 min under agitation. The extent of lipid peroxidation in reaction mixtures and controls (mixtures without penicillamine) was assessed by measurement of TBARS expressed as 1/O.D. at 532 nm.

We observed that 50 μM penicillamine slightly but significantly enhanced the peroxidative process, whereas higher concentrations showed clear inhibition (Fig. 1a). No variation was observed by adding penicillamine (1000 μM) at the end of incubation.

"In vivo" the scavenging effect of the drug was evaluated by administering i.p. D-penicillamine HCl (50 mg/Kg) to Wistar rats in whose right brain cortex a slow lipid peroxidation was induced by iron-saccharate (200 μg Fe^{3+}) injection according to Ciuffi et al. (1988). The extent of lipid peroxidation

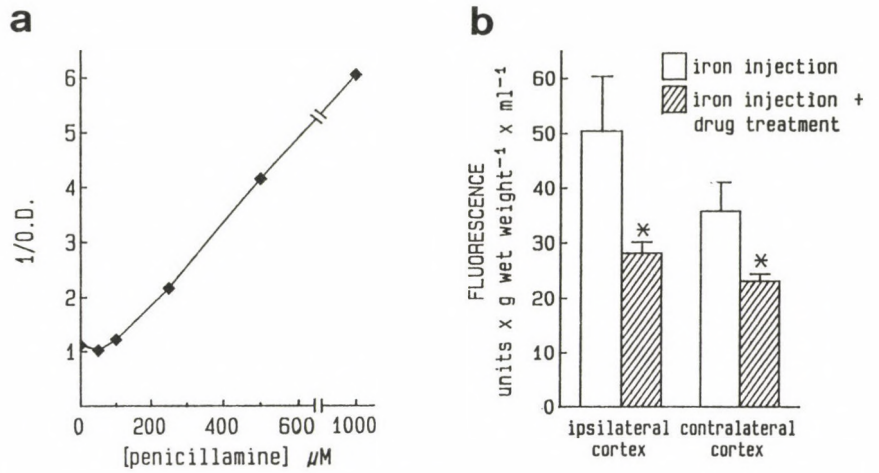

Fig. 1. Inhibition by penicillamine of rat brain cortex lipid peroxidation induced "in vitro" by iron chloride plus ascorbic acid (a) and "in vivo" by iron-saccharate (b). * : $p<0.05$.

in penicillamine treated rats and controls (drug-untreated, iron-injected animals) was assessed by measurements of lipid-soluble fluorescence (Fletcher et al., 1973) in samples from the iron-injected (ipsilateral) and contralateral brain cortex 7 days after operation.

Penicillamine was strikingly effective, causing a significant decrease in lipid soluble fluorescence values in both ipsilateral and contralateral hemicortices (Fig. 1b).

In view of our preliminary data and of the emerging role of metals and particularly iron in the pathogenesis of several ischemic or degenerative CNS diseases via a lipid peroxidation process, it would be interesting to investigate further the effect of penicillamine in the CNS.

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ENERGY OF ACTIVATION OF HETASTARCH IN A LIMITED RANGE OF THERMAL EXPOSITION

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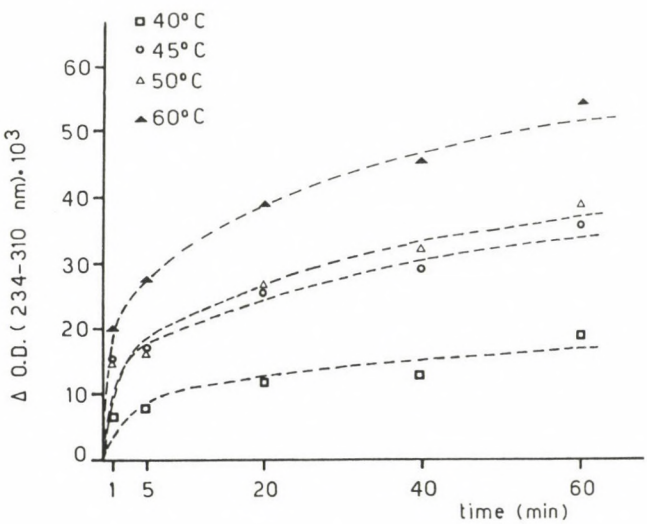
Key words: hetastarch - thermal stability - energy of activation

Conformational and stability analyses of biological linear, simple, complex or chemically modified hydrocarbon polymers appear not to be completely understood, and effectiveness of hetastarch (hydroxyethylstarch 6% in saline NaCl 0.9%; Hespan R.) compared to other plasma substitutes in the resuscitation treatment of patients would seem to call a basic reappraisal. Our preliminary data on the thermal stability of the product had been obtained by spectrophotometric measurements of the commercial product, and its more diluted solutions. Highly significant ($p < 0.01$) 234 vs 310 nm wavelenght differences in different vials of same or different batches had been found, which increased when submitted to sets of thermal expositions such as: one period, one time from 25°C to 100°C (a); one t° until 960 min (b); until 100 times at fixed t° and periods (c) (Al Sawair et al., 1989).

The general principle of finite fluctuations (Piantelli, 1987) had been fitted to the data, the abundance of the molecules with greater energy associated with the increase of the temperatures determined and the discrete distribution of the elements which at a threshold t and energy level reach an observable probability of aggregation with other similar molecules, being their structure modified to the point where it takes on different conformation, estimated. These structures may

partially regain their primary conformation with reversion of the temperature to the initial value(s), part of the structures, however, interacting with similar units, may give raise to new irreversible complexes.

Figure 1 reports the values of the Δ O.D. in one preparation exposed one time, 60 min at the indicated t°. By applying the Arrhenius' log k = Ea / 2.303 RT equation, being k the 234-310 nm Δ O.Ds, the value of Ea equal to 1.22 (r = 0.99) Kcal / M is estimated. An average energy of activation of 1.34 Kcal / mole (standard error +/- 0.17) had been found in three different preparations for the range of t° 25° to 60°C. The value is of the order of that of a charge redistribution transfer and it is compatible with which could happen in different conditions of the product, affecting its stability.



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PHARMACOKINETICS AND TISSUE DISTRIBUTION OF CYCLOSPORIN (CSA) IN METHYLPREDNISOLONE-TREATED RATS

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Key Words: Cyclosporine, Methylprednisolone, Tissue Pharmacokinetics, Rat

We previously observed that the blood levels of cyclosporin (CsA) were modified by methylprednisolone (MTP) in the rats (unpublished data). CsA was assayed by RIA methods. In the present study, we evaluated the role of MTP on the tissue distribution of CsA assayed by high pressure liquid chromatography (HPLC). Male Sprague-Dawley rats were infused intravenously with CsA (1.5 mg/kg) and with CsA (1.5 mg/kg) plus MTP (1.5 mg/kg) during 3 minutes. The CsA levels in blood, lung, liver, kidney and spleen were evaluated from 0.5 to 24 hours after injection. The elimination rate constant, the elimination half-life, the area under the curve of the drug concentration versus time from 0 h to infinity were determined for each group of rats by fitting blood and tissue profiles for each animal to a sum of exponentials by using an extended least-squares method. The percentage of penetration was calculated as ratio between tissue and blood AUC. Statistical analysis of the differences between groups was performed by "t" Student test. The results are shown in Table 1.

Analysis of the area under the curve of the drug concentration in the blood and different tissues versus time showed a higher accumulation of CsA in the methylprednisolone infused rats than in the rats treated with CsA alone. Methylprednisolone was associated with an increase in CsA half-life in liver, spleen ($P>0.05$) and kidney and lower elimination rate constant. The percentage of tissue CsA penetration was modified by methylprednisolone in kidney ($P>0.05$). These results demonstrate that MTP modifies the blood and tissue pharmacokinetics of CsA. In our experiment, CsA accumulated in tissues 0.5 h after it was injected and the tissue of MTP-infused rats accumulated more CsA than did the tissues of rats treated with CsA alone. MTP infused in our animals enhanced the blood levels of CsA and

then the distribution and the uptake in the tissues.

Table 1. Pharmacokinetic parameters of cyclosporin in rats treated with cyclosporin alone and associated with methylprednisolone.

Rats	Fluid or Tissues	Elimination half-life(h) ± SD	Percentage of Penetration ± SD	AUC (ng.h/ml) ± SD
CsA	Blood	12.47 ± 1.27	-	21,897 ± 1,524
	Liver	12.14 ± 1.71	79.0 ± 9.59	17,260 ± 1,483
	Spleen	9.1 ± 1.64	41.2 ± 6.65	8,965 ± 1,016
	Kidney	11.1 ± 1.98	45.8 ± 8.64	9,930 ± 1,674
	Lung	12.4 ± 3.54	31.8 ± 5.81	6,939 ± 1,070
CsA + MTP	Blood	11.02 ± 1.74	-	25,786 ± 4,273
	Liver	16.63 ± 4.37	80.2 ± 12.6	20,444 ± 2,836
	Spleen	13.99 ± 3.35*	40.2 ± 10.73	10,181 ± 2,314
	Kidney	15.94 ± 4.5	51.0 ± 3.67*	13,219 ± 2,257
	Lung	15.94 ± 1.90	35.8 ± 12.99	8,828 ± 1,961

* $P < 0.05$ "T" Student

Klintmaln and Sawe (1984) observed an increase of blood CsA levels in patients after MTP treatment and explained this result with an inhibition of the CsA metabolism induced by the steroid since both CsA and MTP probably utilise the hepatic microsomal enzymes for their metabolism.

The persistence of CsA in some tissues (liver, spleen and kidney) might be the result of an altered tissue binding of the drug induced by MTP.

As alterations in glucose and lipid metabolism are induced in some tissue by MTP (Jensen et al 1987), we cannot eliminate the possibility that it could have acted directly on the tissue uptake of CsA.

In every case, although the mechanism by which MTP interfers with tissue pharmacokinetics of CsA is not clear, the increase in CsA within the tissues might be associated with an higher incidence of toxicity.

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MULTICENTER STUDY OF HOSPITAL ADVERSE DRUG REACTIONS

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Key words: Hospital monitoring - Adverse drug reactions

Clinical trials carried out before licensing new drugs are designed primarily to assess efficacy and generally do not involve more than 3000 patients. Rare adverse drug effects are unlikely to be detected at this stage and there are many examples of drug withdrawn from the market because of serious adverse reaction not detected before (hepatotoxicity of benoxaprofen, epidermic necrologies of isoxicam, oculomucocutaneous syndrome of practolol). Postmarketing drug surveillance studies (PMS) have been developed with the aim to screen unknown adverse drug reactions, determine the incidence of known adverse reactions in patient populations, evaluate the role of factors that influence drug efficacy and toxicity. From some years we are carrying out PMS studies primarily addressed to adverse drug effects in hospitalized patients (Leone et al., 1988; Conforti et al., 1989).

The present study was performed with the cooperation of medical, geriatric and neurological wards of some Hospitals of Regione Veneto, and had a length of three months. The patients of 33 medical and geriatric wards assuming calcium antagonists, angiotensin-converting enzyme inhibitory and antimicrobial agents, and those of 14 neurological wards assuming antiparkinsonism drugs and platelet aggregation inhibitors were admitted to the study. Data were collected by doctors using a special form requiring patient informations related to possible adverse drug effects, pharmacological therapy during hospitalization, suspected adverse reactions and some data to assess drug imputability. The ATC classification system was used. The total number of forms was 5355.

The percentage of side effects was 3.7 in the medical and geriatric ward patients and 8.3 in the neurologic patient group. In both groups there was a positive correlation between previous and present adverse drug effects ($P < 0.01$, Chi-square test). Figure 1 shows the incidence side effects of the five groups of drugs referred to the number of administrations.

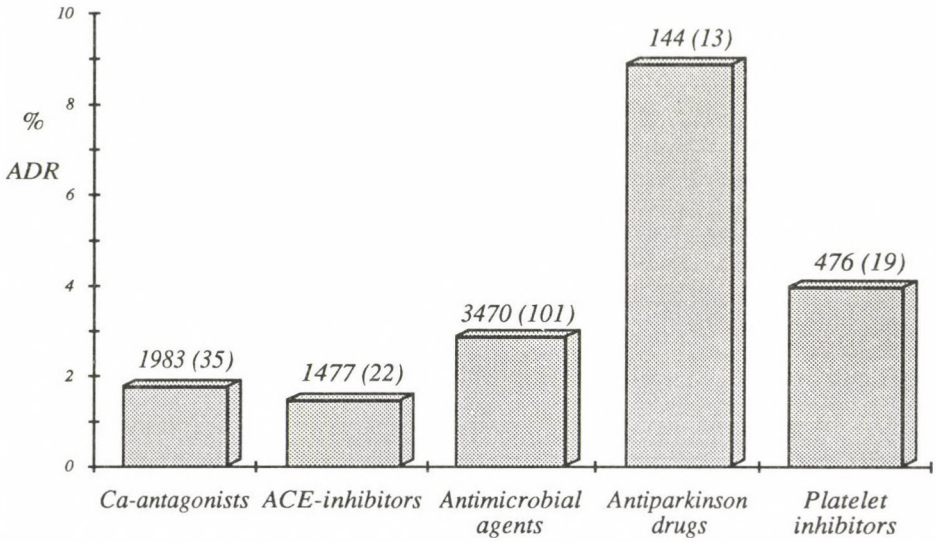

Figure 1. Adverse drug reaction (ADR) incidence in hospitalized patients. On columns number of drug administrations, in brackets number of patients with ADR.

In certain cases the analysis of the single chemical substances revealed in the some group of drugs differences in the incidence of side effects. For example among macrolides erythromycin lactobionate and among Ca-antagonists gallopamil showed the highest incidence of side effects. However in this last case the small number of patients requires further confirmations. Eight patients showed adverse effects unknown for the suspected drug. In two of these patients the relationship between drug and effect resulted uncertain. In 14.4% of medical ward patients and in 4.8% of neurologic patients the side effects were considered "serious" by the doctors.

We think that the main value of this study, beside the obtained results , is to awaken doctors towards the necessity of monitoring side effects of drugs and more in general towards the problems of pharmacoepidemiology.

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PHARMACOLOGICAL STUDIES ON NEW GASTROKINETIC DRUGS.

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Key words: benzamide fluoro-derivatives; gastrokinetic activity; anti-dopaminergic activity.

Metoclopramide is in clinical use as antiemetic and gastrokinetic agent.

Its usefulness is limited by central side effects such as extrapyramidal reactions, parkinsonism, hyperprolactinemia; these effects are considered a consequence of central dopaminergic D_2-receptors antagonism. Many researches have been made toward the modification of metoclopramide in order to separate gastric therapeutic effects from central undersiderable side effects.

During these research we obtain three new interesting compounds: P1435 {2-methoxy-3-amino-5-fluoro-N-[(2-diethylamino)-ethyl]-benzamide}, P1439 {2-methoxy-4-fluoro-5-amino-N-[2-diethylamino)-ethyl]-benzamide} and P1450 {2-methoxy-4-fluoro-3-amino-N-[(2-methylcyclopropylamino)ethyl]-benzamide}.

In the rat gastric emptying time (Amberlite pellets) test (Brodie and Kundrats, 1965), the activities, in decreasing order, were: metoclopramide > P1450 > P1439 > P1435 (ED_{50} were 1.16; 3.54; 5.28; 6.32 mg/kg/os respectively).

In the central anti-dopaminergic activity test by using apomorphine in rat (Janssen et al., 1965), the activities, in decreasing order, were: metoclopramide > P1435 > P1439 > P1450 (ED_{50} were 5.50; 48.74; 67.01; > 80 mg/kg/os respectively).

In the mouse cataleptic activity test (Athee and Buncombe, 1974) the ED_{50} of metoclopramide was 84.0 mg/kg/os whereas all three compounds showed an ED_{50} > 640.0 mg/kg/os.

The oral LD_{50}s in mouse of P1435, P1439 and P1450 were>1600; ca. 1200 and>1600 mg/kg respectively whereas that of metoclopramide was ca. 350 mg/kg.

The new compounds, in which the 5-chloro group of metoclopramide was substituted by fluoro group in position 4 or 5, show, in comparison with the standard, less gastrokinetic activity but very low central dopaminergic activity and oral acute toxicity.

In the reserpinized (5 mg/kg i.p.) rat the most interesting compound P1450, administered at 0.2 mg/kg/os 18 h after reserpine, showed no variation in plasma prolactin (RIA dosage) (Cocchi et al., 1985) whereas metoclopramide, at the same dosage, shows a significant increase.
The table summarized the results obtained with P1450, in comparison with metoclopramide as standard.

Compound	Rat gastric emptying time ED_{50} (mg/kg/os)	Central anti-dopaminergic activity ED_{50} (mg/kg/os)	Cataleptic activity ED_{50} (mg/kg/os)	Plasma prolactin levels Variations (%)	Oral acute toxicity LD_{50} (mg/kg/os)
P 1450	3.54	80.0	640.0	+ 46.4	> 1600
Metoclopramide	1.16	5.50	84.0	+ 85.6*	ca. 350

* $p < 0.05$ Dunnett "t" test.

The new compound P 1450 is slightly less active than metoclopramide as gastrokinetic but it is also pratically devoid of central dopaminergic activity.

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SENSITIVITY TO ENDOTHELIN-1 IN MESENTERIC BEDS AND AORTIC RINGS OF 4-WEEK-OLD SPONTANEOUSLY HYPERTENSIVE RATS

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Key words: endothelin, hypertension, vascular reactivity

Endothelin is a very potent vasoconstrictor agent isolated from the supernatant of cultured porcine endothelial cells (1). The role of endothelin in the development and/or in the maintenance of high blood pressure is not yet understood. Studies from our laboratories in adult 16-week-old spontaneously hypertensive rats (SHR), indicated that endothelin may not be involved in the maintenance of hypertension (2). The objective of these studies was to investigate whether potential early functional abnormalities to endothelin-1 (ET-1) occur in young (4-week-old) SHR, which may contribute to the development of high blood pressure in genetic hypertension.

The vasoconstrictor effects of ET-1 were studied in perfused mesenteric vascular beds (MVB) and aortic rings of 4-week-old SHR and age-matched Wistar Kyoto rats (WKY).

Initial mean blood pressure in SHR was significantly higher than in WKY rats, although the values are not hypertensive (124 vs.97 mmHg resp., $n=12$, $p<0.01$). Compared to WKY, reactivity to ET-1 was increased in MVB's of SHR, as indicated by the maximum perfusion pressure obtained (223 vs 155 mmHg resp., $p<0.001$). Sensitivity, however, was not significantly different between the two strains, (similar ED_{50}s: 50 and 80 pmol resp., $n=10$).

In aortic rings, in contrast, reactivity to ET-1 was reduced in SHR, whereas sensitivity was similar.
As with ET-1, reactivity to noradrenaline was increased in MVB's, but not in aortic rings of SHR.

The lack of increased sensitivity in both the mesenteric and aortic vasculature, indicates that there are no functional abnormalities to ET-1 in SHR at a pre-hypertensive age. The increased reactivity in the perfused MVB are most likely due to vascular changes. These results indicate that ET-1 may not be a primary hypertensive mechanism in genetic hypertension.

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INTESTINAL BACTERIAL FLORA AND LESIONS IN ARTHRITIC RATS: EFFECT OF ANTIINFLAMMATORY DRUG

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Key words: Lesions, bacterial flora, arthritic rats, NSAIDs

The observations concerning the presence of pathological intestinal lesions or the increased sensitivity of the gastrointestinal mucosa against NSAIDs in arthritic rats, are controversial. Bjarnason et al (1987) referred that patients with rheumatoid arthritis, untreated, have normal intestinal permeability. Schleyerbach et al. (1984) referred that the injection of Freund's adjuvant in rats induced not only polyarthritis but also histological changes of the viscera and alterations in the secretory capacity of gastrointestinal tract. Rainsford (1978) did not find significant differences between the number and occurrence of lesions in adjuvant-arthritic compared with normal rats given single or repeated doses of aspirin. Bjarnason et al (1987) found that NSAIDs caused small intestinal inflammation in arthritic patients, unrelated to the type and dose of drugs and previous or concomitant second-line drug treatment. Di Pasquale (1973) referred similar results in rats. Our purpose was to study whether complete Freund's adjuvant in rats induced intestinal lesions and alterations in bacterial flora pattern. Moreover we evaluated if, in presence of intestinal lesions, there were alterations of bacterial flora after indomethacin and SOD-PEG administration, antiinflammatory drugs with different mechanism of action, in arthritic rats.

Adjuvant arthritis was induced in male Sprague-Dawley rats, by injection of 0.6 mg of heat-killed Mycobacterium butyricum (Difco), suspended in 0.1 ml of mineral oil into the plantar region of hindfoot. After 14 days rats

received indomethacin (3 mg/kg), SOD-PEG (10 mg/kg) and indomethacin plus SOD-PEG (3 mg/kg and 10 mg/kg) i.m. for 14 days. Faecal samples were collected from all rats 24 h after the last drug administration and the microbiological analysis was performed using the standard techniques. Then animals were killed and intestinal mucosa was everted, rinsed under water and macroscopically examined. Arthritic rats showed no ulceration. Moderate inflammation was observed in indomethacin treated rats: 4 on 10 animals showed some small ulcerations. In animals receiving SOD-PEG and SOD-PEG plus indomethacin, no alterations of intestinal mucosa were evident. No changes of intestinal aerobes and anaerobes were observed in arthritic rats compared to controls. Indomethacin and SOD-PEG treatments did not induce significant alterations in bacterial flora pattern, even if SOD-PEG plus indomethacin induced an increase of Escherichia coli 1, Bacteroides and anaerobic lactobacilli.

In conclusion, we have shown that the functional integrity of the intestinal barrier, in arthritic rats, was not correlated with alterations in bacterial flora pattern. Moreover, the moderate intestinal inflammation induced by indomethacin, was not correlated to bacterial flora alterations. Then, the mechanism of drug action, route of administration, kinetics and the changes of the intestinal acidity due to the alterations in the gastrointestinal functions during the development of adjuvant disease may be important factors inducing intestinal damage. Further investigations are needed to evaluate the influence of long-term antiinflammatory therapy on intestinal mucosa and bacterial flora in arthritic rats.

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ARE BACTERIAL TOXINS INVOLVED IN ARTHRITIS INDUCED IN RATS?

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Key words: bacterial toxins, arthritic rats, NSAIDs

In previous works (Zaphiropoulos, 1986) it has been suggested that the disruption of the intestinal barrier in untreated patients with rheumatoid arthritis is important in the aetiology of the disease.

Bjarnason et al (1986) have also shown that non-steroidal anti-inflammatory drugs themselves increase intestinal permeability. This effect, combined with NSAID effects on chemotaxis and neutrophil function (Warne et al., 1978) may overwhelm the local mucosal immunological defense system and result in the long term in the bacterial invasion of the mucosa.

Previously (Benoni et al., 1987), we observed in normal rats treated with high doses of indomethacin, intestinal lesions and an overgrowth of bacteria, particularly an increase of Clostridium perfringens enterotoxin.

The aim of this work was to evaluate in arthritic rats treated already from the third day with antiinflammatory drugs, the intestinal lesions and the presence of Clostridium perfringens enterotoxins.

Adjuvant arthritis was induced in male Sprague-Dawley rats, by injection of 0.6 mg of heat-killed Mycobacterium butyricum (Difco), suspended in 0.1 ml of mineral oil into the plantar region of hind foot. At the third day from inducing arthritis, a group of rats was treated with indomethacin (1.5 mg/kg i.m.) and another group with SOD-PEG (10 mg/kg i.m.) (Veronese et al., 1989). The treatment was continued until the 28th day. Feces were collected for Clostridium perfringens enterotoxin determination at 14th, 21st and 28th day of drug administration. The enterotoxin was determined by means of the late agglutination text of Sakaguchi et al. (1973), by using a

commercial kit (Oxoid). The results were expressed as 1-5 scores on the basis of concentration ranges 0-40 to 640-1280 ng/g feces. The animals were killed 24 h after the last drug treatment and intestine was examined macroscopically for the presence of lesions.

The results of enterotoxin concentrations are shown in the following table (values as mean ± S.D.):

Treatment	Enterotoxin score 14th day	21st day	28th day
Arthritic untreated rats	0.75±0.46	0.63±0.74	0.75±0.46
Indomethacin 1.5 mg/kg	1.00± 0	0.86±0.69	1.14±0.38
SOD-PEG 10 mg/kg	0.50±1.07	1.38±0.74 *	2.88±1.13 *

*P<0.05 as compared to arthritic untreated rats

No macroscopic lesions were observed in all groups. It is known (Griffiths, 1987) that the low iron concentration enhances the production of several toxins, among which Clostridium perfringens enterotoxin. It is also known that the metabolic reactions involving SOD-PEG are iron-mediated. The treatment with this drug may therefore further increase the iron deficiency, common in rheumatic patients, and then the overgrowth of toxins.

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PHENTOLAMINE ANTAGONIZES THE HYPOXIA-INDUCED SUPPRESSION OF MYOCARDIAL CONTRACTILITY AND SINU-ATRIAL PACEMAKER ACTIVITY

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Key words: adenosine, hypoxia, myocardial contractility, phentolamine, sinu-atrial pacemaker activity

It has been previously shown that phentolamine antagonizes some physiological actions of ATP (BURNSTOCK, 1976). It was also described that a competitive non-competitive antagonism exists between phentolamine and adenosine (CSEPPENTŐ et al., 1983). In addition, an important role of adenosine releasing under hypoxia in the functional impairment of myocardial contractility (SZENTMIKLÓSI et al., 1979) and sinu-atrial pacemaker activity (SZENTMIKLÓSI et al., 1986) was also suggested.

In the present experiments, isolated, electrically driven left and spontaneously beating right atria of guinea pigs were used to analyse the action of phentolamine on the hypoxia-induced suppression of contractile force and sinus rate. Atrial preparations were mounted in an organ bath containing Krebs solution (95% O_2 and 5% CO_2; 37^{o}C). Hypoxia were induced by gassing of the nutrient solution with 95% N_2 and 5% CO_2. During hypoxia (16 minutes) the isometric tension of left atria gradually declined, but this cardiodepression could has been partially antagonized by various concentrations of phentolamine (3,10 and 30 μmol/l; Fig 1A). When spontaneously beating right atria were exposed to a long-lasting hypoxia (80 minutes), the sinu-atrial rate reduced by about 80%. In the presence of phentolamine (30 μmol/l) this hypoxia-induced sinus slowing was strongly antagonized (Fig. 1B). The results presented here confirm the previous suggestion, according to which, endogenous adenosine released under hypoxia, could play an important role not only in regulation of coronary vascular tone (BERNE, 1963),

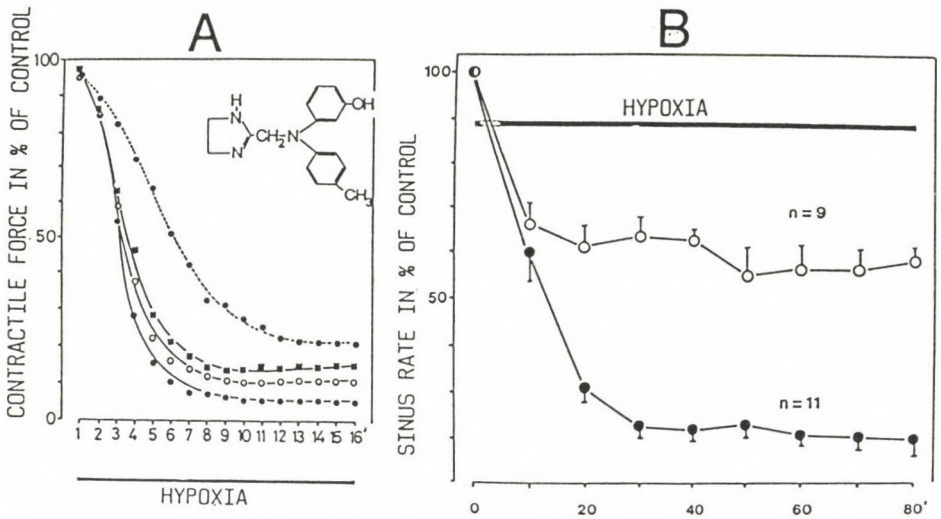

Fig.1. Action of 3 μmol/l (o——o), 10 μmol/l (■——■) and 30 μmol/l (■----■) phentolamine on the hypoxia-induced decrease of contractile force (■——■ before exposure to hypoxia) in electrically driven atrial preparations (Fig. 1A). Effect of hypoxia on the sinu-atrial pacemaker activity in the absence (■) and in the presence (o) of 30 μmol/l phentolamine in spontaneously beating right atrial preparations of guinea pigs Fig. 1B; means±S.E.M.)

but in decrease of functional activity of atria (contractility, sinu-atrial pacemaker activity), as well. In addition, our results could serve as experimental basis for explanation of the clinical observations, according of which, phentolamine has a beneficial effect on the impaired mechanical performance during acute myocardial infarction.

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THE APHRODISIAC EFFECT OF (-)DEPRENYL IN MALE RATS

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Key words: (-)Deprenyl, aphrodisiac effect

The effect of (-)deprenyl, the selective inhibitor of monoamino oxidase (MAO) B form (for review see Knoll 1983) was tested in male rats having low and high baseline sexual activity. CFY male rats (body weight 400-450 g) were exposed to receptive females and the male copulatory patterns (mounting, intromission, ejaculation) were tested once a week (test duration 30 min) for four weeks. At the end of the fourth week (selection period) male copulatory performances were categorized according to Dalló, Lekka and Knoll 1986 as follows): 1. males which failed to show any copulatory patterns as non copulators; 2. males which displayed at least one mounting during the selection period as only mounting males; 3. males which displayed intromission at least once without ejaculation as sexually sluggish; 4. males which displayed full copulatory repertoire including ejaculation in at least two out of four weekly mating tests as copulators. 280 males went over this procedure and 37 proved to be sexually inactive, 82 as sexually sluggish and 20 males were sexually active (copulators). (-)Deprenyl treatment started after four weeks selection period. The males were treated with single (0.25 mg/kg s.c.) and repeated doses of (-)deprenyl, and the mating behavior of the males were checked weekly. Control non-copulators failed to reach ejaculation during the observation period (17 consecutive weeks) but mountings and

intromissions were detectable during repeated testings. Single dose of (-)deprenyl elicited ejaculation in non-copulator males which effect lasted for several weeks and facilitated intromission and mounting as well. Repeated doses of (-)deprenyl (three times a week) exerted a more pronounced stimulation of mating patterns in non-copulator male rats: ejaculations and intromissions were consistently produced during observation. These findings are consistent with the ones described on sexually sluggish males (Knoll, Yen and Dalló, 1983). Single dose of (-)deprenyl stimulated the sexual activity in copulator males, it decreased the number of intromissions preceding ejaculation and decreased the ejaculation latency, one week after the administration. Thus (-)deprenyl proved to be a potent aphrodisiac in male rats both at low and at high baseline sexual activity. (-)Deprenyl was shown to facilitate the activity of the nigrostriatal dopaminergic neurons with high selectivity (Knoll, 1987). This might explain the aphrodisiac effect of the drug in male rats, as the activation of the dopaminergic system is known to facilitate male sexual activity.

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NON-ADRENERGIC NON-CHOLINERGIC INHIBITORY INNERVATION OF THE GASTRIC FUNDUS IN STREPTOZOTOCIN-DIABETIC RATS.

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Key words: diabetes, neuropathy, NANC innervation, stomach.

Alterations of gastric motility have been described in diabetic patients (Loo et al., 1984). A complexity of progressive changes occurs in adrenergic, serotonergic and peptidergic nerves of the gastrointestinal tract from diabetic rats (Belai et al., 1988). The aim of the present study was to investigate whether diabetic neuropathy occurs in non-adrenergic non-cholinergic (NANC) inhibitory innervation of the gastric fundus from streptozotocin-diabetic (D) rats at different stages of disease. Since VIP, and possibly Peptide Histidine Isoleucine amide (PHI), contribute to the NANC inhibitory innervation in the rat gastric fundus (D'Amato et al., 1990), the effect of VIP and PHI was also studied.

Diabetes was induced in adult male Wistar rats (300-400 g) by a single i.p. injection (65 mg/kg) of streptozotocin. Controls (C) consisted of untreated animals of the same initial weight range. Auxotonic responses were measured from longitudinal muscle strips from the gastric fundus 12 and 25 wk after induction of diabetes. The strips were suspended in Krebs solution at 37°C, bubbled with 95% O_2 and 5% CO_2. Atropine 1 μM, guanethidine 50 μM and Prostaglandin $F_{2\alpha}$ 1 μM were present throughout the experiment. All relaxatory responses were expressed as a percentage of the relaxation induced by SNP 35 μM administered at the beginning of the experiment to normalize the results, expressed as mean ± S.E.M. Data from

C and D from 12- and 25-wk groups were compared by ANOVA. A level of probability <0.05 was considered to be significant.

Electrical field stimulation (EFS, rectangular biphasic pulses, 1 msec, 120 mA, supramaximal voltage), induced a frequency-dependent relaxation (0.25-8 Hz) in all groups of preparations. The amplitude of the relaxation was significantly smaller in D than in C at each, but the highest frequency of stimulation both in 12- and 25 wk groups. No differences were observed between 12- and 25-wk groups of D rats. VIP (0.3-300nM) and PHI (10nM-1μM) induced a concentration-dependent relaxation. At equieffective concentrations, VIP was about 30 times more potent than PHI.

The present study measured the NANC relaxation of gastric fundus at different stages of diabetes. The defective relaxation found only during EFS of the NANC inhibitory neurones, but not on application of their putative neurotransmitters VIP and PHI, clearly indicate the occurence of diabetic autonomic neuropathy of the NANC inhibitory neurones from the rat gastric fundus. The nerve damage is accomplished by functional impairment that might thus account for the alterations of gastric motility described in diabetic patients. As no differences were observed between 12- and 25-wk groups of D rats, we might speculate that the functional impairment does not increase progressively during the course of the disease.

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EXOCYTOSIS AND MEMBRANE RECYCLING IN ADRIAMYCIN-TREATED RAT PERITONEAL MAST CELLS.

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Key words: adriamycin, exocytosis, membrane recycling, monensin, mast cells

During the process of secretion by exocytosis, secretory granule membranes fuse with the plasma membrane of the cells; subsequently, the excess membrane must be retrieved from the cell surface to restore the normal dimension of the plasma membrane, by an endocytosis like process. This process is known as exocytosis-endocytosis coupling. The mast cell provides a cellular system in which secretion can be induced by numerous stimulants under controlled conditions. Recently it has been shown that the antineoplastic drug adriamycin is a potent noncytotoxic histamine liberator (Decorti et al., 1986). In addition, adriamycin exhibits a very high affinity for mast cells, and adriamycin uptake and histamine release are strictly correlated (Decorti et al., 1989).

The aim of the present study was therefore to evaluate if, during exocytosis, a specific adriamycin-binding site is exposed on the cell surface, and if this phoenomenon could be responsible for the high affinity of adriamycin for mast cells.

Purified rat peritoneal mast cells were used for all experiments. Histamine realease and adriamycin binding (0 °C) and uptake (37 °C) were measured by the spectrofluorimetric methods of Shore et al. (1959) and Bachur et al. (1970) respectively.

Incubation of mast cells at 0 °C with adriamycin 50 ug/ml for 10 min resulted in a limited binding of the antineoplastic drug. However, if mast cells were stimulated to secrete with compound 48/80 (1 ug/ml) at 37 °C for 10 min, chilled and then treated with adriamycin (50 ug/ml) at 0 °C, more than 5-fold greater binding of the drug could be observed. Permeabilization of mast cells with saponin resulted in a high binding of adriamycin, even if

the uptake was prevented (0 °C, prefixation with p-formaldehyde). Similar results were obtained when mast cells were disrupted by sonication, and hence the granule membrane was exposed.

The carboxylic ionophore monensin, which mediates proton movements across membrane, had no effect on adriamycin binding (0 °C, sonicated cells), but blocked, in a time and concentration dependent fashion, receptor mediated uptake of the antineoplastic drug. Adriamycin uptake was an energy dependent process, and was completely inhibited by antimycin A (1 uM); on the contrary antimycin A had no effect on the binding of the anthracycline.

These results indicate that the great majority of adriamycin binding sites in mast cells are not exposed in basal conditions, but become evident when these cells are stimulated to exocytate, either by adriamycin or by other secretagogues, permeabilized by saponin, or disrupted by sonication.

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IDAZOXAN AND CH-38083 AFFECT GASTRIC SECRETION BY INHIBITING ALPHA-2 ADRENOCEPTORS AT VAGAL AND SYMPATHETIC PATHWAYS

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Key words: Idazoxan, CH-38083, alpha-2 adrenoceptors, gastric secretion

Introduction - The concept that alpha-2 adrenoceptors are involved in the regulation of acid secretion is mainly based on the inhibitory effects of alpha-2 agonists (Del Tacca et al., 1982). However the mechanisms by which alpha-2 adrenoceptors influence acid secretion are still unclear. The present study investigates the effects of idazoxan and CH-38083, two selective alpha-2 antagonists (Doxey et al., 1983; Vizi et al., 1986), on acid secretion during vagal and sympathetic activation.

Materials and Methods - Experiments were carried out in stomach lumen-perfused rats (200-220 g, 24 h fasting) anaesthetized with urethane (1.2 g/kg i.p.). Under these conditions urethane potentiates basal sympathetic activity (Emas, 1964). The vagus nerves were cut at the cervical level and the acid secretion was evoked by continuous electrical stimulation of the left vagus nerve (0.5 msec, 3 Hz, 10V). The same procedure was performed in a group of animals treated with reserpine (2 mg/kg i.p. 20 h before the experiments). The significance of differences between means was evaluated by Student's t-test for unpaired data. Each value refers to 8-10 experiments.

Results - Electrical vagal stimulation rapidly increased acid output (from 5.3 ± 1.2 to 31.4 ± 4.2 $\mu Eq H^+$/15 min; n=10; $p < 0.05$). Both idazoxan and

CH-38083 (1 mg/kg i.p.; n=8 for each drug) caused a significant increase in vagal hypersecretion (from 31.4±4.2 to 42.1±3.9 and to 52.3±5.3 μEqH^+/15 min, respectively; $p < 0.05$). At the dose of 3 mg/kg i.p. both the antagonists gave erratic results. When a combined i.v. infusion of prazosin (0.5 mg/kg/h) and propranolol (0.5 mg/kg/h) was given, both idazoxan and CH-38083 (3 mg/kg i.p.; n=10 for each drug) significantly potentiated vagal hypersecretion (from 31.4±4.2 to 45.5±5.2 and to 56.6±5.9 μEqH^+/15 min, respectively; $p < 0.05$). In reserpinized rats both idazoxan and CH-38083 (1 mg/kg i.p.; n=8 for each drug) did not affect vagal hypersecretion.

Discussion - The present potentiating effect of both idazoxan and CH-38083 at low doses on vagal hypersecretion was prevented by reserpine; this may indicate that during a sympathetic activation alpha-2 adrenoceptors play an inhibitory role on acid secretion at vagal peripheral sites. Moreover, the results obtained in the presence of a combined treatment with prazosin and propranolol suggest that higher doses of both idazoxan and CH-38083 might potentiate an inhibitory influence on acid secretion through the blockade of alpha-2 adrenoceptors on sympathetic nerve pathways.

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INTERACTION OF ADENINE NUCLEOSIDES IN THE CANINE MESENTERIC CIRCULATION

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Keywords:adenosine,inosine,mesenteric blood flow,intestinal vasodilation

Inosine (INO) in small doses markedly potentiates the adenosine (ADO)--induced coronary vasodilation(a) and "immunizes" the latter effect against methylxanthine blockade(b); larger doses of INO are capable of dilating the coronaries maximally(c). This triplet of INO-actions is thought to be central to the general nucleoside theory of vascular adaptation in the heart muscle (Kékesi 1987). Presupposing distinctly different interactions in the splanchnic circulation where peptidergic neural mechanisms of adaptation seem more important than direct metabolic coupling (Rózsa and Varró 1989), the actions of adenine nucleosides were investigated in the mesenteric bed.

Methods In dogs anesthetized with pentobarbital a large segment of the small bowel (167±29 g) was exteriorized and a flow probe (Statham SP) was positioned on its artery. ADO and INO were infused intraarterially to 5 dogs through a thin (23 gauge) indwelling needle in dose ranges of 6-200 μg min^{-1} and 1.25-20.0 mg min^{-1}, respectively. To block purinoceptors, aminophylline was injected in a single dose (6 mg kg^{-1}, i.v.). Responses to drugs were characterized by changes of mean flow and calculated vascular conductance. The threshold INO dose was determined in each dog according to the latter term. Homogenity of vasodilation was controlled by computer-aided infrared thermography using an AGA 750 camera.

Results and Discussion The resting levels of blood flow (0.74±0.12 ml min^{-1} g^{-1}) and mean blood pressure (151±8 mmHg) remained essentially unchanged throughout the interventions. Moreover, since systemic pressure was little affected by the nucleosides, percent changes in flow and conductance were practically identical. The mesenteric vascular responses to ADO, INO, and ADO + the threshold dose (2.5-5.0 mg min^{-1}) of INO are shown in Fig. 1.

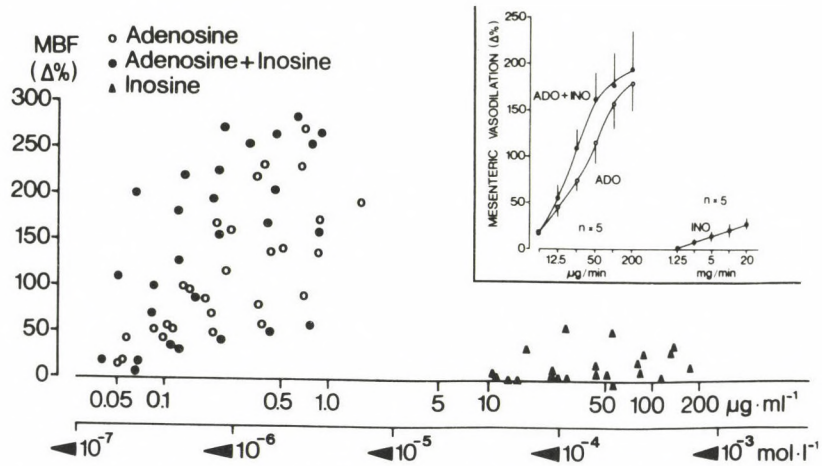

Fig. 1. Changes of mesenteric blood flow (MBF) plotted against calculated nucleoside levels in the arterial blood. Molar plasma concentrations (arrowheads) are also indicated. Insert: Dose response curves. INO-induced potentiation was not significant ($p > 0.05$) at any of the points.

Individual measurements as well as dose response curves indicated that INO was nearly ineffective, and that the trend for INO-induced potentiation of the ADO effect was slight. This is in sharp contrast with the coronary effects of INO. At the same time, ADO, in itself, elicited very great intestinal flow increases at concentrations which were less, by about one order of magnitude, than those obtained in the artificially perfused mesenteric bed. (Granger et al. 1978). Thus, the efficacy of ADO in the mesenteric circulation was comparable to that in the coronaries. Aminophylline was found to block similarly both control and INO-backed flow responses (86±8 % vs 81±6 % blockade $p > 0.5$). Taken together, in spite of the great potency of ADO for inducing vasodilation, the lack of specific INO-actions renders highly improbable the crucial role of adenine nucleosides in the metabolic autoregulation of the intestinal vessels.

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CHRONOTROPIC AND INOTROPIC EFFECTS OF MILRINONE AND CONGENERS: COMPARISON WITH PDE III INHIBITION.

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Key words: milrinone analogs - guinea pig atria - PDE III.

Amrinone and milrinone are bipyridine derivatives with well established positive inotropic activity. An antagonism towards endogenous adenosine and a selective phosphodiesterase (PDE III) inhibition are part of their complex mechanism of action. However, the extent to which each of these mechanisms is involved in the cardiac effects of these drugs is still unclear. In the attempt to obtain a new compound with defined mechanism of action and with consequent more specific therapeutic indications, milrinone was taken as the parent drug for the synthesis of various analogs. The present study was undertaken in order to evaluate the effects of these new molecules on heart preparations and on PDE III activity.

For the evaluation of inotropic and chronotropic effects, spontaneously beating atria obtained from reserpine-treated guinea-pigs (2 mg/Kg i.p. daily for 2 days) were used. The atria were suspended in organ baths containing 30 ml of Webb solution maintained at 29° C (pH 7.5). For the evaluation of drug effects on PDE, PDE III was prepared according to Kariya et al (1982) and assayed according to Thompson et al (1974).

Compounds I and II are esters of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acid. Compound III is a 6-substituted of 5-aryl-1,2 dihydro-2-oxo-3-pyridinecarbonytrile. Compounds IV and V are a 7,8-dihydro-2,5(1H,6H) quinoline-dione and a 5-aryl-2(1H)-pyridinone derivative, respectively.

The results are reported in table I and can be summarized as follows :
- like milrinone, compounds I-IV increased the force and the rate of contraction in guinea-pig isolated atria and inhibited PDE III activity. By contrast compound V exerted negative inotropic and chronotropic effects and didn't modify PDE III activity. The relative potencies (EC_{50}s) and the maximum activities of these molecules indicate that:

Table I. Potency and activity of milrinone and milrinone analogues.

Compound	EC_{50} (μM)			E_{max} (% of control)	
	Inotropism	Chronotropism	PDE III	Inotropism	Chronotropism
milrinone	32	80	2	+ 48	+ 30
I	13	120	10	+ 63	+ 31
II	17	240	65	+ 43	+ 33
III	60	240	23	+ 65	+ 23
IV	70	320	760	+ 46	+ 26
V	120	200	-	- 46	- 41

- milrinone and its derivatives affected cardiac inotropism more than the rate of contraction, as indicated by the differences in EC_{50}s and E_{max} for the two effects;
- comounds I and II, with a -CN group in 5 position, were the most potent in terms of cardiac effects, but not as inhibitors of PDE III activity;
- the order of potency as inotropic or chronotropic agents was different from the one as inhibitors of PDE III activity;

These data suggest that, if PDE III inhibition is related to the inotropic effect of these compounds, it is not the sole mechanism involved.

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CONTINUOUS MEASUREMENT OF LOCOMOTOR ACTIVITY OF SMALL LABORATORY ANIMALS

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locomotor activity, neurotoxicity

Because of the increasing demand of continuous measurement of chemical-induced motor activity of rats and mice (Schaeppi, 1988) a monitoring system was developed and evaluated enabling registration of experimental activity without experimentator's intervention. The plastic rat's cages are placed on the spring-mounted plates with vibration-sensitive detectors similarly to those proposed by Cizadlo and Bown (1980). The converted signals are accumulated by a microprocessor based on Intel 8085 for the preset intervals. The equipment consists of 8 separate units. There is an option to differentiate the total movements from gross one. The measures were conducted in a conventional animal room.

Some factors influencing on motor activity were studied. It was found that the adaptation of experimental rats to a new cage lasted for 4 hours. There are more advantages of registration of motor activity of animals in group then those in singly held animals. The system is suitable to the circadian rhythm analysis of motor activity. The accumulated values for 4 hours epochs in dark period proven to be 300-400 % higher than in light span. The physiological variations remained within 20 % during the 2 weeks continuous observation.

The reproducibility of measurements were tested by amphetamine and chlorpromazine treatment. The relatively small doses (1.5

and 3 mg/kg) of amphetamine administered by i.p. injection enhanced the locomotor activity over 3 hours after treatment. 6 mg/kg dose produced repetitive stereotyped behavior and increased the activity up to 3.5 times. Dose-dependent decreasing was observed in chlorpromazine administered rats.

Our aim was to demonstrate the extent to which locomotor activity was affected by toxic chemicals. The motor activity measurement was followed immediately the acute i.p. exposure to carbon tetrachloride (2.0 g/kg), hexachlorophene (50 mg/kg) and cadmium chloride (1.0 mg/kg). Continuous measurement was performed throughout 3 days after treatment. After transient enhance caused by carbon tetrachloride in the activity comparing to the baseline (pre-exposure) value a considerable decrease was observed in 3 hours time after treatment. On the 3rd day only hexachlorophene produced significant reduction in the activity.

Our results emphasize the potential value of using 24-hour continuous home cage locomotor activity measure for studies of the neurobehavioral effects of toxicants. The characteristic daily motor activity pattern indicates that the time of testing may be important in determining the effects of chemicals on motor activity.

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NEGATIVE FEEDBACK MODULATION OF NORADRENALINE RELEASE FROM THE SPLEEN

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Key words: noradrenaline release, spleen, alpha-2 receptors

The stimulation evoked release of noradrenaline (NA) from noradrenergic axon terminals is subject to presynaptic negative feedback modulation. (E.S.Vizi, 1979, 1984; K.Starke, 1981). It was shown that this feedback modulation is mediated via presynaptic alpha-2 adrenoreceptors. This fact indicates that NA, released from the axon terminals is able to reduce its own release evoked by subsequent stimulation.

The modulation of NA release from isolated rat spleen strips was investigated. Rat spleen strips, kept in Krebs solution, were loaded with ^{3}H-NA, superfused and stimulated electrically (8 Hz, 480 pulses). The effect of drugs on ^{3}H-NA release was studied on S_2/S_1 ratio. The drugs were administrated 15 min before S_2. A computer programme was used to calculate fractional release. The tissue content of radioactivity was 439431 Bq/g $\pm$ 47194 (n=3). Electrical stimulation released 3.20 $\pm$ 0.21 % of the total radioactivity (n=3).

The effects of different alpha1 and alpha2 agonists and antagonists on NA release were studied. Table 1 shows the data

obtained. CH-38083 (0.02 uM - 2 uM), a highly selective alpha-2 antagonist (E.S.Vizi et al.1986) enhanced the stimulation-evoked release of NA in a concentration dependent manner. L-phenylephrine, an alpha-1 agonist enhanced both resting and stimulated release of NA. In addition it was found that the M_2-muscarinic and nicotinic receptors are present on the axon terminals and exert an inhibitory and stimulatory effect respectively. Oxotremorine (M_2 agonist;1 uM) reduced the release of NA enhanced by alpha-2 antagonist (CH-38083;0.02 uM).

It is suggested that alpha-2 and M_2- receptors are present on noradrenergic neurons and located on the same nerve terminals.

Table 1.

Effect of different agonists and antagonists on ^{3}H-NA release

Drugs	(uM)	n	S_2/S_1	Significance
Control	-	3	0.94 $\pm$ 0.03	
CH-38083	0.02	3	1.98 $\pm$ 0.18	$p < 0.01$
CH-38083	0.1	3	2.12 $\pm$ 0.18	$p < 0.01$
CH-38083	0.5	3	2.49 $\pm$ 0.24	$p < 0.01$
CH-38083	0.02			
+ Oxotremorine	1.0	1	0.80	-
Oxotremorine	1.0	3	0.44 $\pm$ 0.02	$p < 0.001$
DMPP	100.0	2	3.53 $\pm$ 0.85	$p < 0.01$

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PAPAVERINE BLOCKS THE EARLY OUTWARD CURRENTS IN HELIX NEURONS

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Key words: papaverine, fast potassium current, snail neurons

Papaverine has been reported to exert either blocking effects on fast Na^{+} and slow Ca^{++} currents in guinea pig heart, Schneider et al.,/1975/ or facilitatory actions on the c AMP-induced transmembrane current in Helix neurons, Kononenko et al., /1986/. Papaverine is structurally retated to verapamil. However, the latter does not possess phosphodiesterase inhibitor activity but can modulate the potassium currents in molluscan neurons, Kostyuk et al., /1975/.

The effects of papaverine on the resting membrane potential, action potertial and fast outward currents were examined in snail neurons under current- and voltage-clamp conditions. The detailed methods concerning the physiological preparation, the electrophysiological recording and the solutions were published previously, Erdélyi and Such, /1986/.

As can be seen in Figure 1A, papaverine /1 mM,10 min/ slightly depolarized the neuronal membrane and prolonged the action potential duration. Under voltage-clamp papaverine decreased the amplitude of the A-currents in dose-dependent way /K_d= 0.8 mM, n_H= 0.6 / and increased the rate of the late decay component /Fig. 1B and C /. Papaverine did not influence the voltage-dependence of the steady-state activation and inactivation or 0 potential of the A-current. Other voltage activated membrane ionic currents, including leakage current were in much less extend influenced by papaverine in comparison with the effects found on the A-currents.

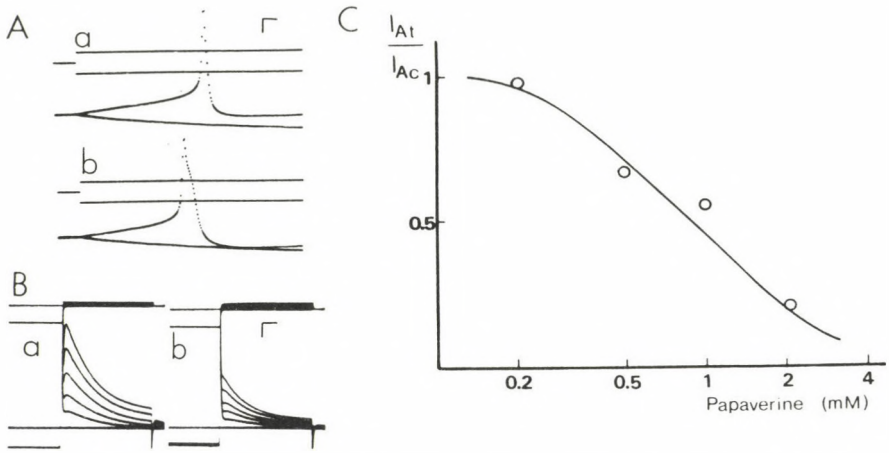

Figure 1. Effects of papaverine on the resting membrane potential, action potential, membrane resistance / 1 mM, 10 min, A / and the early outward currents / 0.5 mM, 10 min, B /. A-currents were estimated by depolarizing voltage steps from a holding potential of -50 mV to -25 mV in increments of 5 mV with preceding hyperpolarizing pulses of 500 ms duration to -100 mV. a= control responses; b= test responses. Papaverine induced attenuation of the A-currents is a dose-dependent event / K_d= 0.8 mM, C /. Calibrations: 20 mV, 10 ms and 25 nA for A; 50 mV, 100 ms and 25 nA for B.

The experiments show that papaverine has an inhibitory action on the A-currents in Helix neurons which corresponds with a moderate prolongation of the APD and a decrease of the spike threshold voltage. The verapamil-induced actions differ from the papaverine evoked events on the A-currents, Kostyuk et al., /1975/. Both drugs contain methoxybenzene groups which determine the effectiveness of the verapamil molecule but does not the papaverine one on the A-currents, Erdélyi and Such, /1989/.

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EFFECT OF AMPRILOSE ON VARIOUS IMMUNOLOGICAL AND INFLAMMATORY ANIMAL MODELS

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Keywords: amprilose, macrophag function, antirheumatic action

Amprilose, a low molecular weight immunopotentiator (1,2-O-isopropylidine-3-O(3'-(N,N'-dimethylamino)-propyl)-D-glucofuranose HCL) has been found effective in the treatment of rheumatoid arthritis without considerable side effects (Caldwell et al., 1987).

The aim of our experimental studies was to find out the dose dependent spectrum of immunological and antirheumatic effects of this compound on animal models.

The effect on T and B lymphocyte functions was measured in mice by means of the following tests: delayed type hypersensitivity, SRBC induced heterohemolysin and hemagglutination titers. Amprilose failed to influence the functional responses of lymphocyte. Supression of macrophage functions (phagocytosis and chemiluminescence test) was, however, detected at 200 mg/kg ip. dose level of amprilose. These data are in accordance with those described by Hopkins (1985) in his survey.

The antiinflammatory and antirheumatic studies were performed using the carrageenan edema test (Winter et al., 1962) and the adjuvant arthritis model (Newbould, 1963) in rats. In short term study amprilose failed to inhibit edema formation but in the case of subacut treatment high, 300 and 1000 mg/kg ip. doses resulted in a significant inhibition. In further studies amprilose produced a dose dependent inhibitory effect on the FCA induced adjuvant arthritis model. In a three week study oral doses of 30 mg/kg/day caused a stimulation while 1000 mg/kg/day exerted a marked inhibition on the development of immunarthritis (Table 1.)

Table 1.

Effect of amprilose on the paw wolume of rats on adjuvant arthritis test

Treatment	(n)	Dose mg/kg p.o.	Means of pawvolume increase ± S. E. (unit)		Action (%)
Control	(15)	-	R: 100.6 ±	7.37	
			L: 40.9 ±	6.40	
Amprilose	(13)	30	R: 103.9 ±	8.39	+ 2.7
			L: 58.6 ±	8.90	+ 43.4 *
Control	(11)	-	R: 103.9 ±	8.44	
			L: 56.2 ±	5.85	
Amprilose	(12)	300	R: 107.8 ±	9.23	+ 3.7
			L: 48.3 ±	0.57	- 14.1
Control	(8)	-	R: 112.9 ±	10.86	
			L: 49.8 ±	9.24	
Amprilose	(10)	1000	R: 94.5 ±	7.24	- 16.3
			L: 23.4 ±	8.48	- 53.0 **

R = right leg: primer inflammation; L = left leg: immuno-inflammation; *: $p < 0.1$; **: $p < 0.05$ on Duncan's test

In conclusion the study revealed the selective macrophage directed as well as antirheumatic effects of amprilose wich failed to have any action on the T and B lymphocyte dependent immunological and on the prostacyclin dependent short term antiinflammatory tests.

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THE DOSE-RELATED HYPOTENSIVE EFFECT OF HIGHLY PURIFIED BOVINE LIVER ANGIOHYPOTENSIN IN NARCOTIZED NORMOTENSIVE RATS

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Keywords: angiohypotensin, rat blood pressure, hypotensive effect

Angiohypotensin (AH) is a blood-borne selective inhibitor of the release of noradrenaline from the nerve terminals of resistance vessels. The existence of the substance in the blood which inhibits the release of noradrenaline with high selectivity in resistance vessels, the fact that the effect of AH is readily reversible, the onset and offset of the effect is extremely rapid, no tolerance to this effect develops and blood pressure can be decreased to a reasonable limit only, led to the hypothesis that AH is a continuously acting, highly specific brake in resistance vessels operating as a putative tuning mechanism for controlling peripheral resistance (Knoll, 1987, 1988).

Recently highly purified AH preparations (containing 3000-3500 units/kg) were elaborated from bovine liver. The aim of the study was to measure the hypotensive effect of bovine liver angiohypotensin on normotensive rats anaesthetized with urethane (1 g/kg).

Animals of both sexes weighing 300-350 g were used in this study. AH was injected in single doses into the femoral vein. Blood pressure was measured in the carotic artery.

Blood pressure decreased within a few seconds after the in-

travenous injection of highly purified bovine liver AH. The magnitude of the effect, as well as, its duration depended on the dose given. The lowest dose range which exerted a visible decrease in blood pressure was found to be between 1-3 units/kg. After finding the lowest detectable dose we checked the effect of 10-50-100-250-500-1000-3000 units/kg. The initial blood pressure was usually between 105-125 Hgmm. The blood pressure decreased in response to the injection of AH in a dose-dependent manner between the 10-250 units/kg dose range. In response to the 250 units/kg dose blood pressure usually declined to 75-80 Hgmm, as the lowest, level and the hypotensive effect disappeared within a few minutes. Normal blood pressure (105-120 Hgmm) was reestablished after each injection of AH. The further rise of the dose (500, 1000 and 3000 units/kg) did not lower blood pressure significantly more than the 250 units/kg dose, though, the recovery was a bit longer lasting. Even after these high doses of AH, blood pressure was resumed to the initial level. After the reestablishment of blood pressure from the decrease caused by the 3000 units/kg dose, we checked again the effect of 10 units/kg AH and found it to be as effective as it was at the beginning of the experiment.

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COMPUTERIZED INTERACTIVE CONTINUOUS MONITORING OF CARDIAC ARRHYTHMIAS WITH THE SM 2000H SYSTEM

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The arrhythmia-analysing system developed by Rohla et al. (3rd Dept. of Medicine, Semmelweis University Medical School, Budapest) has proved of value in observations on intensive care patients and in clinical pharmacological studies. The arrhythmia program can be run on a personal computer (IBM AT /40 Mbytes, Winchester/, C language) and is suitable for the simultaneous real-time monitoring of one patient to satisfy scientific requirements. The program is based on recognition of the QRS complexes, their identification, measurement of the RR intervals, filtering of the T wave and observation of the ST segment. The new program variant also permits visualization of the P waves with an optional level of magnification of the recalled ECG. The individual arrhythmic events can be documented in automatic or interactive operating mode, and the system is able, after redefinition of the QRS categories, to separate the 'aberrantly' conducted supraventricular beats and the ventricular extrasystoles, for instance. It is possible to store the various QRS morphologies, and to observe and calculate all of the arrhythmia categories of importance in clinical practice. From the aspect of the inter-

pretation and treatment of ventricular arrhythmias, it is often of decisive importance to recognize some systematic correlation ("string") in the apparently chaotic extrasystolic activity. The computer program and the mathematical model permit recognition of the ventricular parasystole and simulation of the modulated parasystolic activity with given initial conditions. The arrhythmic events can be stored on the basis of previously specified criteria (saving on hard discs or floppy discs), they may be analysed retrospectively at will, the stored events may be scrolled forwards and backwards in time, and the more interesting periods may be surveyed again, with printing-out in optional size. Slides can be prepared from the coloured monitor.

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EFFECTS OF RESTACORIN[X] (B-GYKI-38233) A NEW ANTIARRHYTHMIC AGENT IN A CORONARY OCCLUSION-REPERFUSION CANINE MODEL

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Key words: Restacorin,B-GYKI-38233,arrhythmias,antiarrhythmic agent

Restacorin (1-(2,6,-dimethylphenyl)-4,4-dimethyl-aminoguanidine hydrochloride) is a newly synthesized antiarrhythmic compound. In vivo studies in rats, guinea pigs and dogs have demonstrated that Restacorin given by oral or parenteral route has a potent and long lasting inhibitory action against supraventricular and ventricular arrhytmias (Varro et al., 1987; Rabloczky et al., 1988; Ohmura et al., 1988).

We studied the effects of Restacorin in open chest dogs. A 15-min ligation of ramus descendens anterior (LAD) was applied and when no ventricular fibrillation occured, the myocardium was reperfused for 60 minutes. The protective effects of B-GYKI-38233 was evaluated by comparison of a control group I (n:17) and two treated groups II (n:7) and III (n:8).

Group II: 0.2 mg/kg/min rate infusion for 45 minutes (from 15 min before coronary occlusion to 15 min after reperfusion)

Group III: 4.0 mg/kg iv. bolus administration 5 min before occlusion.

Restacorin did not result in significant change in hemodynamic parameters: AMBP, LVSP, +dP/dtmax and HR. Regional contractile function (measured by strain gauge) was reduced significantly by 22% and 28%, respectively (negativ inotropic action) and myocardial oxigen demand (PRP) decreased at 15 and 5 minutes, respectively after the treatment.

There was no significant difference between control and treated groups in contractile function after reperfusion of severe ischemic segment. Recovery was 50% of preischemic value after 60 minutes of reperfusion in all three groups.

Pretreatment was unable to protect against incidence of early severe arrhythmias (VT-VF) induced by coronary occlusion,(I.6/17 vs. II.4/7 vs. III.4/8) but reduced ectopic activity and the severity of existing arrhythmias. Reperfusion VF did not occur in group III compared to group I: 5/11 (45%).

Our data suggest that 4 mg/kg iv. pretreatment can increase the survival, but treatment of existing arrhythmias (ongoing study) can be more effective when VT or VF does not occur immediately at 3 minutes or between 10-15 minutes of the occlusion period.

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x Synthesized by GYKI(Budapest,Hungary) as B-GYKI-38233 and licensed to Toyobo(Osaka,Japan) as TYB-38233

THE BEHAVIOR OF CH-38083 ON PUTATIVE ALPHA-2 ADRENORECEPTOR SUBTYPES

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(CH-38083,alpha-2 adrenoceptor subtypes,h. platelet,neonatal rat lung)

CH-38083 is an antagonist with high selectivity and specificity on alpha-2 adrenoreceptors, Vizi et al., (1986).

However, there are several reports on the heterogeneity of alpha-2 receptors. It was shown that the displacement pattern of [^{3}H]Yohimbine by Prazosin can be characterized by two dissociation constants (K_{d1} = 7 nM; K_{d2} = 273 nM) in rat brain, i.e. two subtypes of alpha-2 adrenoreceptors were identified. According to the Prazosin binding characteristics these subtypes are present in equal ratio in the rat brain. Human platelets contain mainly the low affinity type (α_{2A}) and the neonatal rat lung has only high affinity sites (α_{2B}), Bylund (1985).

In the present work our purpose was:

1.) to identify by binding studies the putative alpha-2 adrenoreceptor subtypes in rat brain, neonatal rat lung and human platelet membrane preparations.

2.) to clear whether CH-38083 possesses subtype specificity on alpha-2 adrenoreceptors.

Rat brain (minus cerebellum) and neonatal rat lung membranes were prepared by homogenization in 20 vols of buffer (50 mM Tris/HCl; pH = 7.7, 25 ^{o}C, used throughout), filtration through cheese-cloth, centrifugation at 1000 g for 1 min and (2x) at 12000 g for 10 min. Human platelets were prepared according to the method of Enyedi et al., (1986). The membranes (0.2-0.4 mg protein) were incubated at 25 ^{o}C with 0.5 nM [^{3}H]Rauwolscine (Amersham, 85 Ci/mmol) for 45 min. Phentolamine (10^{-5} M)

was used to define the ratio of specific binding. The bound radioactivity was trapped on Whatman GF/C filters and washed with 3x5 ml ice-cold buffer. The compounds were tested in a concentrations range of 10^{-5}-10^{-11} M.
The experiments were evaluated by a non-linear curve fitting program, Batke and Gaál (1986).

The results are summarized in the following table:

TISSUE	[^{3}H]Rauwolscine Prazosin (K_d, nM)	CH-38083 (K_d, nM)
HUMAN PLATELET	2000±86	0.8±0.036
RAT BRAIN	250±25	2.7±0.17
RAT LUNG	90±3.3	1.0±0.04

Altogether,
in agreement with the results of Lanier et al, (1988) we were not able to identify high affinity binding sites to Prazosin in rat brain, only the α_{2A} type was detectable. Using human platelet and rat lung preparations it was possible to demonstrate binding sites of high and low affinity, revealing a considerable (22 x) difference.
However, CH-38083 was bound to the tested tissues with equally high affinity. Therefore, like other alpha-2 antagonists, CH-38083 does not have alpha-2 adrenoceptor subtype specificity.

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Changes of GTP binding in hormone induced opiate or adrenergic sub sensitivity

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First in 1983 it has been published that a prolonged treatment with glucocorticoids or ACTH decrease the opiate agonist induced pituitary hormone release (Kanyicska et al. 1983). The glucocorticoid induced decreased sensitivity is also demonstrable measuring 2 adrenergic responses. Prolonged stress situation mimiced the effects of prolonged gucocorticoid treatment (Fekete et al. 1984.a). Certain nonendocrine effects, as well as changes of dopamine metabolism induced by morphine were also inhibited by glucocorticoids (Fekete et al. 1984.b). The decreased effectivity of icv given opioid peptide agonists as well as receptor binding studies suggest glucocorticoid induced changes of receptor sensitivity, which could be demonstrated by selective kappa, mu and delta agonists measuring pituitary hormone release (Kiem et al. 1987). The daily alterations of endocrine responses evoked by opoid peptides run in dependence of the presence of adrenals in case of prolactin and ACTH/corticosterone release (Kiem et al. 1988), while in case of growth hormone release the daily rhythm seemed to be related to the testosterone release in male rats (Fekete et al. in press). Similarly the changes of adrenergic sensitivity under physiological conditions were

mainly attributable to the endogeneus testosterone (Kiem, Do, T. et al. 1990). In heterologue binding studies the high affinity binding site of alpha$_2$ adrenergic receptors was not demonstrable and the amount of low affinity binding increased following hydrocortisone treatment. The high affinity site reappeared in the presence of GTP, in contrast the high affinity binding was depressed by GTP in the control preparates. In case of mu opiate bindig DAGO-morphine competition has been measured to reveal the posible altered binding characteristics. The binding affinity was decreased by the addition of GTP to the assay medium of membranes derived from saline treated rats. This effect of GTP disappeared when measuring the opiate receptors in cortisol treated animals. It is suggested that the biochemical mechanism of hormone induced receptor alterations is mainly explainable by changes of GTP binding during the transfer of information by the certain mediators. Decrease of adrenocortical reactivity similar to that in glucocorticoid treated animals were described in depressed patients. We suggest that further investigation of the above described phenomena may lead to better understanding of biochemical mechanisms of illnesses.

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OPIATE PEPTIDERGIC NEUROTRANSMISSION AND CARDIOVASCULAR AND RESPIRATORY APPARATUS: EXPERIMENTAL RESEARCH WITH β-ENDORPHIN AND DERMORPHIN ON NORMOTENSIVE AND HYPERTENSIVE RATS

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Key words: β-endorphin, dermorphin, cardiovascular-respiratory apparatus

β-Endorphin and related opiod peptides are involved in both central and peripheral cardiovascular regulation (Donatelli and Marmo, 1981). The dermorphin are a class of opiods isolated from the skin of some South American frogs (Montecucchi et al., 1981) and from mammalian brain (Negri at al., 1981). The cardiovascular and respiratory effects of β-endorphin and dermorphin have been evaluated in freely moving or anaesthetized (natrium pentobarbital 25 mg/kg ip) male rats which were normotensive, spontaneously hypertensive and rendered hypertensive by DOCA administration. Icv injections (3rd and right lateral ventricles) of β-endorphin and dermorphin induced a transient arterial hypotension with sinus bradycardia. This was significantly antagonized by an iv pretreatment with naloxone, a competitive antagonist of μ and k receptors. ICI 174864, a selective δ- opiod receptor antagonist, did not reduce the cardiovascular effects of β- endorphin and dermorphin. Atrial natriuretic antipeptide IgG, iv administered 5 min before, considerably reduced the cardiovascular changes caused by icv β- endorphin and dermorphin. In hypertensive rats (both spontaneously and DOCA-induced) the cardiovascular effects of 2 opiate peptides were more intense and

lasted longer than those seen in normotensive rats.

Icv injections (3rd and right lateral ventricles) of β- endorphin and dermorphin elicited a significant respiratory depression which was antagonized by naloxone. In contrast, a 5 min pretreatment with ICI 174864 or with atrial natriuretic antipeptide IgG did not modify the respiratory depression triggered by β- endorphin and dermorphin.

In conclusion, the cardiovascular and respiratory depression induced by β-endorphin and dermorphin involve the activation of μ and k opiod receptors without any significant effect on δ- receptors. Since naloxonazine, an antagonist of μ_1-receptors, does not antagonize the respiratory depression caused by β- endorphin and dermorphin, it is likely that the respiratory effects seen in the 2 opiate peptides involve μ- receptors different from μ_1- receptors. In addition, the cardiovascular and not the respiratory effects caused by β- endorphin and dermorphin are also likely due to the release of atrial natriuretic peptide. Our findings show that dermorphin is about 20 times stronger than β- endorphin.

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SOMATOSTATIN AND COGNTIVE FUNCTIONS: BEHAVIORAL, NEUROCHEMICAL AND MOLECULAR BIOLOGY STUDIES

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Key words: somatostatin, aging, cognitive functions

Brain somatostatinergic neurotransmission has been reported to be greatly impaired in patients affected by the dementia of the Alzheimer type (Davies 1980). We evaluated the role of brain somatostatin in the modulation of cognitive function in the rat by means of behavioral, neurochemical and molecular biology approaches. For this purpose we used cysteamine (CSH) a thiol agent able to reduce the brain somatostatin content in both young and old rats. The administration of CSH (300mg/kg,sc) to a group of conditioned young rats (more than 90% of conditioned avoidance responses, CARs, for at least three consecutive days) caused a progressive reduction of CARs in both active and passive avoidance conditioning, showing a maximal efficacy after 4h from the injection. After 24h the deconditioning effects of CSH disappeared. The administration of somatostatin or its analog SMS 201-995 (5ug and 100ng/10ul,icv, respectively) almost completely abolished the deconditioning effects of CSH at 4h. This observation strongly suggests that the behavioral effects of CSH are primarily due to the impairment of somatostatinergic neurotransmission (Schettini 1988). In old rats CSH also reduced the acquired CARs, but the icv injection of SMS 201-995 did not revert the

CSH effect. The lack of recovery after somatostatin administration in the old rats could be related to the reduction in rat brain somatostatin receptors during the aging process, as previously reported (Sirvio 1987). We demonstrated that somatostatin receptors are coupled, in an inhibitory way, with the adenylate cycalse system in young rat brain (Schettini 1989). Here we reported an age-dependent alteration in the somatostatin receptor coupling with the adenylate cyclase enzyme. In aged rats, somatostatin inhibition of cAMP production both in the frontal cortex and in the hippocampus was significantly less marked than in the young animals in basal or stimulated conditions. Finally, we evaluated the expression of pre-prosomatostatin gene expression in the frontal cortex of 2, 6, 12 and 25 months old rats. The pre-prosomatostatin mRNA content increased from 2 to 6 and 12 months of age, but was greatly reduced in the 25 months old rats. In conclusion, our data show that the impairment of somatostatin neurotransmission caused impairment of cognitive functions in the rat, and that both the pre-prosomatostatin gene expression and the coupling of the somatostatin receptor to the intracellular effectors are altered during the aging process.

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DIFFERENT CHANGES ON HEPATIC DRUG-METABOLIZING SYSTEM CAUSED BY NON-STEROID ANTI-INFLAMMATORY DRUGS

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Key words: NASAIDS - Hepatic metabolizing system - Cyt. P-450.

In the course of studies of side effects caused by non-steroid anti-inflammatory drugs (NSAIDS), in the rat, we noted different changes in the hepatic and renal drug-metabolizing system. In fact, the treatment of rats with indomethacin led to decreases in the renal and hepatic enzymatic activities cytochrome P-450 dependent (Fracasso et al., 1987); the treatment of rats with piroxicam reduced the same parameters in the livers, even if in less dramatic extent, while the renal drug-metabolizing components were not affected (Fracasso et al., 1988). In the light of these results we have investigated the mechanism whereby these two NSAIDS induced different changes on hepatic drug-metabolizing system.

Male Sprague-Dawley rats (150-200 g) were treated for 3 days with an oral suspension of indomethacin (10 mg/kg) or piroxicam (50 mg/kg) or vehicle. The animals were killed by cervical dislocation 24 h after the third drug administration. The livers were homogenized with cold solutions and the hepatic microsomal fractions (105,000 g) were isolated by differential centrifugation following usual techniques. Cytochrome P-450, P-420 and b_5 levels in the microsomes were determined by the method of Omura and Sato (1964). The amount of complexed cytochrome P-450 was determined as the difference between the amounts of cyt. P-450 that were able to bind CO in microsomes before and after ferricyanide treatment. The total protohaeme content was determined by Omura and Sato (1964) as the pyridine haemochrome.

Indomethacin caused a significant loss in the liver of cyt. P-450 and b_5 (69% and 46% respectively compared to control group), no significant loss (30%) of

cytochrome P-450 was caused by the piroxicam treatment and b_5 content did not change. The reduction of cyt. P-450 levels in the two treatments was not due to the complexed form of hemoprotein, the treatment of microsomal suspension with ferricyanide did not modify the concentrations CO-binding cyt. P-450. The loss of the total protohaeme was consistently lower (45% vs control) after indomethacin than after piroxicam treatment (13% vs control); and moreover, a P-420 peak was detected in indomethacin- and piroxicam-microsomes, while this peak was not observed in any of the microsomal suspension of control.

The most likely interpretation of these findings is that, at these doses, the two drugs cause a different change in the hepatic drug-metabolizing system, in fact, piroxicam treatment reduces the cyt. P-450 only while indomethacin treatment induces an important decrease in both cytochromes (P-450 and b_5) and in the total protohaeme. Probably indomethacin treatment, leads in part to an inhibition of haemoprotein synthesis and in part to a conversion of cyt. P-450 to an inactive form so-called cytochrome P-420, while the piroxicam treatment causes denaturation of cyt. P-450 to cyt. P-420, a derivative deprived of functional activity. In fact we noted that the enzymatic liver activity was greatly affected by indomethacin than by piroxicam treatment.

Treatment	% Loss			Enzymatic activity
	P-450	Tot. haem.	b_5	
Indomethacin	69	45	46	↓↓
Piroxicam	30	13	0	↓

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"IN VITRO" CYCLOSPORIN AND METHYLPREDNISOLONE INTERACTION WITH CYTOCHROME P-450.

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Key words: Cyclosporin - Methylprednisolone - Cyt. P-450 - Interaction.

Cyclosporin (CsA) is a powerful immunodepressive agent which has been demonstrated useful in a wide variety of clinical situations, particularly in the field of organ transplantation. Moreover, post-trasplant patients rarely receive CsA alone but require multiple drug therapy. An important cause of side effects of CsA results from drug interactions. Indeed it has been demonstrated that CsA is extensively metabolized in the liver of both animals and man (Maurer et al., 1984). The many potential sites for oxidation by cytochrome P-450 lead to the speculation that CsA could interfere with other drugs which are metabolized by system P-450 dependent. Particularly conflicting are the data concerning the interaction between CsA and glucocorticoids.

Following the observation (Fracasso et al., 1989) that "in vivo" CsA reduced the hepatic metabolizing system P-450 dependent and that MTP restored this function, we studied the "in vitro" interaction of these two drugs with cyt. P-450 using hepatic microsomal preparations from MTP- and PB-treated rats.

Hepatic microsomal fractions (105,000 g) were isolated by differential centrifugation following usual techniques. Cytochrome P-450 levels in microsomes were determined by the method of Omura and Sato (1964). The binding spectra of CsA and MTP with P-450 were carried out on rat liver microsomes from control animals, pretreated with phenobarbital (80 mg/kg x 3 days) or MTP (0.7 mg/kg x 3 days). The binding spectra of CsA (50 μM) and MTP (100 μM) were measured in a suspension of microsomes containing 2.0 μM of cyt. P-450. Various amounts of drugs were added in 10 μl dimethyl sulfoxide to microsomal suspension in the sample cuvette. Binding spectra were

recorded between 510 and 360 nm in a Perkin-Elmer Lambda 5/15 dual beam spectrophotometer.

The binding spectra obtained upon addition of CsA (50 μM) to PB-microsomes showed that CsA-cytochrome P-450 complex was located at 390 nm and the absorbance minimum at 420 nm. Thus, by convention, CsA can be classified as a type I compound. The binding spectra obtained upon addition of MTP (100 μM) to PB-microsomes showed a reverse type I spectral change. The apparent Ks (spectral dissociation constant) and Δ OD_{max} (maximum spectral change) for CsA were 0.017 and 0.0092 mM respectively in microsomes isolated from MTP-rats. A double reciprocal plot of Δ 420-390 nm/nmol P-450/ml against MTP concentrations revealed a Ks= 0.053 mM and a Δ OD_{max}= 0.0186.

CsA interacts directly with ferricytochrome P-450 leading to spectral change of type I (λ_{max}= 390 nm, λ_{min}= 420 nm), whereas MTP exhibits a reverse type I spectral change; hence the two drugs do not compete for the same hydrofobic binding site of cytochrome. CsA shows a Ks value lower with P-450-PB than P-450-MTP. This means that CsA "in vitro" shows higher affinity for PB-cytochromes P-450 (P-450_b and P-450_e of rat liver) than that shown for MTP inducible cyt. P-450 (glucocorticoids-inducible P-450_p). "In vivo", following the combined treatment (CsA + MTP), CsA increases its own metabolism using the induced MTP-cyt. P-450. The above process accelerates the metabolism of CsA into products which are non-toxic to the metabolizing system P-450 dependent. Following these studies we could say that, "in vitro", there is a different type of interaction between CsA and MTP with cytochrome P-450 in the rat liver.

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40% BUT NOT 20% ETHANOL IS AN IRRITANT ABLE TO EVOKE GASTRIC RELEASE OF PGE2 IN MAN.

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Key Words: cytoprotection/prostaglandins/ 20% and 40% alcohol.

Studies in the rat have shown that gastric mucosa can be made more resistent to acute exposure to potent necrotizing agents e.g. 70% and 100% alcohol, if previous contact with a mild irritant (20% alcohol) is made (Robert 1979). This defensive phenomenon called "adaptative cytoprotection" seems mediated by endogenous prostaglandins (PGs) (Robert 1983). Recently, the role of prostaglandins has been questioned, since it has been demonstrated in the rat that the pre-administration of 20% alcohol, does not increase the release of PGs (Hawkey 1988). Aim of this study was to verify in healthy volunteers and in chronic alcoholics, if acute exposure to alcohol at two concentrations (20% and 40%) could modify the levels of gastric PGE2 and whether chronic prior exposure of gastric mucosa to alcohol could influence this response.

We studied 10 healthy volunteers (9 men and 1 woman: mean age 28.2±2 years) and 11 chronic alcoholics (10 men and 1 woman: mean age 44.9±10.2 years) with daily alcohol intakes of > 160 g/day for al least two years, not affected by cirrhosis. They were submitted to the following experiment: collection of gastric juice (90 min) during i.v. infusion of pentagastrin (0.75 mcg/kg/hr); sample 0-15' not considered; 15-20': intragastric administration of 100 ml of saline and collection of juice up to 45'; 45-60': wash out; 60-65': intragastric administration of 100 ml of 20% or 40% ethanol and collection of the juice up to 90'.PGE2 was assayed in gastric juice using a highly sensitive PGE2 ^{125}I RIA kit (DuPont). Results of gastric PGE2 assay were expressed as nanograms per output (ng/30'). Statistical analysis was performed by Student's t test.

The results indicate that acute intragastric administration of 20% alcohol was not able to provoke gastric release of PGE2 in healthy volunteers (after saline: 35.31±10.84 ng/30'; after 20% alcohol: 37.88±12.42 ng/30', P: N.S.). Howerver 40% alcohol was found to be a potent stimulant of gastric PGE2 production (after saline: 38.34±10.40 ng/30'; after 40% alcohol: 85.18±33.95 ng/30', $P<0.01$). The behaviour of gastric prostaglandins in chronic alcoholism, both at basal levels and after acute alcohol stimulation, appears to be substantially different from that observed in healthy non alcoholics. Infact, PGE2 levels in the basal gastric juice of alcoholic subjects were 2 to 3 times higher than those found in healthy subjects and were not modified following acute exposure to 20 and 40% alcohol (after saline: 112.01±70.51 ng/30'; after alcohol 20%: 106.06±83.96 ng/30', P: N.S.; after saline:79.94±52.89 ng/30'; after 40% alcohol: 87.84±45.83 ng/30', P: N.S.).

Our findings suggest that while acute alcohol exposure in the human stomach can evoke a PGE2 response, it does so only in undamaged mucosa (healthy subjects) and in the presence of alcohol concentrations (40%) twice those considered mild irritants in experimental animals. Long term exposure to alcohol, as occurs in chronic alcoholics, produce elevated intragastric PGE2 levels, which are not subject to further increase. This could indicate a gastric mucosal functional adaptation to chronic alcoholic abuse.

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THE IMPORTANCE OF POSITION SIX IN THE RING C OF MORPHINE IN MODULATION OF AGONIST/ANTAGONIST INTERACTION WITH MULTIPLE OPIATE RECEPTORS

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Key words: opiate receptors, chemical modification, azidomorphines

A large body of evidence has accumulated suggesting that, the mode of interaction with the receptor/s/ is largely the function of the chemical constitution and geometric disposition of groups interacting with opiate receptors of morphinomimetics. 6--deoxy-6-azidodihydroisomorphine (AM) and its 14-OH derivative are extremely potent analgesics, as it was previously reported by us (Knoll,Fürst,Kelemen,1973). Analogs in this family were synthetized with various N-substituents: methyl, allyl, cyclopropylmethyl (CPM). The results obtained show, that while the formal inversion of the 6-OH group of morphine to α-isomorphine or α-isocodeine does not unduly alter the analgesic properties, the 6-methyl, 6-ethyl or 6-butyl analogues are modest antinociceptive agents. The significance of the linear azido-group which has a fixed conformation, and that of its steric (β) orientation is well documented by the fact, that dihydroisomorphine proved to be much weaker analgesic than AM (hot plate ED_{50} > 20 mg/kg and 0.023 mg/kg, resp.). The 6-azido-substitution and the subsequent conformational change induced in ring C seemed to be important for the analgesic activity not only in the case of 3-OH derivatives, but also in 3-ethered members, since they proved to

be 40 times and 50 times more active analgesics, than the parent molecules (codeine and ethylmorphine, resp.). Furthermore, our experiments strongly suggested, that AM interacted with mu receptor, since its agonist effects are readily antagonized by naloxone (AD_{50} estimated in algolytic test: 0.037 mg/kg) both in vivo and in vitro (isolated guinea pig ileum and mouse vas deferens) and also it was supported by the receptor binding study of Horvath and Wollemann (1986). Furthermore, AM appeared to us a pure agonist, since we were not able to detect any antagonist activity. Furthermore, among the N-substituted azidomorphines we have found strong antagonists (Knoll,Fürst,Makleit,1977) as N-allyl-norazidomorphine and its 14-OH derivative, when estimating their antagonist activity in oxymorphone righting test (AD_{50}=0.031 and 0.033 mg/kg,resp.). When N-CPM was the substituent, we have observed a kappa agonist activity, which was demonstrated by diuretic action, by the strong antinociceptive effect in mouse acetic acid stretching test, or agonist activity in rabbit vas deferens, which is devoid of other opiate receptors, than kappa ones etc (Fürst, Friedmann,Knoll,1988). Moreover, this compound showed strong mu receptor antagonist activity not only in analgesia (AD_{50} against morphine in the algolytic test: 0.035 mg/kg) but in catalepsy, respiratory depression as well. The simultaneous 14-OH-substitution in this structure resulted in a marked reduction of agonist type actions, while retaining strong antagonist efficacy.

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COMPARISON OF THE CARDIOPROTECTIVE EFFECT OF NITROGLYCERIN, MOLSIDOMINE AND SIN-1 IN RATS WITH CARDIAC HYPOXIA

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(cardioprotection, hypoxia, nitroglycerin, molsidomine, SIN-1)

Nitroglycerin, molsidomine and its active metabolite SIN-1 have been widely used as antianginal agents. Molsidomine is an effective long-acting, and SIN-1 is a very strong-acting antianginal drug i.v.

We tested the relative cardioprotective potencies and time course of nitroglycerin, molsidomine and SIN-1 in the rat isoproterenol (ISO) model according to Körmöczy et al., (1987).We evaluated the developed hypoxia quantitatively by histological methods. To follow the time course of cardioprotection nitroglycerin, molsidomine and SIN-1 were injected daily i.p., molsidomine 60, 120 and 240 min, and i.v.SIN-1 60 min before ISO to groups of ten rats, respectively. Cardioprotection was defined as the reduction of hypoxic areas, and was expressed as percentage change compared to the control saline treated group.

The comparative cardioprotective effect of nitroglycerin, molsidomine and SIN-1:

Substance	dose mg/kg	pretreatment time (hour)	mean % of hypoxic area	S.D.	delta %
control	-	1	36.7	4.3	-
nitroglycerin	1	1	11.5	2.9	64.8^{x}
		2	14.9	3.2	59.4^{x}
		4	22.3	4.4	39.2^{x}

Substance	dose mg/kg	pretreatment time (hour)	mean % of hypoxic area	S.D.	delta %
molsidomine	2	1	12.6	2.5	65.5^{x}
		2	8.2	5.0	77.7^{x}
		4	15.6	3.1	57.4^{x}
SIN-1	0.25	1	1.7	1.1	95.5^{x}

The values represent percentage change in hypoxic areas compared to control: x = $p < 0.001$

Nitroglycerin and molsidomine showed nearly equipotent cardioprotective effects but the effect of molsidomine was more prolonged. Treatment with SIN-1 prevented the development of cardiac hypoxia.

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POSSIBLE INVOLVEMENT OF THE ANTISEROTONINERGIC AND ANTIHISTAMINERGIC ACTIVITY IN THE ANTICATALEPTOGENIC EFFECT OF EGYT-2509

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Keywords: atypical neuroleptic, EGYT-2509, noncataleptogenic

In animal experiments it was revealed that EGYT-2509 is a new atypical neuroleptic agent. The putative therapeutic gain of the compound is the abscence of extrapyramidal and endocrine side effects (Gacsályi et al., 1988). In rodents it showed no cataleptogenic activity, moreover it inhibited the catalepsy provoked by different neuroleptics (haloperidol, perphenazine). The molecule posseses moderate central antihistaminergic, and considerable central antiserotoninergic activity. It was found that drugs with marked central antihistamine , antiserotonin and/or anticholinergic activity inhibited the catalepsy induced by neuroleptics (Balsara et al., 1979). These findings were supported by our experiments (Table).

Table. Pharmacological profile of some anticataleptogenic drugs

DRUG	ANTICAT. ACT. ID50 MG/KG PO. (haloperidol)	OXOTRM.ANT. ID50 MG/KG PO.	RECEPTOR BINDING Ki (nM)	
			H1	S2
EGYT-2509	43.8	105	18.26	156.4
CHLORPROMAZINE	-	4.13	195.27	15.17
CLOZAPINE	14.8	13.2	8.60	16.0
PROMETHAZINE	38.2	36.3	15.05	144.89
CYPROHEPTADINE	9.09	2.43	6.7	0.85
SETASTINE	40.02	162.6	70.1	9338.0

The compound EGYT-2509 antagonizes the oxotremorine induced tremor only in higher doses (ED50=105 mg/kg po.), which indicates low anticholinergic potential. This result suggests that anticholinergic action may not play a role in the anticataleptogenic activity of the compound.

On the basis of our findings we suggest that the central antiserotonin and antihistamine activity of EGYT-2509 may be responsible for the absence of unwanted extrapyramidal side effects.

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COMPARISON BETWEEN THE EFFECTS OF TETRODOTOXIN AND OF COOLING ON GUINEA-PIG ILEUM CONTRACTIONS INDUCED BY VARIOUS AGENTS

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Key words: tetrodotoxin, temperature, smooth muscle, intestine

INTRODUCTION

Tetrodotoxin (TTX) is widely used to identify nerve-mediated effects of drugs on guinea-pig ileum and other intestinal preparations. This toxin, by acting on Na^+ channels, blocks the conduction of action potentials in nerve cells, without affecting the electrical and mechanical activity of smooth muscle cells (Gershon, 1967). Similarly low temperature, by a still unidentified mechanism, affects all nervous structures of the ileum, thus preventing neurotransmitter release (Innes, Kosterlitz and Robinson, 1964).

The aim of the present study was to make a direct comparison between the influence of TTX and of low temperature on the responses of guinea-pig ileum to various agents that are known to exert direct and/or nerve-mediated effects in this preparation. For this purpose acetylcholine (ACh), 5-HT, PGI_2 and KCl were used to contract intestinal smooth muscle.

METHODS

Segments of the terminal portion of guinea-pig ileum were mounted in organ baths containing 10 ml of Tyrode solution of the following composition (mM): NaCl 136, KCl 2.7 $CaCl_2$ 1.4, $MgCl_2$ 0.49, NaH_2PO_4 0.32, $NaHCO_3$ 12 and glucose 5. The bath temperature was 37°C. In low temperature experiments concentration-response curves were first obtained at 37°C. The preparations were then allowed to equilibrate at 20°C for 60 min before repeating the same treatment. All concentration-response curves were obtained cumulatively, except for PGI_2 that is very unstable in aqueous solutions. TTX was added to the bath fluid 10 min before treatment with the agonists. Other details of the experimental procedure have been previously described (Gaion and Trento, 1983; Gaion and Gambarotto, 1987).

RESULTS AND DISCUSSION

ACh contracts guinea-pig ileum by stimulating muscarinic receptors on smooth muscle cells. TTX (0.5 μM) did not cause any significant change in the concentration-response curve for ACh (0.01-2 μM). Similarly, in preparations maintained at 20°C, contractions induced by ACh were comparable to those obtained under control

conditions (37°C).

5-HT increases ACh and substance P release from enteric neurons. Moreover, at higher concentrations, it interacts also with specific smooth muscle receptors (Buchheit, Engel, Mutschler and Richardson, 1985). The contractile effect of 5-HT (0.01-10 μM) was markedly depressed both by TTX and by low temperature.

Contractions induced by submaximally effective concentrations of PGI_2 (0.005-0.1 μM), that act by increasing ACh release from enteric neurons (Gaion and Trento, 1983), were abolished by TTX and by cooling. With both treatments a residual, quantitatively comparable response was obtained at PGI_2 concentrations of 1 μM and higher, that exert also a direct effect on smooth muscle (Gaion and Gambarotto, 1987).

KCl, by depolarizing cell membranes, allows the entrance of Ca^{2+} into nerve terminals and smooth muscle cells, thus triggering neurotransmitter release and contraction, respectively. TTX inhibited contractions evoked by 5-30 mM KCl. The extent of this inhibition was inversely related to the KCl concentration used and maximum contractions induced by 50 mM KCl were insensitive to TTX. By contrast, in preparations maintained at 20°C the effect of KCl was potentiated at the lowest salt concentration (5 mM) and markedly inhibited at the higher ones. As a result, in these preparations the concentration-response curve for KCl was flat.

These results indicate that TTX and low temperature do not impair the ability of smooth muscle to contract in response to ACh and that they exert comparable inhibitory effects on contractions induced by agents that cause a receptor-mediated increase of propagated activity in neurons. When transmitter release occurs as a result of membrane depolarization, TTX blocks only impulse propagation along axonal structures, while low temperature may counteract the effect of KCl also at nerve endings, possibly by reducing neuronal Ca^{2+} availability.

The mechanism by which low temperature potentiates contractions induced by 5 mM KCl remains to be elucidated.

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ESORUBICIN CARDIOTOXICITY IN VITRO: ANTAGONISM BY PIMOBENDAN

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Key words: Esorubicin - Cardiotoxicity - Guinea pig atrium - Pimobendan

Most of the pathological alterations induced in human heart by anthracycracy have been reproduced in animal models for anthracycline cardiac toxicity: myocardial alterations have been reported to occur following administration of single high dose of anthracyclines in animals and in isolated heart models in vitro (Taylor A.L. et al., 1984).

In attempts to alleviate or prevent anthracyclines toxicity we have recently reported that amrinone and sulmazole (Bossa R. et al., 1986; 1988), new cardiotonic agents, markedly reduced the negative inotropic effect of adriamycin in isolated guinea pig atria in normodynamic and hypodynamic conditions.

The present study reports the effects of pimobendan, a close analogue of sulmazole (Wetzel B. et al., 1988), on guinea pig atrium treated with esorubicin, a newly synthetized adriamycin derivative (Cummings J. et al., 1987).

The experiments were carried out on electrically driven (3 Hz, 5 msec, at supramaximal voltage) left atrium of guinea pigs. Exposure for 60' to esorubicin (100 ug/ml) caused a depression of contractile force and of maximal rate of tension development (df/dt) on normodynamic and hypodynamic preparations. After addition for 5' of pimobendan (30 ug/ml) the contractile force and the df/dt of atrium increased to the value of basal condition; there is a partial recovery in normodynamic conditions and a full recovery in hypodynamic conditions (Table I).

Like amrinone and sulmazole, pimobendan antagonizes the cardiac toxicity of anthracyclines; since these compounds exert inotropic effects probably

through calcium influx, the protective activity against toxic effects of anthracyclines is consistent with a mechanism involving calcium movement.

Table I: Effect of esorubicin on the contractile force and maximal rate of tension development of electrically driven left atrium of guinea pig: antagonism by Pimobendan.

Compound	Conc ug/ml	N	a 0'	b 0'	30'	60'	c Pimobendan 30 ug/ml
Esorubicin	100	5	cf 1.16±0.12		××d 70.6±8.8	×××d 59.0±9.1	×××f 82.2±10.4
		5	cf 0.91±0.18	53.0±19.8	××e 35.2±13.8	×e 31.0±9.6	×××f 60.8±7.1
Esorubicin	100	5	df/dt 25.60±2.68		××d 67.8±9.2	×××d 56.8±8.6	×××f 80.1±10.2
		5	df/dt 20.03±3.39	55.7±18.7	××e 36.2±11.9	××e 30.7±9.2	×××f 60.9±5.6

cf: contractile force; df/dt: maximal rate of tension development: absolute value (g and g/sec) at 0' time in normodynamic conditions and % value of it in hypodynamic conditions and after drug treatment. Esorubicin was added at 0' time in normodynamic or hypodynamic conditions; Pimobendan was added 60 min after antitumor drug. Data are expressed as mean ± S.D. and analyzed by Student's t-test. N: number of preparations; a: normodynamic conditions (Tyrode with CaCl2 0.2 g/l); b: hypodynamic conditions (Tyrode with CaCl2 0.1 g/l); c: cf and df/dt were measured 5' after Pimobendan administration; d: statistic significance is referred to 0' time in normodynamic conditions; e: statistic significance is referred to 0' time in hypodynamic conditins; f: statistic significance is referred to 60' time; × $p<0.05$, ×× $p<0.01$; ××× p 0,001

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PREVENTION OF DIISOPROPYLFLUOROPHOSPHATE (DFP)-INDUCED LETHALITY BY MEPTAZINOL AND 9-AMINO-TETRAHYDROACRIDINE (THA) IN THE MOUSE.

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Key words : acetylcholinesterase; meptazinol; 9-amino-tetrahydroacridine.

We have recently demonstrated that the opioid analgesic meptazinol is endowed with considerable protective activity against acute DFP poisoning in the mouse (Galli and Mazri 1988). In the present study we have extended our investigation to the anticholinesterase agent THA and we have compared the protective effects of the two compounds with those, well documented, of the carbamates physostigmine and pyridostigmine (Gray 1984).

Table 1. Protection against DFP-induced lethality in the mouse

Pretreatment	(mg/kg)	Protection ratios (95% confidence limits)	
		Without atropine	With atropine
Physostigmine	(0.1)	2.2 (1.9-2.5)	9.2 (8.3-10.1)
Pyridostigmine	(0.1)	1.3 (1.0-1.5)	8.1 (7.4-9.0)
Meptazinol	(30)	4.8 (4.4-5.3)	4.7 (4.2-5.3)
Meptazinol	(45)	9.7 (8.2-11.5)	8.1 (6.8-9.8)
THA	(5)	3.1 (2.7-3.3)	8.1 (7.2-9.1)

Protection ratio = DFP LD_{50} (treated animals) / DFP LD_{50} (untreated animals). Pretreatment was carried out 15 min before poisoning. Atropine sulfate was 17.4 mg/kg.

The results in table 1 show that under our experimental conditions both meptazinol at high doses and THA exerts a protective action against DFP intoxication comparable for intensity to that of physostigmine and pyridostigmine. However, while meptazinol's action is not affected by the co-administration of atropine, that of the other protective agents

is clearly increased by this drug.

In separate experiments, the time-course of acetylcholinesterase (AChE) activity recovery was evaluated in whole brain and diaphragm tissues of mice pretreated with meptazinol (30 mg/kg), THA (5 mg/kg), physostigmine (0.1 mg/kg) together with atropine sulfate (17.4 mg/kg) 15 min before poisoning with DFP (8 mg/kg). At 10 min after DFP administration, residual AChE activity in whole brain tissue averaged 8, 49, 29 and 23% of controls in the animals pretreated with atropine alone (unprotected animals), meptazinol, THA and physostigmine, respectively. At 24 hours after poisoning, brain AChE activity averaged 39, 56 and 53% of controls in the mice protected by meptazinol, THA and physostigmine, respectively. The data from the diaphragm closely paralleled those from the brain.

On the whole these data point to different protective mechanisms for meptazinol and the carbamates physostigmine and pyridostigmine. Our results appear to be consistent with meptazinol being able to exert a short-lasting shielding action on AChE against DFP attack. The experiments carried out so far do not permit us as yet to offer definite interpretations of THA protective action.

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COMPARISON OF THE ULCER HEALING EFFECT OF DIFFERENT CYTOPROTECTIVE AND ANTISECRETORY DRUGS IN GASTRIC ULCER PATIENTS

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Key-words: ventricular ulcer, ulcer planimetry, protective agents

The deasease peptic ulcer does not represent a uniform clinical entity. Many factors - known and unidentified - can be incriminated in the development of peptic ulcer. It also seems to be evident that the natural history of ventricular and duodenal ulcer is different. Caused by these facts there is no uniform therapeutic response.

A continous work has been done in our institute to compare the ulcer healing effect of different antisecretory and protective agents in experimental ulcer models as well as in ventricular and duodenal ulcer patients. The authors summarize their results in the field of the treatment of ventricular ulcer patients by comparing the ulcer healing effect of cimetidine (1000 mg/day), Pirenzepine (150 mg/day), Sucralfat (1000 mg/day), vitamin A (50 000 I.U./day) alone and in combination with cyproheptadinum chloratum, De-nol (3X5 ml/day) and Tisacid (Al-Mg antacidum in different doses). Patients were randomly allocated in the different treatment groups. A detailed case report preceeded upper gastrointestinal endoscopy. Gastroscopy with biopsy was repeated in every two weeks. Size of ventricular ulcer was recorded and a list of complaints and possible side effects were registered by the patients. Different laboratory examinations were carried out on the day of endoscopy. Pharmacodynamic effect of the drugs was compared by the mentioned parameters.

It has been found that:

1. Among the agents listed above De-nol resulted in the highest complete healing rate after four weeks; 2. Pharmacodynamic effect of different drugs was compared by measurement of ulcer size in incompletely healed patients after two weeks: vitamin A with and without cyproheptadinum chloratum and De-nol treatment significantly increased the speed of ulcer healing in comparison to the less effective antacid group; 3. Vitamin A was also able to decrease ulcer pain as well as Tisacid which is an important aspect of drug treatment from the clinical point of wiew. 4. Antacid consumption was much higher in cimetidine-group, than in other treatment groups. Higher healing rate in cimetidine group may be a combined effect of cimetidine and antacids.

It has been concluded that the gastric cytoprotective agents (De-nol, vitamin A, Tisacid) have superior ulcer healing effect than antisecretory compounds (cimetidine,Pirenzepine)

THE ACTION OF ANGIOTENSIN II ON THE ARACHIDONATE CASCADE OF BRAIN MICROVESSELS

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Key words: angiotensin II, arachidonate cascade, microvessels

Introduction

Evidence indicates that various prostanoids participate in the vascular and renal mechanisms controlling blood pressure. Prostaglandins (PGE_2, PGD_2 and PGI_2) subsurve antihipertensive processes by opposing pressor mechanisms that bring about vasoconstriction and conservation of salt and water. On the other hand, thromboxane A_2 (TxA_2) causes vasoconstriction, PGF_{2alfa} reduces venous complience, and PGE_2 and PGI_2 stimulate renin secretion. This dual nature of prostanoids in blood pressure control seems to be very important mechanism (Mistry and Nasjletti, 1988). Arachidonic acid can be metabolized in addition to the cyclooxygenase enzyme by the lipoxygenase pathway resulting in the release of metabolites which might have regulating function in cellular responses. The present study was designed to investigate the effect of angiotensin II on the arachidonate cascade of rat brain microvessels.

Methods

Blood-free cerebral cortex microvessels were isolated from male rats of CFY strain, weighing 160-180 g by a micromethod of Hwang et al. (1980) with modifications. An aliquot was examined for purity and viability.

The isolated microvessels were preincubated in TC Medium 199 at 37 ^{o}C for 10 min and then angiotensin II (10^{-11} 10^{-5} M) was added to the incubation mixture, ten minutes later the enzyme reaction was started by the addition of ^{14}C-arachidonic acid (3.7 kBq) as tracer substrate to the incubation mixture. Radioactive arachidonate metabolites were separated an quantitatively determined in a Beckman LS 1800 liquid scintillation counter.

Results and Discussion

The lipoxygenase pathway dominated the arachidonate cascade in the microvessels isolated from the brain cortex of rats. The main cyclooxygenase products were 12-hidroxyheptadecatrienoic acid (12-HHT), PGD_2, TxB_2, followed by PGF_{2alfa}, PGE_2 and 6--keto-PGF_{1alfa}. It is accepted, that 12-HHT elevates arachidonate release from endothelial cells and stimulates prostacyclin synthesis (Sadowitz et al. 1987). Angiotensin (10^{-7} and 10^{-6} M) significantly inhibited the formation of 12-HHT in brain microvessels, which might result in less prostacyclin synthesis. Similar results were observed determining the released TxB_2 quantity from the microvessels in the presence of angiotensin II (10^{-7} and 10^{-6} M). The ratio of lipoxygenase and cyclooxygenase metabolites was not modified by angiotensin II (10^{-11} and 10^{-10} M) while 10^{-9} M concentration significantly reduced the ratio.

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A NEW IMMUNE-MODULATORY SUBSTANCE:CH-27584

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(Immunstimulation, antitumor acitvity, CH-27584)

CH-27584 exerts both tumor cell inhibitory and immunstimulant activities. It enhances antibody production, stimulates T, K and NK cell activity and also affects the function of phagocytes. This complex action may be a result of the stimulation of the early steps of lymphocyte activation. The exact mechanism of the compound has yet to be elucidated.

We tested the immune modulatory activity of CH-27584 in vivo in animal experiments and also in vitro on human lymphocytes. The compound dose-dependently increases:

1/ NK and ADCC activity, the increase is more striking in cases with depressed cytotoxic activity

2/ the migration of leukocytes

3/ mitogen (PHa- and ConA-) induced lymphocyte blastogenesis

4/ the primary and secondary IgM and IgG antibody response

5/ restores immune activity suppressed by cortisone

CH-27584 reduces the number of K-562 and P-388 tumor cells in culture. The effect is due to direct antitumor activity. It has only slight inhibitory action on NK-ly ascites lymphoma, but the immuno genic Lewis-lung tumor is inhibited very

strongly. The effect reflected in the lover number of metastases.

The compound strongly inhibits the spontaneous mammary tumors of dogs.

The oral therapeutic doses are ranging from 0.01-1 mg/kg, and the oral acute LD_{50} 2380±320 mg/kg in mice.

REPEATED ADMINISTRATION OF (-)DEPRENYL LEAVES THE MESOLIMBIC DOPAMINERGIC ACTIVITY UNCHANGED

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Key words: (-)deprenyl, dopamine uptake, dopamine turnover, tuberculum olfactorium

(-)Deprenyl facilitates the activity of the nigrostriatal dopaminergic system with high selectivity (Knoll, 1987). The deprenyl-induced increase of the dopaminergic tone in the striatum was proved by measuring several biochemical parameters of the activity of the nigrostriatal dopaminergic neurons. It was found that in the striatum taken from rats pretreated with 0.25 mg/kg (-)deprenyl for 30 days, the dopamine content and the turnover rate of this biogenic amine was significantly higher than in saline-treated animals. It was also shown that in deprenyl-pretreated rats the uptake of dopamine was significantly decreased.

We checked now the change of these parameters in the limbic system in (-)deprenyl-treated rats, using tuberculum olfactorium as an indicator.

Rats were treated daily with 0.25 mg/kg (-)deprenyl or saline, respectively, for 30 days and the tuberculum olfactorium was prepared for biochemical analysis 24 hours after the last injection. The result of the study are summarized below:

Treatment	DA ug/kg	DOPAC ug/g	HVA ug/g	TR_{DA} ug/g/h	DA uptake ug/min/mg protein
SALINE	10.76±0.3	2.15±0.14	0.72±0.02	0.72±0.02	7.85±0.46
(-)Deprenyl 30x0.25 mg/kg	11.88±0.75	2.37±0.15	0.79±0.05	0.79±0.05	7.39±0.26

These results substantially support the suggestion that (-)deprenyl facilitates the activity of the nigrostriatal dopaminergic system with high selectivity.

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NEUROLEPTIC PROFILE IN BEHAVIORAL TOXICOLOGICAL STUDY

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Keywords: behavioral toxicology, neuroleptics, profile

Behavioral toxicological studies carried out in our laboratory are based on the methods of Irwin (1968), Campbell and Richter (1967), and Alder et al. (1986) with several modifications. The standard procedure finally adapted by us is as follows:

Mice of both sexes are treated intraperitoneally with the test compound 30 min before observation and registration of behavioral symptoms by the means of appropiate checklists. At least three consecutive doses are chosen from the ...-3-10-30-100-... dose array according to the following guidelines: 1. the maximum dose is 300 mg/kg 2. there must be found a dose with at least 50 % lethality (unless it would exceed the 300 mg/kg limit) 3. there must be found at least one non-lethal dose. The registration of the symptoms has a sequence of three phases: 1. observation in the cage 2. observation on the table 3. examination in hand.

Forty-eight behavioral variables and lethality are recorded. The variables are of three types: 1. present/absent parameters (registered as 0 or 1) 2. uni- or bidirectionally rated parameters (maximum range is -2/+2) 3. descriptive variables (denoted by a letter). The zero point score represents the normal behavior. Lethality is determined one hour, 24 hours and 7 days after drug administration. The observed behavioral symptoms are pooled into seven groups (Table).

Neuroleptic compounds (chlorpromazine, levomepromazine, haloperidol, clozapine, thioridazine and pimozide) showed a

characteristic behavioral symptom-profile on the basis of whic they could be distinguished from other psychopharmacons.

Table. Behavioral symptoms of neuroleptic compounds

1. SMA	2. stereotyped behavior	3. arousal	4. neurologic dysfunctions
marked decrease	none	marked decrease	abnormal gait and body posture, decr. muscle tone

5. sensory-motor functions	6. muscular functions	7. vegetative and other symptoms
impaired visual-motor coordination	severe impairment	abnormal respiration hypothermia, ptosis

Effects on SMA and arousal variables can be considered as the "main effect" of neuroleptics. The order of potency to elicit these symptoms was haloperidol = clozapine = levomepromazine > chlorpromazine = thioridazine > pimozide. However, obviously toxic symptoms such as neurologic dysfunctions and impairment of muscular functions and visual-motor coordination appeared in the same dose like in which the "main effect" symptoms did - in the case of all molecules.

These findings indicate that in behavioral toxicological studies neuroleptics can be distinguished from other psychotropic drugs, but the method is too robust to differentiate between these compounds either by their potency or by their therapeutic range.

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THE EFFECT OF DIFFERENT OPIATES ON ACIDIFIED ETHANOL-INDUCED GASTRIC LESIONS IN RATS

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Key words: opiates, gastric damage due to ethanol

Contadictory data are pulished on the effect of morphine on different types of gastric damage. Morphine was found partly to protect (Arrigo-Reina and Ferri, 1980) partly to aggrevate (Gyires et al., 1985) the different experimental mucosal lesions. Recently we found that morphine inhibited the acidified ethanol--induced gastric damage (Gyires and Knoll, 1988). In the present study we examined the effect of morphine, the relatively most selective ligand of μ-opiate receptors, bremazocine, a kappa--opiate receptor agonist and (D-Met^2-Pro^5) enkephalinamide (Bajusz et al., 1977), which binds both to μ and δ receptors (Chang et al., 1989).

We found that both morphine and (D-Met^2-Pro^5) enkephalinamide (DMPEA) were highly effective against acified ethanol induced lesions, the ID_{50} values are 0.07 and 0.04 mg/kg s.c., resp. Morphine was effective given both orally and s.c., while DMPEA was effective only in the case of s.c. administration. Bremazocine (0.01-1 mg/kg s.c. and p.o.) failed to produce any significant, dose-dependent inhibition on gastric lesions.

In the presence of naloxone (0.5 mg/kg, s.c.) the 82% and 88% inhibition of the gastric lesions due to 0.5 and 1 mg/kg s.c. morphine was reduced to 33 and 25%. The inhibitory activity of

DMPEA was less affected by naloxone (0.5 mg/kg, s.c.); the 97% and 93% inhibition induced by 0.25 and 0.5 mg/kg DMPEA was decreased only to 56% and 64%. When morphine (1 mg/kg s.c.) and bremazocine (0.5 mg/kg, s.c.) were combined, bremazocine significantly inhibited the action of morphine. This result is in accordance with the findings that bremazocine exert some μ receptor antagonist activity, too (Leander, 1983).

The protective action of both morphine and DMPEA was higly reduced by indomethacin (10 mg/kg, p.o.) and the sulfhydryl blocking, N-ethylmaleimid (50 mg/kg, s.c.).

These results suggest that the protective action of morphine against ethanol-induced lesions is mediated via opiate receptors. Kappa receptors seems to be not involved in the mucosal protection. Both protaglandin and sulfhydryl system might be involved in the protective action of both morphine and (D-Met^2--Pro^5) enkephalinamide.

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MECHANICAL ACTIVITY OF THE ISCHAEMIC MYOCARDIUM AS AFFECTED BY VERAPAMIL AND FENDILINE

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Keywords: fendiline, verapamil, ischaemia, mechanical activity, papillary muscle.

Several studies have provided evidence to prove that the negative inotropic potency of some of the calcium channel blockers is enhanced under 'in vivo' ischaemic circumstances (Smith et al., 1976; Abrahamson et al., 1985). Since the 'in vivo' drug effects are also influenced by systemic changes induced by ischaemia, studies were carried out to establish the alterations of the negative inotropic activity of calcium antagonists under various 'in vitro' experimental conditions, simulating one or several components of the 'in vivo' myocardial ischaemia (e.g. acidosis, elevated extracellular potassium, hypoxia) (Boros et al., 1984; Papp and Szekeres, 1986; Robertson and Lumley, 1989). Despite the extensive investigations, the available data have so far been obtained at some fixed preload or sarcomer length only, which can not be extrapolated on any arbitrary part of the respective 'preload-maximum contractile force' relationship.

The purpose of the present study was therefore twofold:

1). To establish the effects of 0.4 mg/l verapamil and 2 mg/l fendiline (producing a 50 % depression in the maximum contractile force under physiologic conditions) on the mechanical activity of rabbit right ventricular papillary muscles in the presence of a moderate 'ischaemia' (9 % O_2 and 5 % CO_2 in nitrogen: pO_2 = 330 mmHg; $/K^+/_O$ = 7 mM; pH = 6.8) at a near physiological preload (2 mN).

2) To examine the 'ischaemia'-induced alterations of the inotropic

effects of verapamil and fendiline as a function of preload in general.

In both series of experiments the isolated preparations were electrically driven by field stimulation at a rate of 1.67 Hz.

In 'ischaemia' the negative inotropic potency of verapamil (i.e. the extent of the drug-induced depression of the maximum contractile force) was significantly enhanced (means $\pm$ S.D. = control: 51.43 $\pm$ 4.78 %, 'ischaemia': 23.51 $\pm$ 5.18 %; n = 5) at the preload of 2 mN. However, at the same physiological preload 'ischaemia' did not alter the mechano-depressant activity of fendiline (control: 48.86 $\pm$ 5.27 %, 'ischaemia': 50.73 $\pm$ 6.39 %; n = 5).

In 'ischaemia' the course of the control 'preload-maximum contractile force' relationship (fittable with a modified Hill's equation) was changed to bell-shaped having a maximum at 7 mN. Under these conditions the 'kinetic' constant governing the descending arm was -0.113 ± 0.021 mN^{-1} (n = 5). In the prersence of fendiline and verapamil the constant was -0.012 ± 0.003 mN^{-1} and -0.019 ± 0.007 mN^{-1}, respectively. This reflects a remarkable anti-'ischaemic' action for both drugs at high preload.

On the basis of these findings it can be suggested that both verapamil and fendiline are able to exert some anti-'ishaemic' effect in the myocardium, but they seem to affect inotropy at different cellular entities possessing differential sensitivity to myocardial ischaemia.

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ACID-BASE PARAMETERS IN MORPHINE TOLERANCE

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Keywords: acute morphine tolerance, acid base parameter

Lethal respiratory depression (RD) caused by opiate overdosing is rather typical among drug abusers. In some cases opiates may induce RD without appearent evidence of overdosing (Roerig et al., 1987). The aim of our investigation was to study whether there is a relationship between the pharmacological (i.e. analgesic) tolerance and the tolerance that developes in acid-base parameters. Mice were rendered tolerant to morphine (MO) by a dose of 60 mg/kg. The degree of tolerance was measured 24 hours later. Tail blood was taken to measure blood gas parameters with an AVL-945 type acid-base analyzer. It has been found that acute MO treatment elevated the pCO_2 level in a dose-dependent manner. Lower doses of MO (1 and 5 mg/kg s.c.) did not change the pH, while higher doses of 10 and 60 mg/kg resulted a significant decrease in pH and O_2 saturation as well. All changes could be antagonized with naloxone. One day after 60 mg/kg MO treatment, acute tolerance developed to the analgesic effect but not to changes in acid-base parameters (Fig. 1). It is concluded that development of tolerance is faster to the analgesic (and supposedly to the euphoric) effect of MO than to RD and this difference may play a role in lethal RD.

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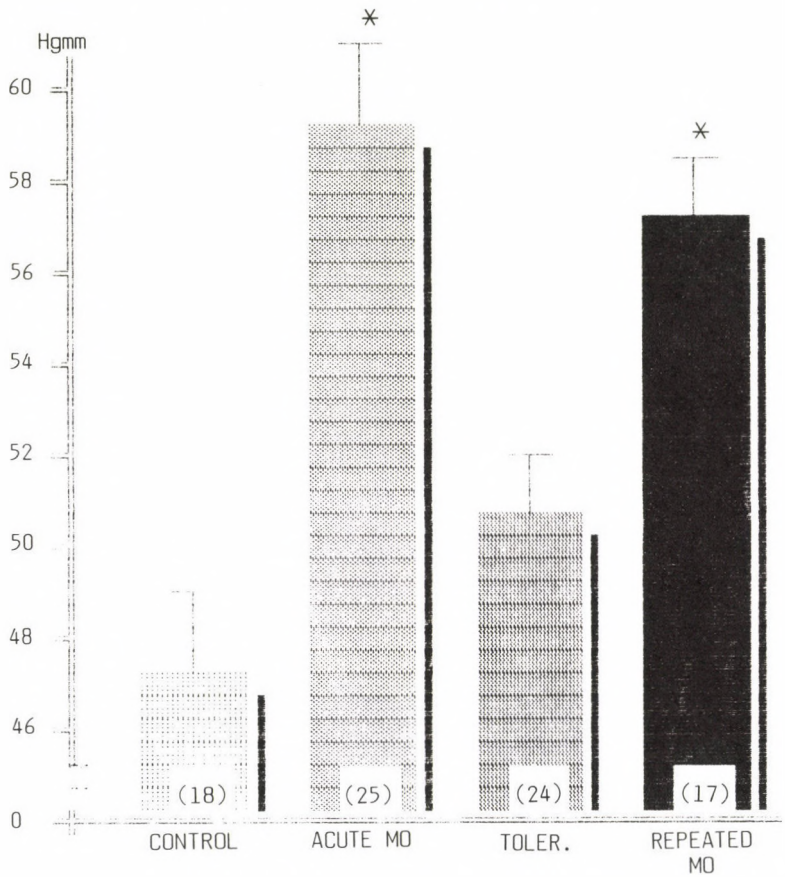
Figure 1.

pCO VALUES IN MORPHINE-TOLERANT MICE



Legends:

Control group

Acute MO (10 mg/kg) in morphine-naive mice

MO tolerant group (24 h after 60 mg/kg MO)

Repeated MO treatment (10 mg/kg) in MO tolerant group

* $p < 0.05$ (significantly different from the control)

RELATIVE SELECTIVITY OF RESPONSIVENESS TO CALCIUM ANTAGONISTS IN PERIPHERAL VASCULAR SMOOTH MUSCLE OF DOGS

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Keywords: peripheral vascular smooth muscle, verapamil, diltiazem, fendiline, KHL-8430, prajmaline.

It has been well established that there is a variability in drug responsiveness of the receptor-operated calcium channels throughout the vascular system (Cauvin et al., 1983; Carmeliet, 1986; Taire, 1987). The evidence for the existence of such a differential sensitivity to drugs in the potential-operated calcium-channels is rather scanty. The present study was therefore undertaken to compare the efficacy of five calcium antagonists (verapamil, diltiazem, fendiline, KHL-8430, prajmaline) in relieving potassium-induced contractions of isolated carotid and femoral arteries.

The blood vessel preparations were obtained from dogs. Segments of the carotid and femoral arteries were cleaned of extraneous connective and fatty tissue, spirally cut into strips and placed in an organ bath containing Krebs solution. Cummulative concentration-response curves for calcium antagonists were constructed on the basis of the ability of the individual agents to inhibit the contractile response to potassium-rich (KCl = 50 mM) nutrient solution, as described earlier (Hof and Vuorela, 1983; Horváth et al., 1987).

The results are summarized in Table I, from which it is obvious that although the rank order of potency (calculated from IC_{50}) is similar for the studied drugs in the carotid and the femoral vascular tissue (verapamil > diltiazem > other calcium antagonists), KHL-8430 is almost 4-times more active on femoral and approximately 4-times less potent on carotid artery than fendiline. A differential sensitivity to prajmaline in the two vascular

tissues is also apparent. Furthermore, the results of our more recent studies have shown that under the same experimental conditions fendiline is some 2-times, prajmaline is almost 5-times more active, as a vaso-spasmolytic, in canine peripheral than coronary blood vessels.

Table I

IC_{50} values and relative activities relating to fendiline for relaxation of contractions induced by potassium (50 mM KCl) in isolated peripheral arteries of the dog

Drug	Carotid artery		Femoral artery	
	IC_{50} (mol/l)	Relative activity	IC_{50} (mol/l)	Relative activity
VERAPAMIL	1.613×10^{-9} (n=5)	5641.42	1.033×10^{-7} (n=5)	84.38
DILTIAZEM	3.422×10^{-7} (n=4)	26.60	1.223×10^{-6} (n=5)	7.14
FENDILINE	9.103×10^{-6} (n=5)	1.00	8.717×10^{-6} (n=5)	1.00
KHL-8430	3.726×10^{-5} (n=5)	0.24	2.241×10^{-6} (n=5)	3.89
PRAJMALINE	3.000×10^{-5} (n=5)	0.30	1.016×10^{-5} (n=5)	0.86

Mean values are given (± S.D. ± 5 per cent). n = number of preparations.

In view of these results it appears that there is a variability of drug sensitivity of potential-operated calcium channels among vascular beds. This may result in a relative selectivity of peripheral vaso-spasmolytic responses to calcium antagonists.

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STUDY OF THE PREDOMINANT VASCULAR POSTSYNAPTIC ALPHA-ADRENERGIC BLOCKING CAPACITY OF GYKI-12 743 IN ISOLATED ORGANS AND PITHED RATS

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Keywords: Postsynaptic alpha-adrenergic antagonism

It has been accepted that vascular smooth muscle was equipped with postsynaptic $alpha_2$-adrenoceptors as affective as $alpha_1$-adrenoceptors and both of them mediated vasoconstriction in the vasculature (Drew and Whithing, 1979; Docherty and McGrath 1980; Fowler et al., 1984). Since vascular tone is controlled predominantly by alpha-adrenoceptors, therefore pharmacological antagonists of alpha-adrenoceptors decrease the elevated blood pressure in most forms of hypertension. Selective blockade of the postjunctional $alpha_2$-adrenoceptors (without strong prejunctional $alpha_2$-adrenoceptor blockade) may offer a new approach to antihypertensive therapy.

The present study has been undertaken in order to characterize the $alpha_1$- and $alpha_2$-adrenoceptor antagonist properties of GYKI-12 743 in vitro (isolated mouse vas deferens (MVD), dog saphenous vein (DSV), rabbit aortic strip (RA) and in vivo (pithed rat). GYKI-12 743 was a competitive antagonist at both subclasses of postjunctional alpha-adrenoceptors. In RA GYKI-12 743 like prazosin produced a parallel shift to the right of dose-response curve to noradrenaline (pA_2-values are 7.-6 and 9.45 respectively). However in DSV using BHT-933 as agonist, GYKI-12 743 showed much higher (10 times more potent) antagonist potency at postjunctional $alpha_2$-adrenoceptor sites (pA_2 = 7.5) than idazoxan (pA_2 = 6.3). In MVD GYKI-12 743 was 5 time weaker (pA_2 = 7.0) while in the case of idazoxan 10 times more potent antagonism was demonstrated in this preparation than in CSV. Studies in pithed rats provided an important in vivo evidence of the predominant postjunctional cardiovas-

cular alpha$_2$-adrenoceptor selectivity of GYKI-12 743,namely it did not increase the tachycardia induced by electrical stimulation of cardioaccelerator nerve.
The above results indicate that, besides a significant alpha$_1$-antagonist activity, GYKI-12 743 induced a postsynaptically selective alpha$_2$-adrenoceptor blockade in the cardiovascular system, thus it might be a potent antihypertensive agent without significant tachycardia in human therapy.

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VASOCORTIN INHIBITS Ca^{2+} INFLUX AND HISTAMINE RELEASE INDUCED BY CONCANAVALIN A IN RAT MAST CELLS

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Key words: Ca^{2+} influx, glucocorticoids, histamine, vasocortin

Vasocortin is a glucocorticoid-induced anti-inflammatory protein that we have recently identified in the peritoneal cavity of dexamethasone-treated rats (Carnuccio et al., 1987). We have recently reported that partially purified vasocortin selectively inhibits histamine release from rat peritoneal cells induced by dextran or concanavalin A (Con A) but does not modify the release induced by calcium ionophore A23187 or compound 48/80 (Carnuccio et al.,1989). Since the histamine release induced by both dextran and Con A is dependent on extracellular calcium we decided to investigate the effect of vasocortin on Ca^{2+} influx into the cells. Vasocortin was generated, collected and partially purified from the peritoneal lavage fluid of dexamethasone treated rats following the procedure described by Carnuccio et al. (1987). Mast cells were purified from peritoneal cells of Wistar rats according to Foreman et al. (1977). Histamine release was induced, quantified and calculated as previouse described (Carnuccio et al.,1989). Ca^{2+} was measured spectrofluorimetrically using the Ca^{2+} fluorescent indicator, fura 2. Results are shown in Fig.1. Ca^{2+} influx and histamine release reached a maximum at about 5 min. and 10 min. respectively. Partially purified vasocortin

inhibited both the Con A induced release of histamine from mast cells as well as the Ca^{2+} influx into these cells. Since Ca^{2+} uptake is essential for histamine release it is coincevable that vasocortin inhibition of histamine release may depend on its ability to prevent Ca^{2+} influx into the cells. However the mechanism underlying the vasocortin inhibition of histamine release deserves further investigation.

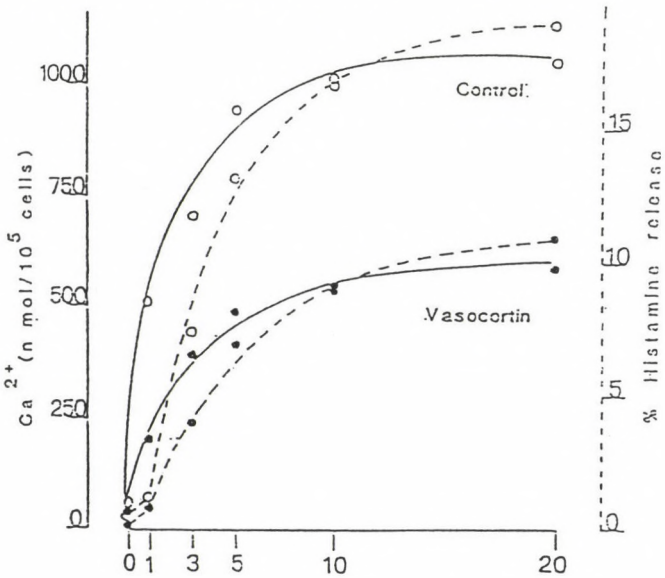

Fig. 1
Effect of vasocortin (50 μg ml^{-1}) on the Ca^{2+} and histamine release induced by con A (5 μg ml^{-1}) in rat mast cells. Purity of mast cell preparation was approximately 90%. Vasocortin was added to the incubation medium 10 min. before Con A challenge. Spontaneous histamine release from the cells (5-6%) was subtracted from experimental values. Each point is an avarage of two duplicate experiments.

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PAIN DURING INJECTION AND VENOUS SEQUELAE: THIOPENTONE VERSUS PROPOFOL.

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Key words: Pain, Thiopentone, Propofol.

Many drugs used in intravenous anaesthesia may produce acute pain during administration and venous sequelae such as trombophlebitis. These complications are often underestimated whereas they can produce a sad memory of anaesthesia and can be harmful for patients (Stark et al.,1985; Henriksson et al.,1987). Many factors may influence the appearance of these complications : first the chemical nature of the drug and the possible presence of the vehicle, then the gauge and the length of the catheter, the vein's size and the speed of injection (Mattila et al.,1985; Mundeleer,1988). The aim of this study was to investigate the incidence of these complications occurring with two drugs commonly used for induction of anaesthesia in minor gynecological procedures. The study was performed on 40 patients aged 17-66 years of ASA grade I-II undergoing minor gynecological procedures. All patients were premedicated with Trazodone and Atropine i.m. and then randomly divided into two groups. The induction of anaesthesia was performed as follows: group A received 5 mg/Kg of Thiopentone, group B received 2.5 mg/Kg of Propofol(2-6 diisopropylphenol). The maintenance of anaesthesia was the same for all patients: N_2O-O_2(2:1) and Isoflurane. 10 patients

of each group were administered the hypnotic drug through a vein of the back of the hand and the remaining through a larger size one of the forearm. A short 18 G teflon catheter was always employed and the time of injection was always not less than 20 sec. Afterwards patients were asked to score the intensity of injection pain by means of a 4 points evaluation scale (no pain=0, mild pain=1, moderate pain=2, and severe pain=3). Moreover all patients were followed up (7 days) for clinical signs of thrombophlebitis. The results of pain score showed important differences between the two groups (Tab.Ia). Some patients presented mild swelling and referred numbness; the average duration of these symptoms was 48 hours (Tab.Ib).

Tab. I

(a)

Score	Gr.A		Gr.B	
	back hand	forearm	back hand	forearm
0	8	10	1	6
1	1	-	4*	2
2	1	-	4*	2
3	-	-	1	-

*= p<0.01

(b)

	Gr.A	Gr.B
swelling	3	5
redness	1	3
numbness	3	5

The two drugs considered in this study showed important differences with regard to the analysed complications. Indeed Propofol showed a greater incidence of these side effects when compared to Thiopentone, yet it is possible to reduce them by choosing a large vein for drugs admministration, by using a short and small catheter and finally by injecting very slowly, at least within 20 sec.

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VARIATION IN THE POTENCY OF EPITHELIAL RELAXANT FACTOR TO MODULATE AIRWAY SMOOTH MUSCLE RESPONSIVENESS IN THE FUNCTION OF DIFFERENT CHOLINERGIC AGONISTS

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(airway epithelial factor,cholinergic agonists,tracheal smooth muscle)

We previously reported that removal of the airway epithelium (ep-)from sheep tracheal smooth muscle (TSM) resulted in a small but significant increased contractile response to carbachol when compared to TSM with epithelium (ep+).This effect was abolished if a TSM (ep-) strip was studied in the SAME organ bath as a TSM (ep+) strip,suggesting that sheep airway epithelium produces a transferable relaxant factor,Abraham et al.,(1988). In this study we aimed to determine whether similar responses could be obtained with acetylcholine (Ach).Single TSM strips were hung under 2g tension in organ baths containing Krebs-Henseleit solution (39° C),equilibrated with 95 % O_2 and 5 % CO_2.

Isometric forces generated in response to increasing concentrations of carbachol and Ach were measured to determine the concentration required to produce 50 % of maximum contraction (ED50).Mean$\pm$SE-log M ED50 values for carbachol (n=7) and for ACH (n=7) in TSM (ep+) tissues were 7.76$\pm$ 0.12 and 5.80$\pm$0.50, respectively.In TSM (ep-) tissues carbachol shifted the ED50 to the left by 0.22$\pm$0.06 units ($p < 0.05$).This leftward shift was slightly greater if Ach was used as the contractile agonist (0.30$\pm$0.04).

When TSM strips (ep+/ep-) were hung together,the increased sensitivity of the TSM (ep-) strip to carbachol was lost (delta-log M ED50 for/(ep-)-(ep+)/=-0.03$\pm$0.08 units,n=6)

However,the increased sensitivity of the TSM (ep-) tissue was maintained if Ach was used (delta-log M ED50=0.36±0.09 units, n=3).

The following Table summarizes the effect of epithelial removal on sheep tracheal smooth muscle contractile responses to cholinergic agonists:

SINGLE TISSUE STUDIES

AGENT	n	EC50 ep(+)	EC50 ep(-)	Delta
Ach	7	5.80±0.50	6.10±0.70 §	0.30±0.04
Metacholine	5	7.38±0.18	7.65±0.20 §	0.27±0.02x
Carbachol	7	7.76±0.12	7.98±0.10 §	0.22±0.06x

PAIRED TISSUE STUDIES

AGENT		EC50 ep(+)	EC50 ep(-)	Delta
Ach	3	5.17±0.33	5.54±0.25 §	0.36±0.09
Metacholine	4	7.64±0.33	7.66±0.28	0.02±0.06x
Carbachol	5	7.72±0.17	7.69±0.15	0.03±0.08x

Values are mean SE for EC50(-log M);§=p 0.05 vs (ep+);x=p 0.05 vs Ach

These observations suggest that in sheep TSM,the degree of increased sensitivity attained by epithelial removal depends on the cholinergic agent used,as does the ability of the epithelial relaxant factor to modulate smooth muscle function under the present experimental conditions

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IN VITRO EFFECTS OF ANGIOTENSIN II ON THE HUMAN DISEASED AND THE GUINEA-PIG GALLBLADDER

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Key words: Human gallbladder - Guinea-pig gallbladder - Angiotensin II receptors

The gastrointestinal effects of angiotensin II have already been studied in detail. On the other hand, there is little evidence on the effects of angiotensin II on the gallbladder isolated strips. For instance, it is reported that angiotensin II contracts the guinea-pig gallbladder in vitro (Janković and Beleslin, 1989). Still, the site and mechanism/s of the stimulatory action of angiotensin II on the guinea-pig gallbladder as well as the effect of the peptide on the human diseased gallbladder are not known. In view of these findings, experiments were performed to investigate the site and mechanism/s of excitatory action of angiotensin II on the guinea-pig gallbladder isolated strips, as well as the effect of the peptide on the human diseased gallbladder isolated strips.

Human gallbladders were taken immediately after cholecystectomy of patients, mostly women. Guinea-pigs were killed and the gallbladder was removed. Strips of the gallbladder body of whole wall thickness, perpendicular on the longitudinal axis of the gallbladder were cut and set up in an isolated organ bath of 15 ml in Tyrode solution, gassed with 95 % O_2 and 5 % CO_2 at 37°C. Angiotensin II was cumulatively applied to the organ bath. Each strip was used for evaluation of the effect of only one concentration of an antagonist. Concentration-response curves were constructed using linear regression according to the method of least squares. A coefficient of correlation (r) of linear regression was used to determine the existence of concentration-response dependence. The pA_2 value was calculated according to the method of Arunlakshana and Schild (1959). Antagonism was regarded as competitive when the slope of the Schild plot was close to unity (1.0).

In the first series of experiments the effect of angiotensin II on the human diseased gallbladder was investigated. Angiotensin II (3.2×10^{-7}M - 2.6×10^{-5}M) did not contract, relax or had any effect on resting tone of the human diseased gallbladder isolated strips.

In the second series of experiments, the effect of angiotensin II on the guinea-pig isolated gallbladder was studied. Angiotensin II (3×10^{-7}M - 2×10^{-5} M) contracted the gallbladder isolated preparations. The contractile effect was concentration-dependent ($r=0.99$; $p < 0.01$).

In the third series of experiments, the interaction of the angiotensin II antagonist saralasin, the antimuscarinic drug atropine, the alpha and beta adrenoceptors blocking drugs phentolamine and propranolol, the H-1 and H-2 histamine antagonists, pyrilamine and cimetidine, and the local anaesthetic agent lidocaine on angiotensin II - induced contractions was investigated. Saralasin

(6.7×10^{-11}M - 6.7×10^{-9}M) depressed or abolished the contractile response of gallbladder strips to angiotensin II and the antagonist shifted the concentration-dependent curves of angiotensin II to the right with maximal contractions not significantly different from those obtained with the peptide. The antagonism gave pA_2 value of 12.5. The slope of the regression line was 0.41. On the other hand, atropine, (9.6×10^{-8}M), phentolamine (2.1×10^{-5}M), propranolol (2.3×10^{-5}M), cimetidine (2.6×10^{-6}M) and lidocaine (9.2×10^{-5} M) had virtually no effects on angiotensin II- induced concentration-response curves.

As shown in these experiments, angiotensin II contracted the guinea-pig gallbladder isolated strips, whereas the peptide did not contract, relax or had any effect on resting tone of the human diseased gallbladder isolated strips. This finding would be consistent with reports that angiotensin II contracts the gallbladder isolated strips (Janković and Beleslin, 1989) as well as the sphincter Oddi of the guinea-pig (Harada et al., 1986). The present experiments further revealed that the angiotensin II antagonist, saralasin, but not atropine, phentolamine, propranolol, pyrilamine, cimetidine and lidocaine, inhibited the peptide-induced contractions. It appears, therefore, that angiotensin II acted on angiotensin II receptors in the guinea-pig gallbladder smooth muscle. However, since the slope of the regression line is significantly less than unity (1.0), the antagonism with saralasin cannot be competitive in nature.

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GYKI-12 743, A NOVEL ANTIHYPERTENSIVE COMPOUND WITH SPECIAL ALPHA-ADRENERGIC BLOCKING PROFILE

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Keywords: Antihypertensive, bradycardia, alpha-blocker

Recently, parallel with the development of new selective $alpha_1$-adrenergic blockers in intensive search for selective postsynaptic $alpha_2$-adrenergic antagonists has emerged in the international drug research (Ruffolo et al., 1987). Predominance of postjunctional $alpha_2$-adrenoceptors have been shown in spontaneously hypertensive rats (Medgett et al., 1984) and their activation should play a role in the maintainance of high blood pressure in patients with essential hypertension (Jie et al., 1984). It became widely accepted that there is an interaction between the change of function of adrenergic nervous system and the ion permeability of smooth muscle cell membrane. In the course of our studies on pyridazine derivatives with potential vasodilating properties, a new group of 2-amino-alkyl-3(2H)-pyridazinonen was synthetized. One member of this class of compounds, GYKI-12 743 (Kasztreiner et al., 1989) decreased elevated blood pressure in different types of conscious experimental hypertensive rats and neurogenic hypertensive cats after acute (3 mg/kg and 0.5 mg/kg respectively) and subacute (3-weeks treatment with 10 mg/kg twice daily in rats) oral administration. Its antihypertensive action manifested without tachycardia even bradycardia was regularly observed. In canine experiments GYKI-12 743 decreased the total peripheral resistance by its strong vasodilator capacity in femoral, renal, carotid and vertebral arteries (0.25-1.0 mg/kg i.v., i.d.). At a very low dose range (25 and 50 ug/kg i.v.) GYKI-12 743 could decrease the coronary vascular resistance before any marked reduction of systemic blood pressure deve-

loped.
According to our haemodynamic studies and on the base of our data on mechanism of action (see posters:Horváth et al., Bakonyi et al., Jednákovits et al.) it might be suggested that GYKI-12 743 could be a potent antihypertensive compound in human therapy. Besides its significant $alpha_1$-antagonist activity, GYKI-12 743 exerts predominantly postjunctional (vascular) $alpha_2$-adrenoceptor blockade and in its vasodilating action the opening of K^+-channels of vascular smooth muscle cells could play role.

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VASCULAR AND CARDIAC ELECTROPHYSIOLOGICAL ACTIVITY OF GYKI--12 743 IN ISOLATED TISSUE PREPARATIONS

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Keywords: Potassium channel, GYKI-12 743

Potassium conductance is known to be an important regulator of membrane potential in cardiac and vascular smooth muscle. BRL-34915 (cromakalim) and pinacidil are two new compounds acting at smooth muscles and they are under clinical investigation as antihypertensive and / or bronchodilator agents (Ward, 1984; Van den Burg et al., 1986; Arch et al., 1988). The opening of potassium channels leads to hyperpolarization of the myocytes. Compounds which initiate relaxation of smooth muscle by opening potassium channels may have therapeutic potential as antihypertensives, as well.

The present study has been undertaken in order to characterize the action of GYKI-12 743 (a predominantly postjunctional alpha-adrenergic blocker) on the potassium conductance in isolated cardiac and vasculr preparations. Intracellular cardiac electrophysiological studies was carried out in dog Purkinje fiber (DPF)and guinea pig papillary muscle (GPPM).

GYKI-12 743 (10^{-5}M) like BRL-34915 (10^{-6}M), pinacidil (10^{-6}M) and nicorandil (5×10^{-5}M) induces a significant shortening of the action potential duration (APD_{50} and APD_{90}) in DPF.

The same effect was demonstrated in case of all compounds tested in GPPM but only in higher concentrations. The shortening of the transmembrane potential was inhibited with TEA but not glibenclamide. We have examined the action of GYKI-12 743 on mechanical activity in isolated rabbit ear artery (REA) and dog coronary artery (DCA). The tissues were precontracted with 5-hydroxytryptamine (5-HT). The relaxing ED_{50} of GYKI-12 743 was 5×10^{-7}M and in this concentration GYKI-12 743 did not influence the dose-response curve to 5-HT. We studied the vas-

cular smooth muscle relaxing activity of GYKI-12 743 in other vascular preparation in order to get more information about its vasodilating effectiveness against potassium-induced vasoconstriction.
Our results may provide further evidence that the potassium channel opening activity could play an important role in addition to its predominant postjunctional alpha-adrenergic antagonist character in the vasorelaxing effect of GYKI-12 743.

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THE EFFECT OF NEUROPEPTIDE Y ON FEEDING AND ITS ALTERATION BY RECEPTOR ANTAGONISTS IN RATS

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Keywords: Neuropeptide Y Feeding Bimodal effect Antagonists

Introduction

Neuropeptide Y (NPY) is a polypeptide hormone from porcine brain consisting of 36 AA residues. It was shown that central administration of NPY caused a robust and long-lasting stimulation of feeding (Clark et al., 1984). This effect is similar to that of centrally administered noradrenaline (Stanley and Leibowitz, 1984). In the present study the role of neurotransmitters were investigated in the food intake-inducing effect of NPY, by using several receptor blocking agents.

Method

Male Wistar albino rats weighing appr. 200 g were used. The intracerebroventricular (icv.) treatments were carried out with 100 ng, 1 μg and 5 μg NPY in a volume of 2 μl/rat, and a preweighed amount of rat chow was provided. Food intake was determined by weighing the remaining food 2 and 4 h after the injection. Pretreatments with the antagonists were carried out icv. 10 min prior to peptide injections. As receptor antagonists prazosine, yohimbine, propranolol and naloxone HCl (all SIGMA) were used.

Results and Discussion

NPY exerted a dose-dependent biphasic effect on feeding; the 100 ng dose significantly suppressed 4-h food intake, but higher, 1 µg and 5 µg doses stimulated feeding. It is possible that NPY exerts its action by altering catecholaminergic metabolism in a biphasic way in the brain, since a close relationship between NPY and the brain catecholaminergic systems was demonstrated (Harfstrand et al., 1986; Jacobowitz and Olschowka,1982), together with the finding that low icv. doses decreased and very high doses increased the utilization of catecholamines in hypothalamic structures (Harfstrand et al., 1987). From the adrenergic antagonists, yohimbine (4 µg icv.) and propranolol (5 µg, icv.) slightly but insignificantly inhibited food intake after NPY treatment. Prazosine (4 µg icv.) exerted a pronounced and selective effect on feeding altered by NPY: it did not modify the food intake-inhibitory effect of a low NPY dose (100 ng) but decreased the food intake elevation of a high NPY dose (5 µg) to the control level. Naloxone (0.5 µg, icv.), similarly to prazosine, selectively inhibited the feeding-stimulatory effect of 5 µg NPY icv. The results suggest the involvement of alpha-1-adrenergic and opiate receptors in the food intake-stimulatory effect of centrally administered NPY.

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GASTROPROTECTIVE EFFECT OF MECLOFENAMIC ACID ON INDOMETHACIN-INDUCED ULCERATION IN RATS

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(Meclofenamic acid, leukotrienes, prostaglandins, gastric ulcer)

Gastric mucosal ulceration and haemorrhage due to nonsteroidal antiinflammatory drugs (NSAIDs) has been ascribed, principally to a prostaglandin "deficiency" created from the inhibition of the biosynthesis of so called "cytoprotective" prostaglandins (PGs), especially PGE_2 and PGI_2.

It is known, however , from studies in nongastric tissues that the inhibition of PG cyclooxygenase by NSAIDs can cause diversion of arachidonic acid through the 5 - lipoxygenas pathway with resultant enhancement in the products derived therefrom. Thus, by analogy with these observations it is possible that the consequences of inhibiting PG cyclooxygenase might instead of reducing the production of gastro-protective PGs, leads to excess synthesis of products of the lipoxygenase pathways i.e. vasoactive leukotrienes (LTs) and hydroperoxy-eicosatetraenoic acids (HPETEs). These perturbations by NSAIDs of arachidonic acid metabolism could contribute to the pathogenesis of gastric injury by NSAIDs.

Meclofenamic acid (Park-Davis) a well known NSAID, at a high dose range (10 - 150 mg/kg) did not cause gastric ulceration, but inhibits indomethacin induced gastric lesions in rats (ED_{50} = 40 mg/kg p.o.).

Meclofenamic acid was found to be strong soybean 15 - lipoxygenase inhibitor (IC_{50} = 110 /uM), potato tuber 5 - lipoxygenase inhibitor (IC_{50} = 50 /uM) and sheep vesicular gland cyclooxygenase inhibitor (IC_{50} = 2.5 /uM).

In human blood platelets and leukocytes Meclofenamic acid inhibits the cyclooxygenase and lipoxygenase pahways. The IC_{50} values for TxA_2 formation is about 48 /uM, and for 12 HETE formation is about 78 /uM in platelets,

while for 5-HETE, PGE_2 and 15-HETE formation in leukocytes are 30 /uM, 8 /uM and 147 /uM, respectively.

Meclofenamic acid inhibits LTC_4 and LTD_4 induced contractions of isolated guinea pig lung parenchymal strips, the IC_{50} value for LTC_4 is 1.1./uM and for LTD_4 is 0.8 /uM. Incubation of the parenchymal tissue strips with 1 /uM Meclofenamic acid resulted in significant shift of the LTC_4 and LTD_4 concentration response curves to the right without affecting the maximal response attained. The effect of Meclofenamic acid appeared to be specific for the leukotrienes.

Meclofenamic acid inhibits TxA_2 and LT formation in gastric mucosa in vitro (IC_{50} = 30 /uM for TxA_2 and 25 /uM for LTC_4).

One hour after the indomethacin - irritation, Meclofenamic acid (40 mg/kg p.o.) decreases the LTC_4 content of rat gastric mucosa by 50 %.

These results suggest, that Meclofenamic acid has dual effect on arachidonic acid cascade: inhibits the cyclooxygenase and lipoxygenase pathways, and it has an antagonistic effect on LT-s receptors.

These effects may play an important role in the gastroprotective effect of Meclofenamic acid in indomethacin induced ulcer, inhibits the overproduction of leukotrienes and other lipoxygenase products after indomethacin treatment.

ROLE OF ARACHIDONIC ACID METABOLITES IN ACUTE GASTRIC LESIONS INDUCED BY INDOMETHACIN IN RATS

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(leukotrienes, indomethacin, gastric ulcerations, lipoxygenase antagonists)

Gastric mucosa is capable of synthesizing various products of arachidonate metabolism, via cyclooxygenase and lipoxygenase pathways, such as prostaglandins (PGs) and leukotrienes (LTs).

A major clinical problem encountered with the use of nonsteroidal antiinflammatory drugs (NSAID) is the high incidence of gastrointestinal irritation. The pathogenesis of NSAID - induced ulceration is still not completely known, but it is possible that these effects are bound to a "cytoprotective" prostaglandin deficiency, especially PGE_2 and PGI_2.

In our experiments we observed, that lipoxygenase inhibitors (BW - 755 C ED_{50} = 4 mg/kp p.o.; NDGA ED_{50} = 35 mg/kg p.o.) and leukotriene antagonists (FPL - 55712 ED_{50} = 5 mg/kg p.o.; SKF 88046 ED_{50} = 4 mg/kg p.o.) protect the gastric mucosa against lesions induced by oral administration by indomethacin.

Ulcerogenic effect of the NSAIDs is bound to the cyclooxygenase inhibitory effect of NSAIDs. Those NSAIDs which have a cyclooxygenase and lipoxygenase inhibitory effect can produce a gastroprotective effect against indomethacin induced gastric ulcers.

In the control mucosa we observed a balance between TxA_2/PGI_2 (0.92), and a very low ratio of lipoxygenase and cyclooxygenase products (0.14). After indomethacin treatment these ratios were shifted toward the direction of TxA_2 and lipoxygenase products, and the ratio of protective products (PGI_2+PGE_2) contra irritation products (TxA_2+LTs) overturned in the ulcerogenic mucosa towards the direction of irritation products:

	TxA_2/PGI_2	TxA_2 + LTs	LTs
		$PGI_2 + PGE_2$	$TxA_2 + PGI_2 + PGE_2$
Control	0.92	0.778	0.14
1 hr after irritation	2.7	1.9	0.69
4 hr after irritation	2.4	2.38	0.65

Table 1

These observations suggest that the overproduction of leukotrienes, thromboxane and other lipoxygenase products due to a protective prostaglandin synthesis blockade, induced by nonsteroidal anti - inflammatory drugs, may play an important role in the development of acute gastric mucosal damage induced by these drugs.

ELECTROPHYSIOLOGICAL EFFECTS OF PLATELET-ACTIVATING FACTOR (PAF) IN GUINEA-PIG CARDIAC PREPARATIONS

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Key words: PAF, cardiac action potential

PAF (acetyl glyceryl ether phosphorylcholine) has been found to be synthesized by a variety of cell (leukocytes, platelets, endothelial cells) and to be implicated in different pathophysiological events (Benveniste et al., 1972; Braquet et al., 1989). This autacoids has prominent cardiovascular actions, including hypotension, decreased coronary flow, depressed cardiac contractility. The mechanism by which PAF induces its negative inotropic effect is not clear. Camussi et al. (1984) have reported that PAF may decrease slow Ca^{2+}-current, whereas Tamargo et al. (1985) have suggested the opposite action. The objective of this study was to examine the effects of PAF-acether on the transmembrane action potential in auricular and ventricular papillary muscles of guinea-pig.

Materials and methods. Experiments were carried out on isolated electrically driven (0.3-1.5 Hz) right ventricular papillary muscle and on left auricular muscle of guinea-pig. The slow response action potential (AP) was elicited with isoprenaline ($5x10^{-7}$M) in partially depolarized (>40mV, by means of 25 mM K^+Tyrode) atrial and ventricular preparations (Kecskeméti, 1978). The transmembrane potential was recorded by means of conventional microelectrode technique.

PAF-acether (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) from Sigma was used.

Results. The effects of PAF-acether on both ventricular and atrial APs at concentrations from 10^{-11} to 10^{-7}M are shown in fig. 1 PAF did not cause any significant alterations in RP, $\dot{V}_{max}$. At 10^{-11}M, it increased the amplitude of atrial and ventricular APs and the duration of ventricular AP. Its effect on the repolarization was not the same in the two types of muscle. While the repolarization phase was dose-dependently shortened in the case of atrium, the duration of ventricular AP was rather increased. PAF was not able to generate slow Ca^{2+}-dependent APs in depolarized (by 25 mM K^+) fibers, when fast Na^+channels were inactivated. PAF failed to prevent isoprenaline-induced APs in K^+-arrested atrial fibers. Our data are inconsistent with those of Tamargo et al. (1985) and Camussi et al. (1984) and suggest that PAF does not affect slow inward Ca^{2+}-current.

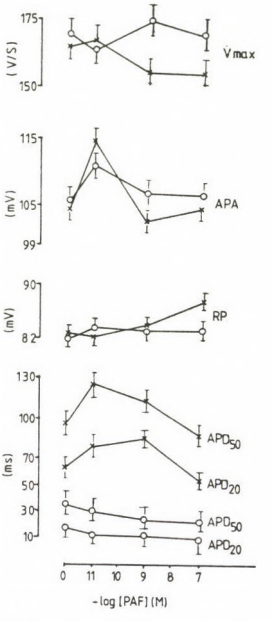

Fig.1. Effect of PAF-acether on AP's characteristics of guinea-pig atrial(o) and ventricular papillary muscles(x) APA=action potential amplitude; RP=resting potential; $\dot{V}_{max}$=maximum rate of rise of the upstroke; APD_{20} and APD_{50}=AP duration measured at 20% and 50% level of repolarization. Each point represents the mean values from 6 experiments; vertical bars= S.E.M.

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PROSTAGLANDIN RELEASING EFFECT OF PYRIDO-PYRIMIDINES IN THE HEART

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Key words: pyrido-pyrimidines, prostaglandins, Na-channel, heart

Prostaglandins have been shown to increase the rate of rise of the cardiac action potential and the amplitude of the fast (sodium) component of the inward membrane current (Kelemen et al.,1980). Paracetamol, known to reduce the the gastric erosive activity of aspirin by stimulating endogeneous prostaglandin production (Seegers et al.,1979) displayed prostaglandin-like electrophysiological effects in the heart (Kelemen and Marko,1988). Prostaglandin-like effect of paracetamol was prevented by indomethacine, suggesting the involvement of endogeneous prostaglandins.

Minor analgesics of pyrido-pyrimidine structure with potent antiinflamatory activity were developed and found to decrease the mucosal damage caused by aspirin and other agents (Knoll et al., 1987, Gyires et al.,1985)). Aim of the present study was to compare the electrophysiological effect of pyrido-pyrimidines with that of paracetamol. Two representatives of pyrido-pyrimidines were selected: rimazolium (3-/ethoxycarbonyl/-6,7,8,9-tetrahydro-1,6-dimethyl-4-oxo-4H-pyrido-/1,2a/pyrimidiniumhydroxide), a clinically approved drug, marketed under the trade name of Probon^{R}) and CH-127 (1,6-dimethyl-4-oxo-1,6,7,8,9,9a-hexahydro-4H-pyrido/1,2a/-pyrimidine-3-carboxamide) also shown to protect intestinal damage in the rat.

Transmembrane ionic currents were measured in the sino-auricular fibers of the frog heart by a voltage clamp technique based on a double sucrose gap arrangement. Drugs were added to the perfusion fluid in the following micromolar concentrations: prostacyclin (PGI_2-Na) - 0.27; rimazolium - 30.0; CH-127 - 30.0; indomethacine - 2.0. Both rimazolium and CH-127 induced a prostaglandin-like effect in the voltage-clamped heart preparation, increasing the maximal amplitude of the fast inward Na-current by 63 and 66% (from 1.64±0.17 uA to 2.59±0.18 uA and from 1.83±0.12 uA to 2.78±0.15 uA, respectively). The changes were statistically highly significant and were comparable with that caused by 270 nmol/l prostacyclin. Pyrido-pyrimidines, similarly to paracetamol and prostaglandins, had no effect on the slow inward Ca-current in the heart. Indomethacine prevented the stimulatory effect of pyrido-pyrimidines on the Na-current, leaving the effect of exogeneous prostacyclin unchanged.

This suggests that the prostaglandin-like electrophysiological effects of pyrido-pyrimidines are due to endogeneous prostaglandin release in the heart.A similar effect in the gastrointestinal tract may be responsible for their anti-ulcer effect.

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EFFECT OF FENOLDOPAM ON ACTION POTENTIAL CHARACTERISTICS IN LEFT ATRIAL AND RIGHT VENTRICULAR PAPILLARY FIBERS OF GUINEA-PIG

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Key words: Fenoldopam, action potential, guinea-pig heart

The type of receptors mediating the cardiac action of dopamine (DA) is not so well defined as that of vascular beds. DA caused a hyperpolarizing effect and it enhanced the overshoot (OS) but did not change the maximum rate of rise (V_{max}) of action potential (AP) on the electrically paced guinea-pig left auricular and right ventricular fibers. These effects of DA were antagonized by pindolol (Kecskeméti et al.,1985) confirming the significance of β adrenoceptors. The aim of this work was to analyse the effect of fenoldopam (selective DA_1 agonist, lack of β agonist activity reported by Hahn et al.) on cardiac transmembrane potential.

Material and methods: Transmembrane potentials were recorded by glass capillary microelectrodes filled in with 3 M KCl. The preparations (left auricules and right ventricular papillary muscles of guinea-pig) were placed into Tyrode solution of 34°C, pH 7.4 bubbled with 95% O_2 - 5% CO_2, and were stimulated by rectangular pulses (0.3-3 Hz, 0.5 ms at voltage 2 times the stimulus threshold). $\dot{V}_{max}$ of AP was recorded as electronically obtained differential (dV/dT) of rising phase.

Results: The obtained results are shown in figure 1. Fenoldopam (more or less selective DA_1 agonist, without β agonist activity) had any effect neither on atrial nor ventricular AP parameters, and increased only OS of the ventricular AP at 10^{-5}M.

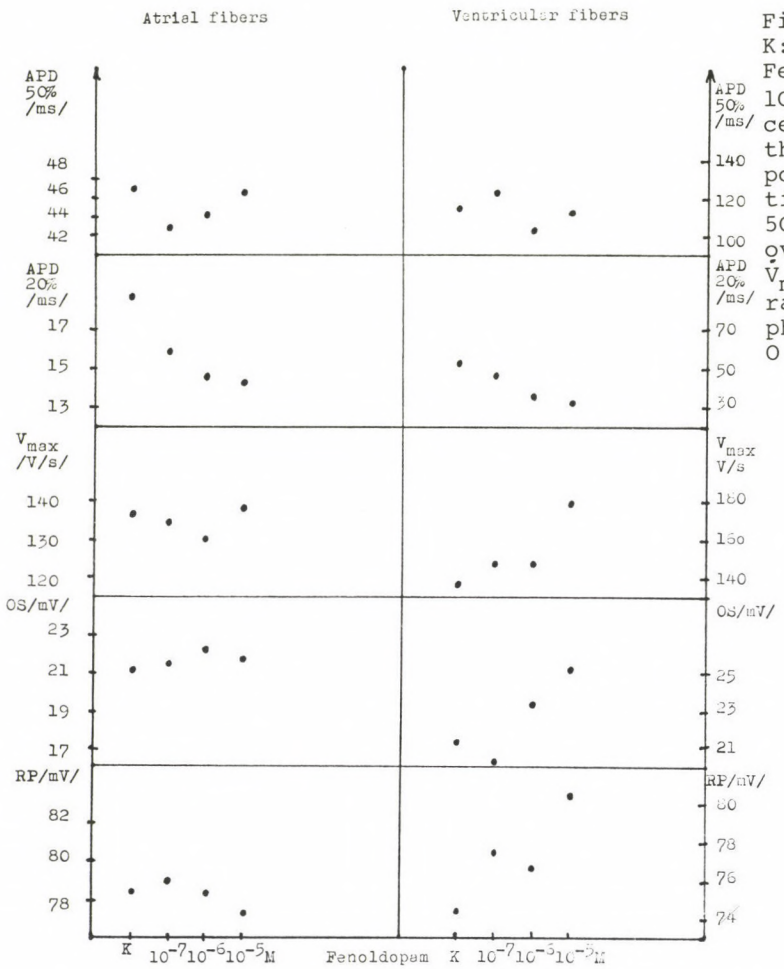

Figure 1. K: Control Fenoldopam 10^{-7} 10^{-6}, 10^{-5}M concentrations on the action potential duration at 20% and 50% repol. OS: overshoot, $\dot{V}_{max}$: maximum rate of rise of phase 0 depolarization

It could not generate slow AP in K^+-depolarized atrial preparation. $4x10^{-7}$M pindolol could not modify the above mentioned action of fenoldopam. These results suggest that DA_1 dopamine receptors are not involved in the cardiac electrophysiological action of dopamine.

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PRECLINICAL STUDIES WITH COMPOUND GYKI-22441, A NEW LONG-ACTING NEUROLEPTIC PHENOTHIAZINE

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Keywords: depot-neuroleptics, new phenothiazine esters

On continuing our earlier research in phenothiazines (Toldy et al. 1964; Borsy et al. 1965) the new aim was to develop a fluphenazine decanoate-like long-acting antipsychotic drug for the therapy of schizophrenic patients.

In order to obtain depot neuroleptic effect several new 2-trifluoromethyl-phenothiazine esters were synthetized by using sterically hindered different acids of lipophyllic character (Király et al. 1980 and 1988).

Among the new esters of fluphenazine the 4-chloro-phenoxy--isobutyrate (GYKI-22441), which is a cristallic substance and readily soluble in sesame oil was selected for preclinical trials.

The specific pharmacological experiments verified the expected long-acting neuroleptic effect of this new phenothiazine ester. Among the classical neuroleptic methods it seemed to be highly active in inhibition of conditioned avoidance reflex (CAR) in rats: it showed a good dose-related and long-lasting inhibitory effect on learning performance already in low doses.

Similarly to other phenothiazine neuroleptic drugs, it possessed direct dopamine receptor antagonistic effect were measured by different methods based on anti-apomorphine activity. In these experiments the relative intensity of this inhibitory effect on different part of central dopaminergic system was determined. We could detected strong effect on nigrostriatal, mesolimbic and tuberoinfundibular dopaminergic system.. The duration of inhibitory effect on intact nigrostriatal and mesolimbic system (measured by apomorphine-induced stereotypy and hy-

permotility in rats) was relatively short (3-8 days), but after the lesion with 6-OHDA the inhibition of supersensitive nigrostriatal dopaminergic system (measured by apomorphine-induced turning behaviour in rats) was long-lasting (14-28 days).
Its long-lasting (above 28 days) inhibitory effect on tuberoinfundibular dopaminergic system appeared already in low doses (measured by apomorphine-induced vomiting in dogs).
By means of the above listed results in specific pharmacological experiments the duration and intensity of its neuroleptic effect was equiactive with fluphenazine decanoate.

On the general pharmacological experiments (cardiovascular, endocrinological, etc.) this compound did not influence the function of other organs of vital importance.

The pharmacokinetic studies supported its long-lasting neuroleptic effect already in low plasma concentration (measured by radioimmunoassay in dogs).

On the base of toxicological studies (acute tolerability and chronic toxicity in rats and dogs, local tissue tolerancy experiments) and teratological as well as mutagenical studies there is no any contraindication of this new drug.

The preclinical studies have already finished and the clinical (phase I and II) trials are in progress now.

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A (-)DEPRENYL-DERIVED NEW SPECTRUM PSYCHOSTIMULANT

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Keywords: Amphetamine, MK-306, catecholamine release, uptake

(-)Deprenyl, the only selective inhibitor of B-type monoamine oxidase (MAO) in clinical practice is a safe drug because, in contrast to other MAO inhibitors, it does not potentiate, but inhibits the effect of tyramine, thus it is free of the cheese effect (Knoll, J., 1987). Based on our observations that MAO and uptake inhibitory effects of (-)deprenyl are independent from each other, we developed a new family of (-)deprenyl-derived substances which do not inhibit MAO but are much more potent than (-)deprenyl in inhibiting the catecholamine releasing effect of indirectly acting amines. As a fruit of structure-activity relationship studies, 1-phenyl-2-propyl-aminopentane.HCl (MK-306) was selected as a reference substance of the family which proved to exert in behavioral tests psychostimulant effects reminiscent to amphetamine (Knoll, B. et al., 1988). MK-306 and amphetamine were found, however, to be essentially different in their mechanisms of action. Amphetamine acts via the continuous release of the catecholamine transmitters from the neurons, whereas MK-306 is devoid of this effect.

For screening of the new derivatives the pulmonary artery strip of the rabbit was used. In this test neither low nor high doses of MK-306 showed noradrenaline releasing properties,

on the contrary, it inhibited the releasing effect of amphetamine and tyramine. The same effect was demonstrated in in vivo experiments on the cat nictitating membrane.

We measured on the isolated striatum of the rat the release of dopamine using reversed phase HPLC and electrochemical detection. Amphetamine (5-15 umol/l) released dopamine from the neurons in a dose-dependent manner, MK-306 (up to 50 umol/l) did not change the release of the transmitter.

Whereas MK-306 was much less potent than amphetamine in increasing motility and basal metabolic rate, it was highly active in enhancing learning and retention in behavioral tests. MK-306 (1-10 mg/kg, s.c.) facilitated the acquisition of a conditioned avoidance response in the shuttle box in a dose-dependent manner and completely antagonized tetrabenazine-induced depression. Amphetamine facilitated the acquisition of a conditioned avoidance response in small doses (1-2 mg/kg, s.c.) but inhibited it in higher doses. In contrast to amphetamines with catecholamine releasing properties the psychostimulant effect of MK-306 was not antagonized by α-methylparatyrosine.

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THE ROLE OF HYPOGLYCEMIC SULPHONYLUREAS IN ARRHYTHMIAS CONTRIBUTING TO THE MORTALITY IN ACUTE MYOCARDIAL ISCHEMIA.

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Keywords: diabetes mellitus, cardiac arrhythmias, sulphonylureas

Sulphonylureas of the two generations are known to exert different cardiac effects. To analyse this phenomenon, on one hand the incidence and mortality of acute myocardial infarction have been compared in 775 diabetic patients according to the different therapies. On the other hand the effect of different sulphonylurea compounds have been studied on arrhythmias induced by coronary artery ligation in rats.

Acute myocardial infarction occured in 20% and with lethal outcome in 13% among the diabetic patients. 10% (lethal: 7%) of diabetics treated with sulphonylureas, 7% (lethal:5%) of those on insulin and 3% (lethal: 1.5%) of all with diet controlled patients suffered from acute myocardial lesion. Among the diabetic patients treated with hypoglycemic sulphonylurea compounds, those with glibenclamide therapy showed the lowest ($p<0.001$) incidence of acute myocardial infarction. From all diabetics with acute myocardial infarction 36% (lethal: 25%) have been on insulin, 52% (lethal: 34%) on sulphonylurea therapies and 12% (lethal: 6%) were controlled with regime alone. Acute myocardial infarction had a lethal outcome in all those treated with glibenclamide in 8%, while in 24% among diabetics on carbutamide and tolbutamide therapies ($p<0.001$).

In order to support this clinical observation about the higher incidence and mortality of acute myocardial infarction in diabetic patients treated with first generation sulphonylurea compounds (carbutamide, tolbutamide) the effects of these compounds have been investigated and compared with that of second generation sulphonylureas (glibenclamide, glipizide), on the incidence of ventricular ectopic beats and on the duration of transitional

ventricular fibrillation in ischemic rat haerts. Tolbutamide, carbutamide and gliclaside (doses: 0.1-1-2-5-10-100-1000 μmol/kg) increased in 105, while glibenclamide and glipizide (doses: 0.1.1.2.5.10 μmol/kg) decreased in 50 rats the number of ventricular ectopic beats and the duration of transitional ventricular fibrillation during the first 30 minutes after ligation of the left anterior descending coronary artery in a dose-dependent manner.

These results seem to support the hypothesis that the first generation sulphonylurea compounds in the antidiabetic therapy may contribute or even provoke arrhythmias in the case of acute myocardial ischemia. Therefore in all those cases, when myocardial ischemia is existing, glibenclamide or glipizide must be preferred, if the good metabolic control can not be achieved by diet or regime only.

POSSIBILITIES OF PHARMACOLOGICAL PREVENTION OF THROMBIN-INDUCED CORONARY VASOSPASM

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Keywords: Vasospasm, thrombin, thrombin-inhibitor, cromakalim

Besides its well known role in blood clotting, thrombin (T) possesses a modulatory role in the regulation of the vascular tone, as well. It can induce either relaxation or contraction of the vascular smooth muscle, depending on the type of blood vessel, and the actual state of the endothelial cells (Haustein et al., 1966; DeMey et al., 1981; White et al., 1984). In dog coronary arteries T elicits a biphasic vascular effect: a transient, dose-dependent relaxation, followed by a slow and irreversible contraction (Ku, 1982). T-induced vasodilatory response is endothelium dependent, while vasoconstrictory effect is endothelium independent and both of the vascular effects are blocked when proteolytic activity of T is inhibited (Ku, 1986). This vascular effects of T could not be inhibited by phospholipase or cyclooxygenase inhibitors, or Ca^{2+}-antagonists (Haver et al., 1984). Their experiments demonstrated that the T evoked contractions are linked neither to the synthesis of prostaglandins, nor to the extracellular Ca^{2+} level. Low concentrations of alpha-T were shown to be present even in the plasma of healthy males. Because of its potent effects,alpha-T is supposed to be involved in the etiology of myocardial ischemia (Haver et al., 1983).

In our experiments the influence of T-inhibitors and K^{+}-channel openers was studied on T evoked vascular responses.

1. Inhibitors of the coagulant and catalytic activity of T were effective in blocking the T evoked contractile responses.
   a./ GYKI-14 766 (N-methyl-D-phenyl alanyl-proline-L-arginine

sulphate) is a specific tripeptide aldehyde type T-inhibitor (Bajusz et al., 1987). Our experiments demonstrated that dog coronary arteries,pretreated with GYKI-14 766 (0.5, 1.0, 5.0, 10.0 ug/ml) caused reduction of vasoconstrictory response to the effect of T.

b./ Making a T-inhibitor complex previously to the addition to the vascular tissue, this preformed complex elicited vasoconstrictions, which did not significantly differ from the control.

2. In our experiments BRL-34915, a specific K^+-channel opener was tested against T-induced vasoconstriction in concentrations of 10^{-7}, 10^{-6}M/l. Dog coronary arteries pretreated with BRL-34915 showed decreased responsiveness to T.

Conclusively, GYKI-14 766, a specific T-inhibitor, seems to be effective in preventing T-induced vasospasm. On the other hand, using T-GYKI-14 766 complex vasoconstrictory activity of T remained at least partially preserved in the absence of its proteolytic activity.

Thus, we found two different newer pharmacological means to attenuate T-induced vasospasm.

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THE SELECTIVITY OF HIGHLY PURIFIED BOVINE LIVER ANGIOHYPOTENSIN TOWARDS RESISTANCE VESSELS

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Keywords: angiohypotensin, capacitance vessels, resistance vessels, vas deferens preparations

The substance, named angiohypotensin (AH), capable of blocking the release of noradrenaline to nerve stimulation in a dose-dependent manner was detected in human and mammalian blood and was found to exert its effect on resistance arteries with high selectivity (Knoll, 1987, 1988). Recently highly purified angiohypotensin preparations containing 3000-3500 units/mg were produced from pig and bovine liver. Bovine liver AH preparations bioassayed on the central ear artery of the rabbit were used in our experiments aiming to check the selectivity of this substance to vascular smooth muscle.

The isolated organs prepared from rabbits, rats and guinea pigs were used in our study. From the rabbit samples of the central ear artery, the saphenous artery, the pulmonal artery and the vas deferens; from the rat samples of the aorta, the rectococcygeal muscle and the vas deferens; from the guinea pig samples of the vas deferens and the longitudinal muscle of the ileum were prepared.

The strips of the organs were mounted in an organ bath (5 ml) and a series of contractions of the smooth muscle were elicited by field stimulation with appropriate time intervals

before and after the presence of AH in the organ bath. The dose of AH was raised up to 50 units, according to the needs. The organ was classified to be insensitive to AH if there was no change in the nerve stimulation induced reponse at the 50 unit/ bath level of the substance.

Out of the organs used in this study only the two resistance artery preparations (the central ear artery and the saphenous artery) proved to be sensitive towards AH. In these preparations AH inhibited with a rapid onset the stimulation-induced vasoconstriction in a dose-dependent manner. The effect could always be washed out rapidly and completely, no tolerance to the effect of AH was observed.

In some of the rectococcygeal muscle preparations the contraction of the smooth muscle to field stimulation was transiently inhibited, but the effect was never dose-dependent and reproducible. The capacitance vessels (pulmonal artery, aorta), as well as the vas deferens preparations, used in our study, proved to be insensitive towards AH.

Studying the selectivity of AH we inevitably selected smooth muscle organs with noradrenergic transmitter machinery. As an example, however, we checked the effect of our highly purified AH preparations on the longitudinal muscle strip of the guinea pig ileum, because of the extremely high sensitivity of this preparation to different types of smooth muscle active peptides. This organ, too, was found to be insensitive towards AH.

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CHANGES IN MONOAMINE OXIDASE (MAO) ACTIVITY IN MIGRAINE

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Key words: platelet MAO activity, migraine, sex differences in MAO activity, brain aging

Platelet MAO activity in migraine was widely studied during the last decades. The interest was stimulated by the observation that "cheese reaction" to certain foodstuffs in patients treated with non-selective MAO inhibitors, was similar to dietary migraine (Glover et al. 1977).

Seventy female and 44 male patients (average: 42.4 years of age) with common or classical migraine were included into our studies. Blood samples were taken from the cubital vein and platelet preparation for measuring MAO activity was carried out by the method of Willberg and Oreland (1976). The platelet MAO activity compared to age-matched controls was measured radiometrically based on the method of Wurtman and Axelrod (1963) and modified by Magyar (1980), both during and between headache attacks. In the incubation media ^{14}C-phenylethylamine was used as a substrate.

The MAO activity in migraine was significantly lower between attacks both in male and female compared to controls, but enzyme activity of the males was more expressed in both groups. In this rerspect we did not find significant differences between patients with common or classical migraine. The MAO activities of the same patient did not change significantly during the attacks and the symptoms-free periods, while in some patients a remarkable decrease was observed in the activity during migraine headache. The platelet MAO activity of 20 patients with migraine was determined every 4 weeks over a period of 36 weeks. Remarkable changes of enzyme activity

were registered in patients with migraine, while in controls a relatively constant level was found. The fluctuation in enzyme activity was independent of platelet functions (counts, serotonin content, aggregability of platelets), hormonal changes and the registered clinical parameters. This observation reflects the instability of platelet MAO in migraine and this finding can throw same light on the contradictory data which were registered in these patients by different investigators. We did not find age dependent significant changes in the enzyme activity in our patients with migraine, but their age was relatively low.

The MAO enzyme produces NH_3, aldehydes and H_2O_2, agents with established or potential toxicity. The lower level of MAO activity in migraine may provide lower risk to the harmful effects of these neurotoxic metabolites. It is known from the literature that the MAO activity of the smokers is also lower, compared to non-smokers and the Parkinsonism is rarer in these population. By analogy, we studied the frequency of Parkinson's disease in the migrainous patients who were registered in our clinic and we did not found Parkinsonism among them. This finding supports the above mentioned hypothesis but in order to obtain convincing evidence further careful studies are needed in this line.

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EARLY REGENERATION OF COLONY FORMING UNITS IN CULTURE (CFUc) OF MURINE BONE MARROW AFTER MODERATE DOSES OF DIBROMOMANNITOL

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Key words: Colony Forming Unit in culture, Dibromomannitol

To study the relationship between the dose of a cytostatic drug and the survival of "Granulocyte-Macrophage Colony Forming Units in culture" (CFUc), mice are usually killed 24 h after treatment in order to estimate CFUc. The importance of the "time interval between drug administration and removal of the target tissue under study" was emphasized, among others, by Lohrmann and Schreml (1982). Our data show that the shape of such a dose-response curve may be influenced significantly by a change in this interval. In the case of 1,6-dideoxy-1,6-dibromomannitol (DBM, Eckhardt & Horváth 1980), this is perhaps due to an early regeneration of the CFUc-population in the bone marrow after moderate doses of DBM.

DBM, suspended in saline with 5% Tween 80, was given ip. to (BALB/c x CBA)F1 mice. Four or 24 h later, soft agar cultures of marrow cells were prepared with L-cell conditioned medium as source of colony stimulating activity. Colonies (groups of ≥50 cells) were counted after 7 days of incubation.

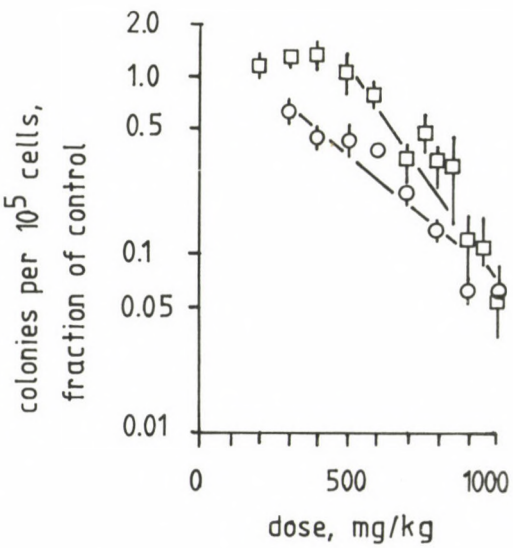

Fig. 1. Frequency of CFUc in bone marrow cells of mice 4 h (○) or 24 h (□) after the ip. administration of dibromomannitol.

The frequency of CFUc in bone marrow cells (colonies growing from 10^5 cells) was not reduced by ≥500 mg/kg of DBM (D_q[1] = 551 mg/kg) but decreased exponentially at higher doses with a D_o[2] of 173 ± 21 mg/kg when estimated 24 h after treatment (Fig. 1). If mice were killed earlier, namely 4 h after the administration DBM, the "shoulder" of the dose-response curve was less wide (D_q = 199 mg/kg) and the exponential portion was significantly ($p < 0.01$) less steep with a D_o of 315 ± 35 mg/kg.

Füzy et al. (1975) estimated the number of proliferating pluripotent stem cells 16 h after treatment with DBM, and found a D_o of 237 mg/kg, which is between the D_o-values found in our studies on granulocyte-macrophage progenitors (CFUc) at 4 and 24 h. Our data are at variance with those of Szamosvölgyi et al. (1989), who reported a D_o of 651 mg/kg and a D_q of ~0 mg/kg for GM-CFU 24 h after treatment with DBM. This might be due to the different strain of mice, vehicle, and source of colony stimulating activity.

The changes in the dose-response curve between 4 and 24 h after treatment (Fig. 1) were probably due to the regeneration of the CFUc-population in the bone marrow during this period, which was more pronounced at doses of ≤600 mg/kg. After larger doses of DBM, the regeneration may start later than 24 h, similarly to that reported by Gulya et al. (1977), who studied the regeneration of pluripotent stem cells after 1600 mg/kg of DBM.

1 Do is the dose-increment reducing the number of CFUc to $e^{-1} \sim 0.37$ at the exponential portion of the curve
2 Dq: "shoulder width" or "quasi-threshold" dose, i. e. the intercept of the exponential portion of the curve with the abscissa

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THE GASTROPROTECTIVE EFFECT OF CHINOIN - 127 ON INDOMETHACIN INDUCED GASTRIC ULCER IN RATS

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(CHINOIN - 127, cytoprotective drugs, indomethacin induced gastric ulcer)

CHINOIN - 127 (1,6-dimethyl-4-oxo-1,6,7,8,9,9a-hexahydro-4H-pyrido-(1,1a)-pyrimidine - 3 -carboxamide) is a potent non - narcotic analgesic and antiinflammatory agent, possessing a remarkable protective effect on ulcerations caused by nonsteroidal antiinflammatory drugs.

CHGINOIN - 127 has a protective effect on indomethacin induced ulcer (ED_{50}= 25 mg/kg p.o.) and on acidified ethanol - induced ulcer (ED_{50} = 26 mg/kg p.o.)

In this study we studied the mechanism of gastroprotective effect of CHINOIN - 127 on indomethacin induced ulcer in rats.

In the mucosa of control animals we observed a balance between TxA_2/PGI_2 (TxA_2/PGI_2 = 0.92). Indomethacin treatment causes disintegration of this balance, shifting the synthesis towaerds TxA_2 production (TxA_2/PGI_2 = 1.92). Increase in the local thromboxane concentration causes contraction in the capillary network of gastric mucosa leading to local ischaemia followed by tissue necrosis and ulcer formation. CHINOIN - 127 (50 mg/kg p.o.) pretreatment improves the deranged TxA_2/PGI_2 balance (TxA_2/PGI_2 = 0.77).

We also determined the activities of the prostaglandin synthesizing enzymes in indomethacin induced ulcer model.In the control mucosa the activity of TxA_2 and PGI_2 synthesizing enzymes is 15 pg/mg prot./min. After indomethacin treatment the activity of PGI_2 synthetase is decreased (10 pg/mg. prot/min) and the activity of TxA_2 synthetase shows a remarkable increase (27 pg/mg. prot./min). Indomethacin shifts the arachidonic acid metabolism towards the production of TxA_2, the balance between the activity of TxA_2 and PGI_2 synthetases overturned. Pretreatment of CHINOIN - 127 restores the overturned balance of TxA_2 and PGI_2 synthetases.

	PGI_2 synthetase	TxA_2 synthetase	ratio
	pg/mg prot./min		
Control	15	15	1
Indomethacin (50 mg/kg)	9	27	3
Indomethacin (50 mg/kg) + CHINOIN - 127 (50 mg/kg)	19	15	1.3

Table 1.

CHINOIN - 127 pretreatment restores the changed TxA_2/PGI_2 ratio and the changed activity of TxA_2 and PGI_2 synthetases, and these effects could be an explanation for their cytoprotective effect.

THE EFFECT OF NEDOCROMIL AND DEXAMETHASONE ON THE ANTIGEN INDUCED CHANGES OF SHEEP TRACHEAL SMOOTH MUSCLE RESPONSIVENESS

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(tracheal smooth muscle,antigen,sensitization,dexamethasone,Nedocromil-Na)

Airway hyperresponsiveness is a characteristic of asthma, however the mechanism responsible is still unclear.The purpose of our experiments was:

1/ To determine if low doses of antigen (i.e. concentrations that do not cause tracheal smooth muscle contraction in vitro) increase smooth muscle responsiveness to acetylcholine (Ach).

2/ To determine if the increased responsiveness can be blocked by pretreatment with compounds that lack anti-mediator or smooth muscle relaxant effect:dexamethasone and nedocromil sodium.

We tested this hypothesis in vitro using the tracheal smooth muscle (TSM) from allergic sheep.TSM strips were hung under 2g tension in separate organ bath (40ml) containing Krebs-Henseleit solution (39° C) equilibrated with 95% O_2,and 5% CO_2.Isometric forces generated in response to increasing concentrations of Ach were measured to determine the concentrations required to produce 25% (EC25) and 50% (EC50) of the maximum contraction.Two Ach dose-response curves (DRC) were performed.Between the DRC-s,the tissues were washed to return to baseline tension and then different strips were subjected to one of the following treatments:

a) buffer (control)

b) Ascaris suum antigen between 0.007-1.06 PNU (subthreshold dose that did not cause contraction)

c) Dexamethasone (9.7x10-7 M) was added 40 min before antigen

d) Nedocromil sodium (3x10-6 M) was added 20 min before antigen

(The tissue which previously served to determine the subthreshold dose of antigen was no longer used in the experiments).

Before adding antigen EC25 and EC50 for Ach in TSM (n=20)(expressed as mean SD,-log M) were 6.60±0.60 and 5.80±0.50,respectively.In the control tissues (no antigen added) tachyphylaxis developed during the second DRC to Ach. However,this was not the case with antigen treated tissues.After antigen EC25 and EC50 values were shifted to the left indicating an increased sensitivity to Ach:

	Delta EC25	Delta EC50
Control (n=20)	+0.30±0.20	+0.20±0.30
Antigen (n=24)	-0.50±0.40 §	-0.30±0.30 §

§=p<0.05 vs respective control; -leftward shift; +rightward shift.

Both nedocromil sodium and dexamethasone prevented the antigen induced increase in TSM responsiveness:

Ascaris suum +nedocromil	n	EC25	EC50	Emax (G)
DRC-1	10	6.1±0.6	5.3±0.6	43±17
DRC-2	10	6.1±0.5	5.4±0.5	44±16
SHIFT		0.0±0.2 §	0.1±0.1 §	1
Ascaris suum+dexamethasone	n	EC25	EC50	Emax (G)
DRC-1	11	6.5±0.3	5.8±0.2	40±4
DRC-2	11	6.5±0.2	5.8±0.1	41±6
SHIFT		-0.1±0.1 §	0.0±0.1 §	1

(Values are mean SD;+ shift to the right (less responsive);- shift to the left (more responsive);§=p<0.05 vs A.suum alone)

Thus,sub-threshold doses of antigen increase the sensitivity of airway smooth muscle.This may be one mechanism that contributes to chronic airway hyperresponsiveness in asthma.

Glucocorticoids and nedocromil inhibit this increase in responsiveness.

TRANSMEMBRANE POTENTIALS OF CANINE CARDIAC PURKINJE FIBRES AS AFFECTED BY PRAJMALINE

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Keywords: Purkinje fibers, prajmaline, transmembrane potential, effective refractory period.

Knowledge of the cardiac microelectrophysiological properties of prajmaline (N-propyl ajmaline hydrogen tartarate), a Class 1 antiarrhythmic drug, is rather limited (Späth, 1983). The present study was therefore designed to obtain quantitative data on the effectiveness and mode of action of the drug in canine Purkinje fibres, when applied in therapeutically meaningful concentrations (Handler et al., 1985).

Right ventricular distal Purkinje fibres were isolated from canine hearts and transmembrane potentials were recorded, utilizing a microelectrode technique, as described earlier (Papp, 1978; Papp et al., 1980).

Prajmaline (0.1-0.5 mg/l) caused a prolongation in the conduction time and a decrease in the action potential amplitude and the maximum rate of depolarization without a significant effect on the resting membrane potential. The action potential duration (APD_{50-90}) and the effective refractory period (ERP) were shortened considerably. The ratio of ERP/APD_{90} was not significantly affected (Table I).

Since prajmaline is thus able to diminish the normally long action potential duration in Purkinje fibres and does not seem to alter considerably the relatively shorter action potential duration in the 'working' myocardium (Virág et al., unpublished), it might be able to decrease the inhomogeneity in regional repolarization within the ventricle, thereby reducing the liability to arrhythmias. To test the validity of such a suggestion, further studies of the microelectropharmacological effects of prajmaline in

ischaemic ventricular muscle and Purkinje fibres would be well worthwhile.

Table I

Effect of prajmaline on the parameters of transmembrane potentials recorded from cells of canine right ventricular distal Purkinje fibre strands

	CT (msec)	$\dot{V}_{max}$ (V/sec)	RP (mV)	APA (mV)	APD_{50} (msec)	APD_{90} (msec)	ERP (msec)	ERP/APD_{90}
CONTROL (n=14)	3.5 ±0.3	344.6 ±24.1	-81.6 ±1.2	101.6 ±1.9	270.2 ±22.8	371.3 ±23.7	337.5 ±18.4	0.89 ±0.01
PRAJMALINE								
0.1 mg/l (n=3)	4.5 ±1.4	282.0 ±27.0	-80.7 ±5.5	91.3 ±5.2	183.5 ++ ±6.5	293.0 ++ ±12.0	283.5 ++ ±4.5	0.95 ±0.04
0.2 mg/l (n=5)	5.4 + ±0.9	202.0 ++ ±26.4	-77.3 ±3.0	80.0 +++ ±1.5	153.6 ++ ±21.6	252.6 ++ ±21.9	243.8 +++ ±2.7	0.95 ±0.04
0.5 mg/l (n=6)	8.0 ++ ±1.4	168.3 +++ ±15.6	-78.5 ±2.3	81.2 +++ ±2.2	156.5 +++ ±12.5	262.0 +++ ±13.6	244.0 ++ ±21.8	0.97 ±0.04

Mean values are given ± S.E. n = number of preparations.
CT = conduction time; V_{max} = maximum rate of depolarization; RP = resting potential; APA = action potential amplitude; APD_{50} and APD_{90} = time for repolarization to 50 and 90%.
Statistical significance: + $P<0.05$; ++ $P<0.01$; +++ $P<0.001$.

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POSITIVE INOTROPIC EFFECTS OF GYKI-52 713 IN CONSCIOUS AND ANAESTHETIZED ANIMALS

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Keywords: GYKI-52 713, positive inotrop, in vivo and in vitro

Major group of newer positive inotropic compounds block the phosphodiesterase enzyme activiy. Recently it became evident that almost all of them had a very limited therapeutic value in patients with cardiac failure. This fact could be explained by (1) the risk of arrhythmias, (2)tachycardiac effect, (3)(4) relative or absolute lumen uneffectiveness, (5)development of tolerance during the longterm treatment. Therapeutically more succesful member of that group remained the compounds which possess some other mode of action (e.g.:calcium sensitizing effect) lack of former undesirable side-effects. Therefore our aim was to develop an experimental schedule for testing and investigating new molecules and thereby select the most effective ones with the hope of the most probable therapeutic usefulness. GYKI-52 713 is a 5H,2,3-benzodiazepine compound with strong positive inotropic and coronary vasodilatory effects.

1. In anaesthetized open-chest cats this compound produced in situ positive inotropic effect after either i.v. or i.d. administrations of 5 mg/kg dose: cardiac effect was equieffective in peak and duration with that of amrinone.
2. In anaesthetized open-chest dogs of intact heart it caused marked increase of the myocardial contractile force already after 0.25, 0.5, 1.0 and 2.0 mg/kg i.v. doses; heart muscle effect was accompained by moderate fall of blood pressure and heart rate.
3. In anaesthetized dogs of intact chest and heart GYKI-52 713 elicited the same positive inotropic effect but, heart rate and blood pressure remained unchanged.
4. In dog experiments the interrelationship of positive inotro-

pic and coronary dilatory effects was shifted to the myocardial effect in the course of the total effective period, while the coronary vascular effect showed predominance in the case of reference compounds (amrinone, forskolin, inosin).

5. 6 weeks after acute myocardial infarction provoked by LAD artery occlusion GYKI-52 713 remained active after 0.25 and 0.5 mg/kg i.v. doses, as well. The predominance of positive inotropic component in relation with coronary vascular effect was achieved in these postinfarcted heart dogs, too.
6. Positive inotropic effect could be demonstrated in chronically catheterized cats of either intact or infarcted heart. In these experiments blood pressure and heart rate never changed.
7. GYKI-52 713 was active in isolated cardiac preparations,too.
8. Studies on the biochemical mode of action and toxicity studies are in progress in the Drug Research Institute,Budapest.

DOPAMINE, DOPEXAMINE AND DOBUTAMINE AS INOTROPIC AND AUTOMATOTROPIC AGENTS

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Keywords: dopamine, dopexamine, dobutamine, inotropy, automaticity.

Dopamine and dobutamine have long been known as positive inotropic and chronotropic agents (Tuttle and Mills, 1975). Dopexamine, a novel structural analogue of dopamine, has also been shown to increase cardiac contractility and sino-atrial automaticity (Brown et al., 1985; Smith et al., 1987). However, a comparison of the inotropic and chronotropic efficacy of these drugs, in order to determine their automatotropic potency at equivalent inotropic concentrations, has not yet been made.

The present study therefore compares the inotropic and automatotropic (arrhythmogenic) activity of dopamine, dopexamine and dobutamine in isolated rabbit cardiac preparations. Right ventricular papillary muscles, the spontaneously firing sino-atrial, A-V junctional and right ventricular Purkinje fibre areas were dissected and placed in an organ bath containing modified Locke's solution, as described earlier (Papp and Vaughan Williams, 1969; Szekeres and Papp, 1986). The muscle preparations were electrically driven (1 msec duration, 100 per min frequency, twice threshold strength) and their contractility was recorded. The electrical activity of the three pacemaker areas was followed by recording surface potentials. The preparations were exposed to increasing drug concentrations, pD_2 values and intrinsic activities relative to dobutamine were determined.

All three drugs exert a concentration-dependent positive inotropic effect, giving a pD_2 value of 6.59 for dobutamine, 6.67 for dopamine and 7.11 for dopexamine. The intrinsic inotropic activity relative to dobutamine is 0.498 for dopamine and 0.324 for dopexamine. Dopamine, dopexamine and dobutamine are able to increase automaticity not only in the sino-atrial, but also in the A-V junctional and ventricular (Purkinje fibre) regions. The effect of the drugs on the relationship between the maximum contractile force and pacemaker activity is shown in Table I. The automatotropic potency relative to dobutamine is significantly larger for dopamine than for dopexamine. The order of the 'arrhythmogenic' potential of the drugs determined in this way is dopamine > dobutamine > dopexamine.

Table I

Effect of dobutamine, dopamine and dopexamine on the relationship between maximum contractile force and automaticity in pacemaker areas of the rabbit heart

	SA-NODE	AV-JUNCTION	PURKINJE FIBRES
DOBUTAMINE	R=1.000	R=1.000	R=1.000
	1.0997 ±0.1521	1.3168 ±0.1227	1.0545 ±0.1909
DOPAMINE	R=1.1372	R=2.0080	R=2.3941
	1.2506 ±0.1545	2.6441 ±0.0563	2.5246 ±0.0522
	(p=0.4150)	(p=0.0001^{x})	(p=0.0003^{x})
DOPEXAMINE	R=0.9333	R=1.3262	R=1.5607
	1.0263 ±0.1708	1.7464 ±0.0934	1.6458 ±0.0797
	(p=0.6395)	(p=0.0806)	(p=0.0119^{x})

Mean values of the slopes of regression lines obtained from five experiments are given ± S.D.
R = automatoropic activity relative to dobutamine.
p = level of significance relative to dobutamine; ^{x} $p<0.05$.

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EFFECT OF DIFFERENT VEGETABLE OILS ON ARRHYTHMIAS INDUCED BY MYOCARDIAL ISCHEMIA IN HYPERCHOLESTEROLEMIC RATS

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KEY WORDS: diet - fatty acid - myocardial infarction - rat

Feeding polyunsaturated fatty acid rich diets has been shown to protect the development of arrhythmias and sudden cardiac death during the acute phase of myocardial infarction in experimental animals (Leprán et al. 1981, 1988; McLennan et al. 1985, 1988). Human epidemiologic studies show positive correlation between the plasma cholesterol level and the incidence of coronary heart disease. The present experiments were devoted to study in experimental conditions the effect of different vegetable oil diets, rich in polyunsaturated fatty acids, on the severity of coronary artery ligation induced arrhythmias in hypercholesterolemic rats.

Male Sprague-Dawley CFY rats, weighing 220-250 g were fed with a standard laboratory chaw, supplemented by 12 % of either pork fat (as saturated fatty acid rich diet, SF), linseed oil (LSO), walnut oil (WNO), or safflower seed oil (SFO). The animals were also given 1 % cholesterol in the diet (+CH).

After 4 week-long diet during ether anesthesia a loose silk loop (Ethicon, 5-0) with a small polyethylene tubing was applied around the left main coronary artery about 2 mm from its origin (Leprán et al. 1983). One week after this preliminary operation acute myocardial infarction was produced by tightening the loose ligature. Continuous recording of bipolar chest electrogram was performed for 15 min or until the death of the animals. The incidence of arrhythmias, e.g. ventricular tachycardia (VT), fibrillation (VF), was registered.

Table 1. Survival rate (SR), incidence of ventricular fibrillation (VF) and tachycardia, and animals showing no arrhythmias (N) during the acute phase of myocardial infarction in conscious rats.

Group	n	SR(%)	VF(%)	VT(%)	N(%)
SF + CH	12	25	75	83	8
LSO + CH	11	73+	27+	45+	36
WNO + CH	11	91+	18+	36+	45+
SFO + CH	14	64+	43	57	43+

+ $p < 0.05$, compared to the SF+CH group.

Coronary artery ligation in conscious, freely moving rats produce severe arrhythmias within 4-6 min and only 25% of the control, saturated fatty acid+cholesterol fed rats survived the most severe 15 min of myocardial ischemia.

Linseed oil, that contains 44% linolenic acid (C18:3, n-3) and 30% linoleic acid (C18:2, n-6), significantly decreased the

incidence of VF and increased the survival rate during the acute phase of myocardial infarction.

Walnut oil and safflower seed oil - containing linoleic acid in 73% and 70%, respectively - produced similar protective effect. The incidence of arrhythmias was decreased and significantly more animals survived the acute phase of myocardial infarction without developing arrhythmias.

The present results show that feeding a polyunsaturated fatty acid rich diet, containing either n-6 or n-3 fatty acids, significantly reduce the incidence of life threatening arrhythmias and increase the chance to survive the most critical phase of myocardial infarction in conscious, hypercholesterolemic rats. Therefore the protective effect presumable manifested not only in decreasing atherosclerotic alterations, but a direct protection against ischemic changes may be involved, as well.

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THE RELEVANCE OF THE CALCIUM CHANNEL BLOCKER NIMODIPINE IN THE THERAPY OF CEREBRAL ANEURYSMS

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Introduction: Significant improvement of microsurgery technique in the treatment of ruptured intracranial aneurysms did not show the suspected satisfaying effects on the neurological dysfunction and mortality rate after surgery. An improvement of the outcome was expected using Calcium antagonists in the pre- and postoperative aneurysm therapy. Methods: 100 patients in the age of 13-75 years underwent aneurysm surgery. All patients received the Calcium channel blocker Nimodipine in a dosage of 2-3 mg/h i.v. before the operation already. In the past 16 month we performed transcranial doppler sonography (TDC) before and after surgery. The timing of the operation date depended on the clinical classification in grades Hunt/Hess. Patients with grade I to III were operated within 72 hours if they had no signs of vasospasm in TCD or angiography. Results: In spite of Nimodipine administration we found in 50 % of the H/H group I-III increased blood flow velocities of 120-200 cm/sec. and in H/H group IV-V in 80 % signs of vasospasm. The results of surgery show excellent and good outcome with full recovery in 70 % after early surgery and in 90 % after late surgery. The mortality rate was 9 % in first group and 3 % in the other group. Patients older than 65 years of age had a significant higher mortality rate. Delayed cerebral ischemia from vasospasm following SAH and surgery was in

the present study also rare as reported in other series (1). Conclusions: Our study demonstrated that the results of early and late aneurysm surgery with nimodipine medication were good. Nimodipine cannot prevent the incidence of vasospasm in all cases but eventually reduce the infarction size improving the outcome. Other factors may be responsible for vasospasm e. g. the amount of initial hemorrhage, chemical mediators and the surgical trauma. Further investigations are to be made in order to answer all the questions in dealing with the vasospasm.

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INFLUENCE OF THROMBOXANE ON ADRENERGIC NEUROTRANSMISSION IN ISOLATED GUINEA PIG ATRIA

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Key words: heart, adrenergic neurotransmission, thromboxane

Conflicting results have been obtained in studies on the thromboxane effects on adrenergic neurotransmission in peripheral preparations. In fact it has been demonstrated that noradrenaline release is potentiated by a thromboxane analogue in preparations obtained from the rabbit (Trachte, 1986; Trachte and Stein, 1989), but that this release is unaffected or reduced in peripheral preparations obtained from other animal species, such as the dog, rat and guinea pig (Makita, 1983, Nakajima and Toda, 1986).

In this study we have evaluated the effect of thromboxane analogues on the cardiac response to adrenergic nerve stimulation in isolated guinea-pig atria. The preparations were stimulated at 4 Hz by point stimulation; the stimulation of sympathetic nerves was obtained by field stimulation applied during the functional refractory period, in the presence of atropine (1 μM). The effect of field stimulation consisted of a transient increase in cardiac contractility, due to noradrenaline release.

The natural substance thromboxane B_2 (0.1-10 μM), and two stable thromboxane mimetics, $cTxA_2$ (1-100 nM) and U46619 (0.1-10 nM) have been studied. All these substances dose-dependen-

tly reduced the cardiac response to adrenergic nerve stimulation, with the following order of potency: U46619 > $cTxA_2$ > thromboxane B_2. The inhibitory effect of U46619 was antagonized by two thromboxane receptor antagonists, namely AH 23848B (0.1-10 nM) and sulotroban (0.1-1 µM), but was unaffected by the prostaglandin receptor antagonist AH 6809 (1µM). The effect of U46619 was not due to interference with postsynaptic noradrenaline receptors, since the agonist was not able to modify the positive inotropic effect induced by exogenous noradrenaline. These findings seem to suggest that thromboxane mimetics are able to reduce the stimulation-induced noradrenaline release. However, in guinea-pig atria loaded with [^{3}H]-noradrenaline, U46619 was not able to modify the stimulation-induced overflow of tritium, although it did reduce the positive inotropic effect of field stimulation.

These findings indicate that the effects of thromboxane mimetics on adrenergic neurotransmission in the guinea-pig heart are mediated by still unexplained mechanisms more complicated than the simple interaction with prejunctional and/or postjunctional cardiac receptors.

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DIFFERENTIAL BINDING OF AGONISTS AND ANTAGONISTS TO $GABA_A$ AND GLYCINE RECEPTORS

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Gamma-aminobutyric acid (GABA) and glycine are major inhibitory neurotransmitters in mammalian brain and spinal cord, respectively. $GABA_A$ and glycine receptors are coupled to chloride channels. GABA receptors were classified by selective antagonists. Bicuculline-sensitive $GABA_A$ binding sites have subpopulations of different affinities. This study seeks different binding modes of agonists and antagonists to these subpopulations. It also attempts to reveal similarities between $GABA_A$ and glycine receptor complexes.

Extensively washed synaptosomal membranes were prepared from rat whole brains and spinal cords (Maksay, 1990) and used for radioligand binding.

Tyrosine-specific reagents such as p-diazobenzenesulfonic acid (DSA) and tetranitromethane were found to decrease selectively the number of the low affinity $GABA_A$ sites (Maksay and Ticku, 1984a), while Eccles' anions (Cl^-, SCN^-) mask the high affinity population. DSA pretreatment and the presence of Eccles' anions shifted the ^{3}H-GABA-displacing potencies of $GABA_A$ agonists and antagonists in the opposite direction (Maksay and Ticku, 1984b). The super-low affinity $GABA_A$ receptors were characterized by the enhancing effect of GABA on ^{3}H-diazepam binding (Maksay, 1988). GABA antagonists SR 95103 and R 5135 shifted and suppressed the dose-response curve of GABA. It contradicts to a pure competitive binding of antagonists to the lower affinity $GABA_A$ sites.

Dissociation of the antagonist ^{3}H-SR 95531 binding was polyphasic. The rapid and slow phases were separated kinetically (Maksay, 1988). The displacing potencies of Table 1 show that the slower phase corresponded to higher affinity SR 95531 binding. This high affinity population for SR 95531 corresponds to the super-low affinity sites of GABA (Table 1).

Table 1. Displacing potencies of SR 95531 and GABA on the rapid and slow phases of ^{3}H-SR 95531 dissociation.

Displacer	IC_{50} (nM) rapid	slow
SR 95531	29±11	12±4
GABA	580±140	2620±1230

The chiral GABA agonist dihydromuscimol displaced high affinity ^{3}H-muscimol binding with great potency and stereoselectivity. In contrast, it displaced the antagonist ^{3}H-SR 95531 binding with low potency and stereoselectivity. The above findings can be explained by a preferential binding of antagonists to hydrophobic accessory sites around low affinity $GABA_A$ receptors.

Dissociation of ^{3}H-muscimol and ^{3}H-SR 95531 binding elicited by dilution was accelerated by excess GABA or SR 95531, suggesting cooperativity in binding (Maksay, 1990). The rapid first phase of dissociation was preferentially accelerated by the agent in antagonistic relationship to the radioligand. Modification of the receptors by DSA selectively decreased the accelerating effect of GABA on the dissociation of ^{3}H-SR 95531 (Maksay, 1990). It suggests that the low affinity $GABA_A$ sites are (partly) due to negative cooperativity in binding.

The dissociation of the antagonist ^{3}H-strychnine binding from spinal cord membranes elicited by dilution was preferentially accelerated by glycine (Maksay, 1990). Similarly, pretreatment with DSA decreased selectively this negative heterotrop (i.e. allosteric) interaction.

In conclusion, "direct" antagonists do not bind to their respective receptors in a pure competitive manner with $GABA_A$ and glycine agonists. Tyrosine residues might be involved in these allosteric interactions similar for the $GABA_A$- and glycine-gated chloride channels.

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A DUAL MEMBRANE STABILZING AND CALCIUM BLOCKING EFFECT OF KHL-8430 (CHINOIN), A FENDILIN ANALOG ON THE HEART

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Key words: KHL-8430, Class I and Class IV antiarrhythmics

The antiarrhythmic mode of action of the compound KHL-8430 (Chinoin), a fendilin analog of diphenyl-propylamine structure has been analyzed by a voltage clamp technique, based on a double sucrose gap arrangement, on the sino-auricular fibers of the frog. Sodium and calcium blocking properties were identified by a pharmacological approach (Kelemen and Marko, 1985.). Calcium-dependent slow inward current was activated by adrenaline (4.6 umol/l) or by histamine (5.4 umol/l) in sodium free solution or in the presence of tetrodotoxin (4.5 umol/l). Sodium-dependent fast inward current was enhanced by prostacyclin (PGI_2-Na, Chinoin, 0.27 umol/l), a physiological stimulator of the fast sodium channel in the heart (Kelemen and Marko, 1988), in calcium free solution or in the presence of D-600 (4.1 umol/l). Calcium blockers, like verapamil, prevent the activating effect of both adrenaline and histamine on the slow (calcium) inward current but leave the stimulatory effect of PGI_2-Na on the fast (sodium) inward current unchanged. Use of both adrenaline and histamine helps to differentiate beta-adrenergic blockers and histamine H_1 receptor blockers, respectively, from calcium blockers. On the other hand, sodium blockers, like lidocaine, prevent the stimulatory effect of prostacyclin on the fast sodium current, without affecting the action of adrenaline or histamine on the slow calcium current.

Effect of KHL-8430 was checked in a concentration range of 5 to 20 umol/l. Threshold concentration was 7 umol/l and maximal effect was reached at 14 umol/l. KHL-8430 had a blocking effect on both components of the cardiac inward current. It prevented, like lidocain, the prostacyclin-induced increase of the amplitude of the fast sodium current and, like verapamil, the adrenaline- and histamine-induced activation of the slow calcium current. Maximal amplitude of the fast inward current (at a depolarization step of +40 to +50 mV against the holding potential) was 1.1 ± 0.6 nA in 20 control preparations, it was increased to 1.9 ± 0.8 nA by 0.27 umol/l prostacyclin ($p < 0.001$), while in the presence of the same concentration of prostacyclin + 14 umol/l KHL-8430 the mean value of the maximal amplitude of the fast inward current was 0.4 ± 0.2 nA ($p < 0.001$ compared to the prostacyclin value). Slow inward current at depolarization steps of +70 to +80 mV against the holding potential could be rarely detected under control conditions but a typical slow inward current of 0.1 to 0.3 nA was consistently activated by either adrenaline or histamine (8 preparations each) which was completely abolished in all preparations by 14 umol/l of KHL-8430.

The results indicate that KHL-8430 combines the properties of Class I and Class IV antiarrhthmics, i.e. the properties of membrane stabilizers and calcium transport inhibitors.

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PHARMACOLOGICAL CHARACTERIZATION OF BENZOTRIAZOLE DERIVATIVES ACTIVE ON THE MUSCARINIC RECEPTOR.

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Key words: **benzotriazole, muscarine, receptor**

In order to explore the stereoelectronic requirement of muscarinic receptor attachment points, a series of benzotriazole derivatives was synthetized (La Rotonda et al., 1989) and investigated by radioligand (3H-N-methylscopolamine) binding experiments for their affinity to the different muscarinic receptors in the cerebral cortex, atria and ileum of the rat according to the method of Yamamura and Snyder (1974). Moreover, the antimuscarinic activity of the more interesting compounds was also investigated in vitro on guinea-pig atria and ileum. The results obtained are summarized in table I.

The affinities to the three muscarinic receptor subtypes differed generally, showing an interesting selectivity toward M_2 receptor (atrium). In vitro pharmacological tests seem to confirm this finding. Among the different benzotriazoles tested, compound III appeared the most active whereas the highest selectivity both on binding and in pharmacological tests was displayed by compound II. It seems that the 2-substituted isomers are more active than the corresponding 1-substituted isomers; moreover, polar effects of the substituents on the ammonium moiety seem to play a role in the capability to differentiate among M-subtype receptors. AF-DX 116, a classical antimuscarinic drug, with high affinity for M_2 receptors (Micheletti et al., 1987), exhibited similar selectivity but higher potency.

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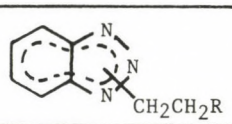
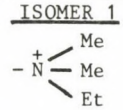
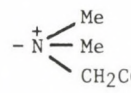
Table I – Affinities of some benzotriazole derivatives for the muscarinic binding sites present in cerebral cortex, atrium and ileum of the rat (radioligand binding studies) and antagonist activity evaluated in vitro on isolated right atrium and ileum of guinea-pig.



N.	R	K_i (M)			IC_{50} (M)	
		CORTEX	ATRIUM	ILEUM	ATRIUM	ILEUM
	ISOMER 1					
I		1.4×10^{-5}	7.5×10^{-6}	1.1×10^{-5}	9.6×10^{-6}	1.5×10^{-5}
II		7.2×10^{-5}	9.0×10^{-6}	2.4×10^{-5}	1.9×10^{-5}	1.1×10^{-4}
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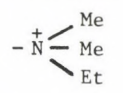
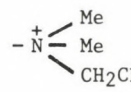
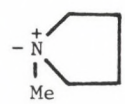
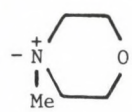
III		2.0×10^{-6}	1.0×10^{-6}	1.5×10^{-6}	1.1×10^{-6}	2.8×10^{-6}
IV		3.1×10^{-5}	9.9×10^{-6}	2.4×10^{-5}	1.2×10^{-5}	3.4×10^{-5}
V		1.2×10^{-5}	2.7×10^{-6}	6.5×10^{-6}	3.6×10^{-6}	1.1×10^{-5}
VI		2.4×10^{-5}	8.4×10^{-6}	1.6×10^{-5}	5.3×10^{-6}	3.2×10^{-5}
	AF - DX 116	8.2×10^{-7}	8.3×10^{-8}	2.4×10^{-7}	8.5×10^{-8}	5.2×10^{-7}

K_i = binding affinity determined as the capacity to induce 50% inhibition in the 3H-NMS binding calculated by the method of Cheng and Prusoff (1973). Values are the mean of 3-4 determinations.
IC_{50} = concentration of drug causing 50% inhibition of the submaximal contractions induced by acetylcholine 5×10^{-7} M (atrium) or 1×10^{-9} M (ileum).

HOW MAITOTOXIN INCREASES CYTOSOLIC CALCIUM LEVELS?

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Key words: maitotoxin, Ca++ channels, PI hydrolysis

Maitotoxin (MTX), a potent marine toxin, is able to increase intracellular calcium levels by a still unknown mechanism. A direct action both on voltage sensitive calcium channels (VSCC) (Freedman et al; 1984) and phospholipids breakdown (Bernard et al; 1988) has been suggested to explain MTX effect on calcium homeostasis. Conversely, MTX was recently supposed to simply act as a pore-forming agent (Sladeczek et al; 1988). We observed that MTX dose-dependently enhances free cytosolic calcium levels, measured by fura 2 and quin 2 fluorescent probes, in different cell types such as normal anterior pituitary cells (Schettini et al; 1984), PRL-secreting cell line 235-1 (Schettini et al; 1988; Meucci et al; 1988) and PC12 cells, while in EGTA containing medium MTX did not cause this stimulatory effect. In order to study the mechanism of MTX action on VSCC, we tested the effect of MTX in presence of organic calcium channel antagonists (verapamil, nicardipine), which specifically block L-type calcium channels. Nicardipine clearly inhibited MTX-induced calcium levels (Table I), and pertussis toxin pretreatment significantly reduced nicardipine inhibition of MTX-induced calcium rise. Furthermore, the addition of Bay-k 8644, a dihydropyridine derivative agonist, one minute before MTX caused a twofold increase of MTX-induced calcium rise. These results support the hypothesis

that MTX could act at L-type calcium channels level.

We also studied the possible involvement of N-type calcium channels in MTX action, by using omega-conotoxin (GVIA fraction), a toxin which blocks N-type calcium channels. In omega-conotoxin treated PC12 cells the effect of MTX was reduced by 48%, thus also suggesting a possible activation of N-type calcium channels by MTX (Table I).

Table I: Nicardipine (Nic)(1uM) and omega-conotoxin (Ctx) (1uM)inhibition of MTX-induced intracellular calcium levels.

	Control	Nic	Ctx	Nic+ Ctx
BASAL	80	--	75	--
MTX (1ng/ml)	299	114	183	99

values represent intracellular calcium level (nM)

Finally, the effect of MTX was evaluated on inositol phosphate production in PC12 cells. We found that MTX caused a dose-dependent enhancement of inositol phosphate production. Nicardipine reduced, and a calcium-free medium containing EGTA abolished, the MTX-stimulation of phosphoinositides hydrolysis. In conclusion, our data suggest that MTX-induced increase of intracellular calcium levels could be ascribed to an enhancement of calcium mobilization that occurs through VSCC.

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EFFECTS OF BISPHOSPHONATES ON CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

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Key words: Bisphosphonates; Osteoclasts; Mononuclear Phagocytes.

Bisphosphonates (BPs) are inhibitors of bone resorption and several studies have shown that they impart potent inhibitory effects on either osteoclasts or their (probably hematopoeitic) precursor cells. We tested the effects of three BPs,dichloromethylenebisphosphonate (CL_2MBP),6-amino-1-hydroxyexylidene-1,1-bisphosphonic acid (AHHexBP) and 4-amino-1-hydroxybutylidene-1,1-bisphosphonate (AHBuBP) on the formation of bone marrow derived osteoclast-like multinucleated cells(MNC). The cells were obtained from the mononuclear fraction of the bone marrow aspirates of rabbits. They were cultivated for a period of three weeks in 16 mm tissue culture wells containing 0,5 ml of medium + test substances. At the end of the incubation period the cells were fixed and stained (Wright's Giemsa). MNC formation was stimulated with 10 nM of 1,25-dihydroxyvitamin D_3 and measured by counting the total number of cells/well containing three or more nuclei using an inverted stage phase microscope.The results obtained show that AHBuBP inhibits the MNC formation at lower concentrations than CL_2MBP and AHHexBP. The results are dose-dependent and statistically significant .For instance,at a concentration of $10^{-7}M$ of BPs the number of MNC per well were reduced from 210 (mean control value) to 110,70 and 50 with CL_2MBP,AHHexBP and AHBuBP respectively. Increasing the BPs concentration to $10^{-4}M$,the number of MNC per well fell progressively to almost null.

Since osteoclasts probably originate from hematopoeitic precursors (a member of the monocyte-macrophage system) various authors have suggested that macrophages(Mϕ) could act as substitutes in "in vitro" model systems,whereby the action of BPs on osteoclast function could be reproduced and easily interpreted (Stevenson,1980).

The three BPs employed on the bone marrow culture system were used to study

some aspects of Mϕ function (phagocytosis,superoxide production,cell viability). The cells were removed from the peritoneal cavity of the rat with heparinized Hank's balanced salt solution. The Mϕ were either resident or elicited by initially injecting thioglycollate intraperitoneally (Gallily,1967).

Cell viability was determined by using the Trypan blue exclusion test. All three substances displayed a dose-dependent increase in cell toxicity,although this effect became significant only at a concentration of 10^{-3}M even with AHBuBP,the most potent of the three: this determined a 10 and 5% mortality rate in the resident and elicited Mϕ,respectively. CL_2MBP and AHBuBP at 10^{-2}M concentration caused respectively a 10 and 15% mortality rate in resident Mϕ.

The phagocytic activity was measured by counting the number of Mϕ that injested opsonised S. Cerevisiae spores. The inhibitory activity was not significant for all the tested BPs up to 10^{-2}M concentration. At this concentration, the number of cells with phagocytosed spores (with respect to 100 cells counted) were drastically reduced from 60 to 20 in the elicited Mϕ and from 50 to 10 for the resident Mϕ. The results were similar for all the BPs.

Superoxide radical production from zymosan stimulated Mϕ was evaluated spectrophotometrically by the ferricytochrome-c reduction technique. Resident cells, cultivated in dishes for 24 hours prior to incubating them with BPs (30 & 60 min.) showed a dose-dependent reduction in O_2^- production but only in the presence of very high concentrations of the compounds. For example,AHBuBP,the most potent of all three,inhibited 20 to 30% of O_2^- release at 1 mM and 5 mM respectively.

The results of our study point out that BPs are only weak inhibitors of Mϕ function or at least they are active only at very high concentrations,not comparable with those that are renowned for osteoclast function inhibition or recruitment. Moreover,the relative potencies of the BPs on Mϕ function inhibition do not correspond to their bone-resorbing capacity.

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A NEW SPECTRUM ANXIOLYTIC: 3-AMINO-4-ETHYLTHIO-7-Cl-QUINOLINE. HCl (EGIS 5278).

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Keywords: Benzodiazepine receptors, chlordiazepoxide, tofisopam EGIS 5278, Miklya-Knoll test.

Tofisopam, a 3,4-benzodiazepine derivative used for its anxiolytic activity in man, is known to be completely ineffective in animal tests, like the Vogel test (Pellow and File, 1986). We described recently (Miklya and Knoll, 1988a,b) a conflict situation by the aid of which we were able to measure the anxiolytic effect of tofisopam and demonstrate that this effect can be antagonized by Ro 15-1788, the selective benzodiazepine receptor antagonist.

The results of a comparison of the effects of chlordiazepoxide and tofisopam in our test suggested that two subtypes of benzodiazepine receptors one with a higher and one with a lower affinity to chlordiazepoxide (named for sake of brevity BR1 and BR2 receptors) were involved in the effect of this drug,whereas tofisopam acts selectively on BR1 receptors.

The way was open for the search of new derivatives acting selectively on BR1 receptors and being much more potent than tofisopam. Structure-activity relationship studies during a 5-year period revealed that some quinoline-derivatives meet the requirements. Out of 63 compounds with essentially similar pharmacological spectrum the thioaminoquinoline derivative

3-amino-4-ethylthio-7-Cl-quinoline.HCl (EGIS 5278), was selected as the reference substance, representing the new spectrum anxiolytic activity.

The analysis of the anxiolytic activity of EGIS 5278 in comparison to reference substances revealed, that Ro 15-1788 left the effect of phenobarbital unchanged and antagonized the anxiolytic activity of chlordiazepoxide, tofisopam and EGIS 5278 which proved to be 20 time more potent than chlordiazepoxide and 3000 times more potent then tofisopam in the Miklya-Knoll test. The ratio between the sedative and anxiolytic dose was found to be 2.5 for diazepam, 8 for phenobarbital 26.6 for tofisopam, 100 for chlordiazepoxide and 500 for EGIS 5278. EGIS 5278 did not exert any significant anticonvulsant activity in mice treated with penthylentetrazol or picrotoxin.

The working hypothesis is forwarded that EGIS 5278 is selectively acting on a subtype of central benzodiazepine receptors and by this mechanism we have a benzodiazepine-receptor-related anxiolytic activity without significant sedative, hypnotic and anticonvulsant effect.

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ONE-DAY KINETICS OF $CaCl_2$ ARRHYTMOGENICITY FOLLOWING ACUTE ISOPRENALINE TREATMENT

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(arrhythmia,isoprenaline,desensitization,$CaCl_2$)

It is well known that those compounds which raise intracellular cAMP level in the myocardium, increase the susceptibility of the heart to arrhythmias (Lubbe et al., 1987).This partially explains the arrhythmia inducing effect of isoprenaline, too. On the other hand, it has been found that chronic administration of isoprenaline reduces the severity of adrenaline induced ventricular arrhythmias (Das et al., 1986). It has however, not been studied how single large doses of isoprenaline (1.0-2.5-5.0 mg/kg) influence the arrhythmic tendency to $CaCl_2$. The objectives of our investigations in that connection were therfore the following:

1/ Does isoprenaline pretreatment influence the development of $CaCl_2$-induced arrhythmia?

2/ If so, what is the kinetics of this effect ?

Experiments were perfomed in male Wistar rats of 250-300 g body-weight. Isoprenaline pretreatment was carried out with different doses (1.0-2.5 and 5.0 mg/kg) 30 min-1-2-3-6 and 24 hours before eliciting $CaCl_2$ arrhythmia.

The $CaCl_2$ solution (200 mg/ml) was injected into the right jugular vein of anaesthetized (40 mg/kg petobarbital-Na, i.p.) rats through a cannula, at a rate of 0.4 ml/min until the appearance of ventricular tachycardia. In the untreated (control) group we compared the dose of $CaCl_2$ required for inducing ventricular tachycardia to that of $CaCl_2$ eliciting tachycardia in the isoprenaline pretreated group. The difference found between the two groups was expressed in percentage form (mean $\pm$ S.E.M.), significance was calculated by Student's t test. The results are presented in the following

table:

(The negative sign before the percentual changes indicate the percent difference between the quantities of $CaCl_2$ required for eliciting tachycardia compared to control group)

PERCENT OF DIFFERENCES BETWEEN THE CONTROL AND ISOPRENALINE-TREATED GROUPS

DOSE mg/kg	n	30 min	n	1 h	n	2 h	n	3 h	n	6 h	n	24 h
1.0	16	-43.2xxx	29	35.0xxx	27	43.2xxx	28	35.2xxx	16	7.1x	23	6.5xx
2.5	18	-44.8xxx	27	-3.3xxx	28	48.3xxx	25	107.3xxx	24	28.8xxx	19	3.3n.s.
5.0	27	-40.1xxx	18	-14.8xxx	16	-4.3n.s.	16	78.1xxx	16	67.6xxx	15	9.6 xx

xxx=p~0.001;xx=p<0.01;x=p<0.05;n.s.= not significant;n= number of animal

At the begining we found increased arrhythmic tendency towards $CaCl_2$ at each dose level. With elevation of the doses the duration of the developed arrhythmia also prolonged. This enhancement of the susceptibility to arrhythmias was followed by significant desensitization, so that arrhythmia could be induced only with considerably higher doses of $CaCl_2$. The arrhytmic tendency, characteristic to the control group, returned to the base level within 24 hours in all cases, independently of isoprenaline dose applied. The early (0.5-1.0 h.) increase in the tendency for arrhythmia may probably be conected with the increase of cAMP level, while the subsequent decrease in arrhytmic tendency (1-3 h) may be related to reduced cAMP formation, meaning that we are faced with a desenzitation process (Blaiklock at al., 1978).

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PHARMACOTHERAPY OF OPIATE ADDICTION IN MARCHE REGION.

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Key words: Addiction Treatment - Follow-up Study - Opioids

The therapeutic approach to drug addiction should be comprehensive of several treatments. Modality settings of individual plan of addiction treatments together with simultaneous resort to several complementary interventions, sometimes synergistic, had been subjected to an integrated evaluation review.

The epidemiological research started January 1989, a collaborative effort of the Regional Department of Health and the University and Hospital Units, focused on the study and follow-up of the various treatment programs, in use both at public and private Regional Centers.

In this note the results of the first step of the overview are reported. The goal to reach detailed knowledge of the therapeutic plans of the 23 public Centers, particularly in reference to the pharmacological therapy, has been hit, as depicted in Table 1.

Tab. I: Pharmacological treatments adopted by 23 public Centers (750 patients for 1989; Marche Region: 1,500,000 of inhabitants).

Only substitutive drugs	8.7%
Only non-substitutive drugs	21.7%
Substitutive and/or non-substitutive drugs	56.5%
Drug-free Centers (n. 3)	13.0%

Among the non-substitutive drugs, the mostly preeminent

use is of sedative-hypnotics and tranquillisers, non-narcotic analgesics, clonidine, naltrexone (after detoxification); muscle relaxants, trazodone, neuroleptics and corticosteroids are less frequently employed.

The treatments with substitutive drugs include the outpatients (ambulatory) detoxification by scalar doses over three weeks, the maintenance on methadone (exceptionally), the hospitalization and rapid detoxification with methadone, and the slow scalar detoxification with methadone or buprenorfine.

In the 96% of cases the pharmacotherapy is associated with non-pharmacological methods (full psychotherapy cycles, family therapy, counseling, social rehabilitation, generic psychological support).

The study of treatment effectiveness includes the specification of the treatment paradigms, the taking into account of the problems of comparison across treatments, the periodic evaluation of ten parameters during treatments and postreatments, the choice of minimal periods of observation, the evaluation of validity of interview data and the characterization of consistency of samplings.

At the present step, emergent problems result from the inadeguate organization and the poor coordination inside and between Centers; a relevant programme managers in the therapeutic group staffings and a better and permanent training towards prevention are urgently requested.

CHEMICAL (ALCOHOL,NaOH,NaCl AND HCl)-INDUCED CHANGES IN THE GASTRIC MUCOSAL MEMBRANE-BOUND ATP-DEPENDENT ENERGY SYSTEMS

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Key-words:chemicals, gastric mucosal lesions, energy systems

Gastric mucosal lesions could be produced by intragastric administration of different necrotizing agents,such as 0,6 M HCl,0.2 M NaOH,25% NaCl or 96% alcohol (Robert et al.,1979). These necrotizing agents produce about the same number and severity of gastric mucosal lesions in rats, at 1 hour after administration of necrotizing agents,however, the developmental mechanisms of mucosal lesions remained unknown (Mózsik & Jávor,1988).

The aim of this study was to evaluate the possible changes in the gastric mucosal membrane-bound ATP-dependent energy systems during the development of gastric mucosal lesions produced by alcohol,NaOH,Nacl and HCl.

Sprague-Dawley (Lati,Gödöllő,Hungary)-strain rats,weighing 180-210 g, were used for this study. The animals were fasted for 24 hours before the experiments. The gastric mucosal lesions were produced by intragastric administration of 0,6 M HCl, 0,2 M NaOH, 25% NaCl or 96% ethanol (1 ml). The animals were sacrificed at 1 hour after administration of necrotizing agents,when the number and severity of gastric mucosal lesions was noted. The tissue levels of ATP,ADP,AMP and lactate were enzymatically, while cAMP by RIA, and their quantities were expressed as means $\pm$ SEM per one 1 mg mucosal protein.

The tissue levels of ATP and cAMP were decreased,while ADP increased at 1 hour after administration of necrotizing agents (Fig.1). The extents of ATP-ADP transformations were increased in assotiation to the decreased ATP-cAMP and cAMP-AMP transformations (Fig.2).

It has been concluded that: 1.The development of gastric mucosal damage associated with an increased energy liberation,obtained by the increased extent of ATP-ADP transformation; 2.The increased ATP-ADP transformation associated with the decreased extents of ATP-cAMP and cAMP-AMP transform

ations; 3.The similar changes were obtained in the gastric mucosal membrane-bound ATP-dependent energy systems,during the development of mucosal injury produced by different necrotizing agents.

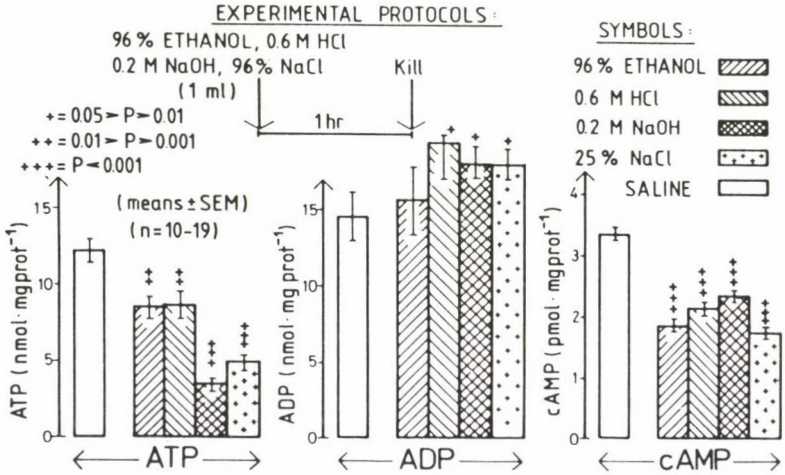

Fig.1.Chemicals-induced changes in the cellular energy systems in the rat gastric mucosa (numbers are 14-16, and severities are about 50 per one rat stomach in all groups of animals).

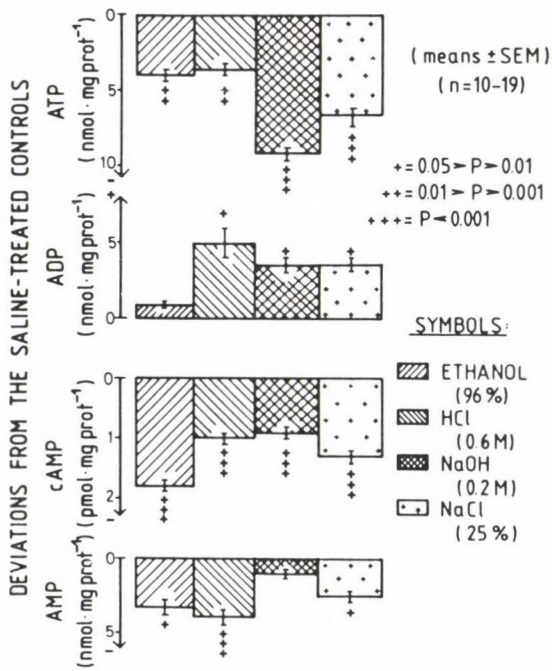

Fig.2.Changes in the feedback between the membrane-bound ATP-dependent energy systems, during the development of gastric mucosal damage produced by different necrotizing agents.

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GASTRIC CYTOPROTECTION MEDIATING IN SH-GROUPS IS FALIED BY SURGICAL VAGOTOMY:

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Key-words:gastric cytoprotection,SH-groups,L-cysteine,NEM,vagotomy.

It has been observed that the gastric cytoprotection,at least partly, mediates via SH-groups of mucosal proteins (Szabó et al.,1981).SH-group (L-cysteine) has gastric mucosal protecting,while SH-blocker compound (N-ethylmaleimide,NEM) has aggravating effect on the development of gastric mucosal damage produced by different necrotizing agents.

The aims of this study were:1.To evaluate the gastric cytoprotective effect of L-cysteine on the number and severity of gastric mucosal lesions produced by intragastric administration of ethanol(ETOH); 2.To study the extent of aggravation of N-ethylmaleimide (NEM) on the ETOH-induced gastric mucosal damage in rats with intact vagal nerve and after acute surgical vagotomy.

The observations were carried out on Sprague-Dawley (LATI,Gödöllő,Hungary)- strain rats,weighing 180-210 g.The animals were fasted for 24 hours before the experiments.The gastric mucosal damage was produced by intragastric administration of 96% ethanol(ETOH) (1 ml). L-cysteine and NEM (in different doses) were given at 30 min before administration of ETOH. The animals were sacrificed at 1 hour after ETOH administration,when the number and severity of gastric mucosal lesions was noted.The results were expressed as means $\pm$ SEM per one rat stomach.

L-cysteine dose-dependently prevented,while the NEM aggravated the number (Fig. 1) and severity of ETOH-induced gastric mucosal lesions in rats with intact vagal nerve, but their effects disappeared after acute surgical vagotomy.

It has been concluded that: 1.The gastric cytoprotective effect of SH-

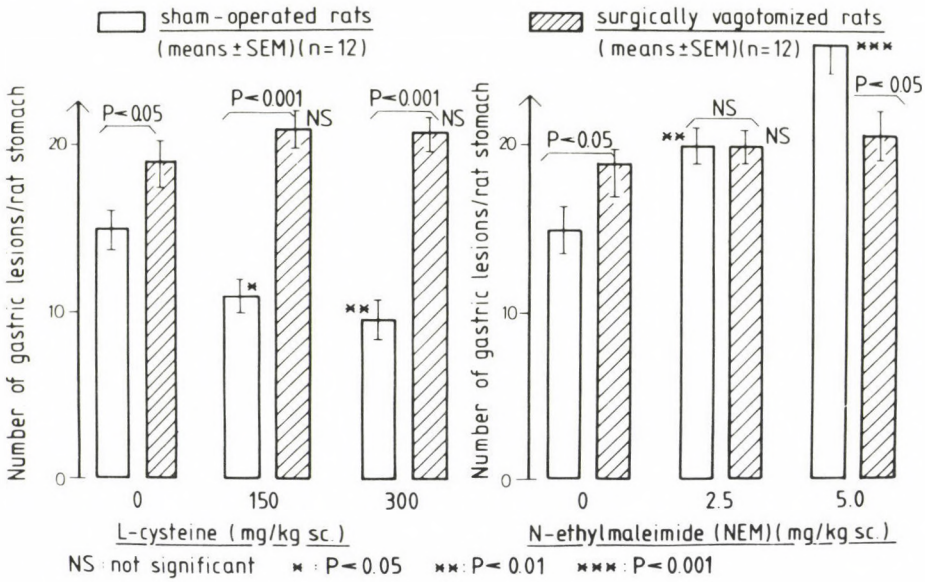

Fig.1.Effects of L-cysteine and N-ethylmaleimide (NEM) on the number of ethanol (ETOH)-induced gastric mucosal damage in rats with intact vagal nerve and in rats after acute surgical vagotomy.

group disappeared after surgical vagotomy;2.The aggravating effect of SH-blocker failed by surgical vagotomy; 3.The acute surgical vagotomy modified the functional properties of SH-proteins in the gastric mucosa.

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THE SHORT TERM COMPARATIVE STUDY OF ANTIULCER DRUGS WITH DIFFERENT MODES OF ACTION IN DUODENAL ULCER PATIENTS

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Key-words: duodenal ulcer, ulcer planimetry, protective agents

Pharmacodynamic studies were carried out with different antisecretory, antacid and cytoprotectiv compounds on duodenal ulcer patients. Patients were randomly selected in different treatment groups. Duodenal ulcer was endoscopically verified and followed. A detailed case report, different laboratory tests were performed at the beginning and the end of treatment. Every patient had a diary card to register the size of ulcer, the complaints, antacid consumption, and possible side effects. Authors investigated the ulcer healing effect of Cimetidine/1000mg Ranitidin/300mg/, Gastrozepine/150mg/, Sucralfat/1000mg/, Tisacid /Al-Mg antacid/ given in different doses, the combination of Atropine/1.8mg/ with Peritol/cyproheptadinum chloratum, 12mg/ supplied with antacid as required, and a combination of Atropine /1.8mg/, Carbenoxolon/500mg/ plus cyproheptadinum chloratum/12mg/. By the mathematical analysis of the results on the healing rates produced by different compounds no significant difference was obtained. The planimetrically measured ulcer sizes were decreased significantly during the first two weeks period, meanwhile this decrease was smaller in the second two weeks period. It can be supposed that from the point of pharmacodynamic action the studiing of the uncompletely healed ulcers can give more infor-

mations. The ulcer size significantly decreased during the first two week period -like in the summarized ulcer size changing, -but no significant difference was obtained among the groups. Comparison of the ulcer sizes to their basic sizes in patients who were not healed during the four week period, showed that the ulcer size were expressively different at the beginning of the treatment, and the reduction in them were found to be significant.

It has been concluded based on our data that there is no evaluable difference among the ulcer healing rates produced by antisecretory drugs, antacids and cytoprotectiv drugs or by their combination in duodenal ulcer patients.

DIFFERENTIAL RESPONSIVENESS TO ANTIARRHYTHMIC DRUGS IN THE RABBIT ATRIO-VENTRICULAR CONDUCTING SYSTEM

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Keywords: atrio-ventricular conduction, calcium antagonists, fast sodium channel blocking antiarrhythmics.

Pharmacological 'mapping' of the regional effects of drugs in the heart have revealed important differences (Papp et al., 1989; Winslow, 1984). It was, therefore, of interest to study the responsiveness of distinct portions of the atrio-ventricular conducting system to some of the representatives of Class 1 (mexiletine, prajmaline) and Class IV (diltiazem, verapamil) antiarrhythmics. Results have also been obtained with a mixed--type (Class 1+4) antiarrhythmic drug, propafenone.

Hearts of young rabbits were removed and a complex cardiac preparation was made which consisted of the inter-atrial septum with the A-V node, the inter-ventricular septum containing the His-bundle, the right bundle branch and papillary muscles and the right distal Purkinje network with attached ventricular muscle. The preparation was put in an organ bath containing modified Locke's solution. Atrium-His bundle (A-H), atrium-Purkinje fibre (A-P), His bundle-Purkinje fibre (H-P) and His bundle-papillary muscle (H-V) intervals as well as A-H, H-P and H-V effective refractory periods were determined in the absence and presence of the drugs, as described earlier (Németh et al., 1986).

Exposure of the preparation for 60 min to drugs with Class 4 antiarrhythmic properties (diltiazem, 0.2 mg/l; verapamil, 0.1 mg/l; propafenone, 1 mg/l) usually resulted in a prolongation of the A-H and A-P conduction times, and an increase in the A-H effective refractory period. On the other hand, agents known to block fast sodium channels i.e. Class 1 anti-

arrhythmics (mexiletine, 2 mg/l; prajmaline 0.5 mg/l; propafenone, 1 mg/l) exert a marked and, for the most part, predominant effect on the H-P and H-V intervals and the respective effective refractory periods (Table I).

It is thus apparent that, as regards their action the atrio-ventricular conducting system, antiarrhythmics classified in the same group of drugs may have a distinctive electropharmacological profile.

Table I

Effect of antiarrhythmic drugs on the rabbit atrio-ventricular conducting system

Parameter	Control (n=40)	Diltiazem 0.2 mg/l (n=6)	Verapamil 0.1 mg/l (n=10)	Mexiletine 2 mg/l (n=5)	Prajmaline 0.5 mg/l (n=5)	Propafenone 1 mg/l (n=8)
CONDUCTION						
A-H	62.9 ±5.3	107.0$^+$ ±9.5	93.5$^+$ ±9.5	71.0 ±4.0	64.6 ±3.6	68.8 ±1.6
A-P	88.2 ±5.6	118.0$^+$ ±8.6	121.2$^+$ ±10.5	91.0 ±6.9	105.2 ±11.7	115.0$^+$ ±12.4
H-P	24.4 ±2.5	27.6 ±4.5	26.7 ±2.7	27.6 ±8.4	46.0$^+$ ±6.8	46.3$^+$ ±5.2
H-V	38.4 ±2.7	39.2 ±3.1	41.8 ±2.3	61.2$^+$ ±4.9	50.6$^+$ ±9.8	58.6$^+$ ±6.1
ERP						
A-H	208.6 ±6.9	342.0$^+$ ±9.2	346.3$^+$ ±11.1	232.0 ±12.4	225.0 ±21.8	242.5$^+$ ±8.6
H-P	193.5 ±6.8	193.3 ±8.1	171.0 ±5.5	238.0$^+$ ±17.2	223.0$^+$ ±7.9	246.0$^+$ ±21.3
H-V	181.2 ±5.1	180.0 ±10.0	162.5 ±5.4	258.0$^+$ ±19.6	217.0$^+$ ±12.4	252.5$^+$ ±17.9

Mean values are given ± S.E. n = number of preparations. $^+$ $P<0.05$
ERP = effective refractory period. See text for abbreviations.

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INHIBITION OF SARCOLEMMAL ATPase AND pNPPase BY CH-103

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Key words: Beta blocking drugs, Na^+,K^+-ATPase, K^+-pNPPase

Beta adrenoceptor blockers, - in addition to their specific drug-receptor interaction, - possess a wide range of specific membrane activity (SMITH, 1982). The beta blocker CH-103 (4-cyclohexilamino/-1-/1-naphtolenyloxy/-2-butanol maleate) has also inhibited the plasma membrane Na^+,K^+-ATPase (SZABÓ et al., 1988). Because the basic ATPase activity in rat myocardium relatively high, it seemed worth studying the effect of CH-103 on the indirect enzymatic manifestation of Na^+,K^+-ATPase, i.e. the K^+-stimulated p-nitrophenylphosphatase (K^+-pNPPase), where a more favourable per cent distribution of enzyme activity could be detected.

Pooled rat heart ventricles were homogenized in 10 volumes of 20 mM Tris-HCl containing 1 mM EDTA and 0.1 mM phenylmethanesulfonyl fluoride /pH 7.0/ (VELEMA and ZAAGSMA, 1981). Protein content was determined according to LOWRY et al. (1951). The ATP and pNPP hydrolysing activities of crude cardiac homogenate were studied with and without beta blockers (AKERA, 1984).

In crude cardiac homogenate only 20% of the total ATPase was the Na^+,K^+-stimulated portion, while the K^+-stimulated portion of pNPPase was higher (35%). CH-103 significantly inhibited the total ATPase and pNPPase activities at concentrations $5x10^{-4}$M and $1x10^{-4}$M, respectively. To inhibit significantly the basic ATPase, higher concentrations of CH-103 were necessary ($2x10^{-3}$M). In the case of basic pNPPase activity we could not achieve a significant inhibition at all. Linewaver-Burk plot of the data by employing different substrate concentrations revealed that inhibitory effect of CH-103 on total ATPase and pNPPase activities was mainly due to a decrease in V_{max} values. In the case of total ATPase the K_m did not change significantly, but a sig-

Table I. Kinetic parameters of ATPase and pNPPase activities in the presence and in the absence of 2 mM CH-103

Enzyme activity	V^{*}_{max}		K^{**}_{m}	
	control	CH-103	control	CH-103
ATPase				
total	14.41 ± 0.94	6.18 ± 0.23^{x}	0.441 ± 0.044	0.384 ± 0.004
basic	13.34 ± 0.59	8.00 ± 0.57^{x}	0.364 ± 0.016	0.425 ± 0.007^{x}
pNPPase				
total	0.98 ± 0.09	0.49 ± 0.02^{x}	0.746 ± 0.094	0.274 ± 0.027^{x}
basic	0.78 ± 0.05	0.64 ± 0.03	0.732 ± 0.061	0.580 ± 0.039

*V_{max} values of ATPase and pNPPase are expressed in 10^{-6}mol P_i/mg protein/hour and in 10^{-6}mol pNP/mg protein/hour, respectively

**K_m values od ATPase and pNPPase are expressed in 10^{-3}M/l ATP and in 10^{-3} M/l pNPP, respectively

$^{x}p < 0.001$ (significantly different from control)

nificant decrease of K_m for total pNPPase activity may indicate an increase in substrate affinity. The basic ATPase activity was also depressed by CH-103 with a decrease in V_{max} and an increase in K_m (Table I).

In spite of the more favourable per cent distribution of total pNPPase, CH-103 has inhibited the Na^+,K^+-ATPase to a higher extent than the K^+-stimulated pNPPase. This effect may originate from the different intramembranal localization of the active sites of enzyme.

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PRESYNAPTIC CONTROL OF STRIATAL TYROSINE HYDROXYLASE ACTIVITY BY CORTICOTROPIN - RELEASING FACTOR.

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key words : CRF ; Tyrosine hydroxylase; striatum.

Corticotropin-releasing factor (CRF), originally identified as a hypophysiotropic peptide, has subsequently been found to affect a variety of brain functions (Aguilera et al., 1987). Recently, we have observed that in vitro CRF causes stimulation of synaptosomal dopamine (DA) synthesis which is associated with an increase of tyrosine hydroxylase (TH) activity in rat and mouse striatum (Olianas and Onali, 1988; 1989). In mouse striatum, CRF maximally increases the enzyme activity by 60 % and this effect appears to consist in an increase of the app. Vmax of the enzyme form with high affinity (Km 80 μM) for the cofactor. The CRF response occurs at nanomolar concentrations of the peptide (EC 50 15 nM), is significantly antagonized by the CRF receptor antagonist α-helical CRF 9-41 (Ki 120 nM) and withstands gel filtration treatment of the synaptosomal extract. Sauvagine and urotensin I, two peptides displaying sequence homology to CRF, mimick the CRF effect with EC 50 values of 5 and 10 nM, respectively. An important property of the CRF stimulation of TH activity is the strict dependence on the concentration of extracellular Ca^{++}. In a Ca^{++}-free medium, the stimulatory effect of the peptide is minimal and is maximal at about 0.5 mM extracellular Ca^{++}. Moreover, polymyxin B, a relatively selective protein kinase C inhibitor, antagonizes the CRF effect (IC 50 30 μM), suggesting a possible role of the second messengers generated by phospholipids hydrolysis in the action

of CRF. To test this hypothesis, we investigated the effect of neomycin, an aminoglycoside which binds to phosphoinositides and inhibits their hydrolysis.

Table 1
Inhibitory Effect of Neomycin on CRF Stimulation of Synaptosomal TH Activity in Mouse Striatum.

		TH activity (pmol CO_2/min/mg prot) basal	CRF-stimulated*
vehicle		20.5 ± 0.5	12.5 ± 1.0
neomycin	0.1 mM	20.2 ± 0.6	9.1 ± 0.8
	0.3	19.1 ± 0.4	7.7 ± 0.6b
	0.6	15.5 ± 0.2a	5.4 ± 0.3b
	1.2	14.8 ± 0.4a	5.5 ± 0.2b

* net increase of TH activity above basal produced by 0.5 μM CRF
a $P < 0.05$ vs vehicle ; b < 0.05 vs CRF alone.

As shown in Table 1, neomycin causes a concentration-dependent inhibition of both basal and CRF stimulated TH activities with maximal effects corresponding to 28 % and 60 % reductions, respectively. The IC 50 value of neomycin in inhibiting the CRF effect is about 0.15 mM. These results indicate that in striatal dopaminergic terminals CRF receptors control the state of activation of TH by a mechanism that requires both Ca^{++} and inositol phospholipids hydrolysis.

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POSITIVE CORRELATION OF CALCIUM CONCENTRATION IN CEREBROSPINAL FLUID AND RECTAL TEMPERATURE IN RABBITS.

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Keywords: Thermoregulation, CSF calcium.

Experiments performed by Feldberg et al. (1970) and Myers and Veale (1971) led to formulate the theory that the chemical composition of the cerebrospinal fluid (CSF) in the 3rd ventricle adjacent to the temperature centres in the hypothalamus exerts an influence on body temperature. It was also postulated that the "set point" of the thermoregulatory system is determined by the balance between Na^{+} and Ca^{++} ions in CSF. A recent study carried out in our laboratory, showed that in rabbits neurones responsible for temperature control are a target of organic calcium antagonists or agonists. In fact, intracerebroventricular injection of Verapamil, Nifedipine and Cinnarizine caused a dose-related hyperthermic response, while the injection of BAY-K-8644, resulted in a dose-dependent hypothermic response (Palmi and Sgaragli, 1989). These findings led us to hypothesize that calcium concentration in CSF might have a relevant role in thermoregulation. If this was the case, when body temperature increases as it happens in fever, there should be a change in (Ca^{++}) of the fluid bathing the brain. To test this hypothesis, in the present study, we checked whether a possible correlation exists between CSF (Ca^{++}) and rectal temperatue in rabbits following recovery from surgical implantation of a cannula into the **cisterna magna**. Conscious, restrained animals (n=29), 2-3 days after surgery had been performed under nembutal anaesthesia, were kept at constant ambient temperature of 20 ^{o}C throughout the experimental session and, one hr after their rectal temperature was stabilized, samples of CSF were withdrawn during a 2 hrs period at 24 min intervals and at a flow rate of 5 ul/min. Total CSF calcium concentration was determined by the method of Stern and Lewis (1957), using ortho-cresolphthalein complexone as Ca^{++} complexing agent and 8-hydroxyquinoline-5-sulfonic-acid to eliminate Mg^{++} interference. CSF protein content was also determined according to the coomassie-blue binding method. Animals exhibited a wide range of rectal temperatures, from normothermia to fever and also their CSF (Ca^{++}) showed a large variability. These two parameters, as shown in fig. 1, were positively and highly significantly correlated. On the contrary, CSF protein concentration (64 ± 4.3 mg x 100 ml^{-1}, mean value $\pm$ s.e.m., n=18) did not seem to be related either to rectal temperature of to CSF (Ca^{++}). Even though a previous study carried out in men failed to evidentiate any correlation between body temperature and lumbal CSF (Ca^{++}) (Nielsen et al. 1973), our findings give support to the hypothesis that brain calcium metabolism plays a role in thermoregulation.

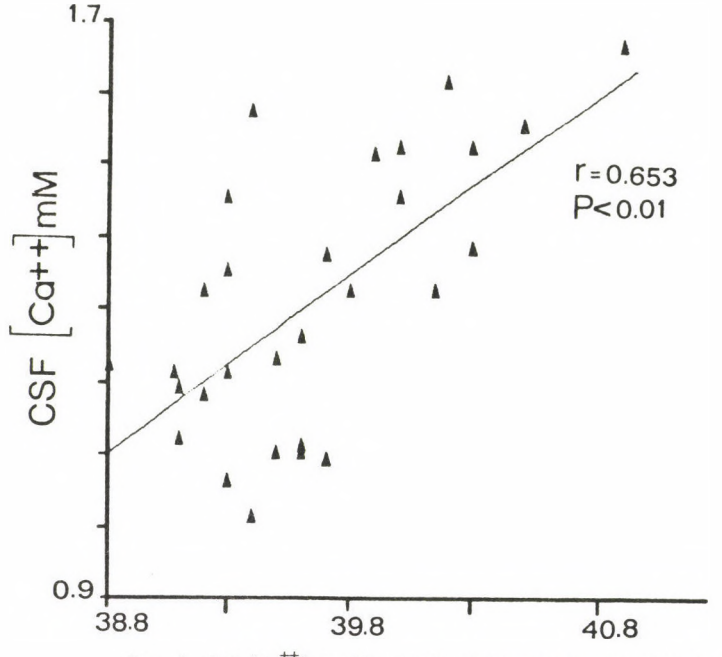

Fig. 1. CSF (Ca^{++}) in 29 rabbits plotted against rectal temperature (Tr). (Ca^{++}) value of each animal is the mean of 5 fractions analysed in quadruplicate. Coefficients of variation of the 5 determinations ranged between 1.4 and 6.7 per cent.

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AN EXPERIMENTAL MODEL TO SELECT DRUGS AGAINST HEAD TRAUMA

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Key words: head trauma, lipid peroxidation, free radical scavanger, Ca-ionophore, Ca-antagonist

Brain ischaemia, aberrant Ca-fluxes and lipid peroxidation are sought to be the most important events after CNS injury (Hall and Wolf, 1986). There are lot of evidences showing the protective effect of antioxidants against cerebral ischaemia (Suzuki et al. 1984; Mizoi et al. 1986). Ca-antagonists were found to be effective in posttraumatic spinal cord ischaemia-model (Hall and Wolf, 1986). We present some experimental evidences proving the role of free radical reactions and intracellular Ca-accumulations in the consequences of head injury in mice. Our acute head trauma modell was also suitable to follow up the effect of drugs on the mortality and neurological status of mice during 3 days of the observartion period.

1. Mortality and neurological deficits following acute head injury were decreased by antioxidants (Selen, Vitamin-E, Mannitol, D-penicillamin)

2. Endogen free radical production can be increased by Paraquat or Fenilhidrasin (Oroszlan et al. 1981). A moderate head injury combining with the administration of a free radical inductors resulted in the neurological status similar to the consequences of serious head trauma. In this way, the selectivity of the antioxidants can be estimated.

3. A Ca-ionophore (A 23187) was able to evoke higher mortality and more serious damage than the acute trauma alone.

4. Drugs having protective effect against cell damage (membrane stabilizers, Ca-antagonists) were able to ameliorate neurological deficits.

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ORAL ZINC SULPHATE TREATMENT IN WILSON'S DISEASE.

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Key Words: Zinc therapy, copper, Wilson disease

Wilson's disease is a inborn error of copper metabolism which promotes an excessive copper accumulation that is fatal if untreated. Most patients with this disease are treated with a highly toxic cupriuretic agent D-penicillamine (D-pen) (Brewer et al., 1987). It has been recently shown that the oral administration of zinc sulphate may well substitute D-pen at least in those patients which have been previously decoppered by chelation therapy (Cossack, 1988; Sanstead, 1987).

We present the results obtained in three patients affected by Wilson's disease and submitted to a zinc sulphate treatment. In the first moment and then periodically the efficacy of zinc sulphate, orally administered at the dose of 200 mg 3 times daily, was verified by means of copper balance study. Furthermore the patients were controlled by measuring copper and zinc plasma concentrations, 24 h copper and zinc urinary excretion and serum ceruloplasmin (S-Cp) levels.

One patient, a 29 year old female with serious neurological symptoms, after 10 years of D-pen therapy was shifted to oral zinc and manteined on this regimen since three years. After the beginning of the substitutive treatment the patient was submitted to a two copper balance studies: the first, after three months, was negative for 0.09 mg/day; the second, after 30 months, was negative for 0.24 mg/day.

The second patient, a 25 year old man, presenting a neurological form of the

disease, was treated from the start with zinc sulphate and he has been on this therapy for two years. The copper balances under zinc sulphate have shown a negative copper balance for 1.16 mg/day after one month and 0.59 mg/day after 12 months.

During zinc treatment, in both patients, the clinical conditions and S-Cp levels remained unchanged, while a significant decrease of the cupruria and cupremia was observed.

The third case was a 11 year old child in seemingly good health with no symptoms of neurological disorders but showing a remarkable increase of serum transaminases. He received oral zinc sulphate as the primary and sole therapy for Wilson's disease. The basal copper balance was found positive (0.04 mg/day); on the contrary the copper balance immediatly after the beginning of zinc sulphate therapy resulted negative (-0.56 mg/day). The results obtained during two years of treatment have shown a decrease of SGOT, SGPT and SGGT, a progressive decrease of total copper in both 24 h urine and plasma and stationary S-Cp concentrations.

Considering that the only side effect seen during the therapy of the three patients was an initial mild gastric irritation, which disappeared after few days, in the first patient, these data may suggest the convenience to use oral zinc as both primary and manteinance therapy in Wilson's disease.

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SYSTEMICALLY APPLIED RUTHENIUM RED INHIBITS THE STIMULATION OF SENSORY RECEPTORS BY CAPSAICIN

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Key words: capsaicin, ruthenium red, Bezold-Jarisch reflex

Introduction

The inorganic dye ruthenium red (RR) selectively inhibits the effects of capsaicin in vitro (Maggi et al., 1988) and prevents capsaicin-induced nociceptor stimulation in the perfused rabbit ear (Amann and Lembeck, 1989). Topically administered RR inhibits both reflex and local efferent responses evoked by capsaicin (Maggi et al., 1989). Since there are no data available about in vivo administration of this dye, the aim of the present study was to examine the effect of systemically applied ruthenium red on reflex responses evoked by capsaicin.

Methods

Female albino rats of the Wistar strain weighing 230-320 g were anaeshetized with urethane (1.0-1.2 g/kg i.p.). The trachea, the right carotid artery and the right jugular vein were cannulated. Arterial blood pressure, myocardial contractility (dp/dt) and breathing movements were recorded by a Beckman dynograph. Rectal temperature was kept at 38°C by means of a heating pad. Capsaicin and veratridine were administered by intravenous bolus injections of 0.1 ml.

Results and discussion

Intravenous injection of capsaicin excites the pulmonary J receptors and evokes the Bezold-Jarisch reflex of bradycardia, fall in arterial blood pressure and apnoe (Paintal, 1973). The threshold dose of capsaicin was 0.8-1.6 μg/kg. RR (0.5, 1.0 and 2.0 mg/kg), injected intravenously within 10 to 30 seconds inhibited the triple response evoked by capsaicin in a dose--dependent manner. The respective dose ratios were: 2.0 (n=7),

2.2 (n=4) and 6.8 (n=4) by calculating the identical effects of capsaicin on the heart rate before and after the treatment. Slow intravenous injections of RR (0.5, 1.0, and 2.0 mg/kg) produced no significant cardiovascular changes. In some cases, particularly after the highest dose apnoe or tachypnoe was observed.

Capsaicin excites the endings of vagal sensory C-fibres and the efferent fibres to the heart run also in the vagus nerve (Szolcsànyi, 1982). The RR-induced inhibition of the reflex is not due to an effect on parasympathetic fibres and neurotransmission. Bradycardia induced by electrical stimulation of the peripheral stump of the cut vagal nerve was not inhibited by RR (0.5-2.0 mg/kg, n=4-4).

Evidence against a site of action in the central nervous system was also obtained. Stimulation of the preterminal part of vagal afferents by veratridine also elicits the Bezold--Jarisch reflex (Paintal, 1973). In contrast to the capsaicin--induced response the vagal reflex evoked by veratridine (8, 16 μg/kg i.v.) was not abolished by RR (0.5-2.0 mg/kg, n=4-4).

It is concluded that RR inhibits the effect of capsaicin at the level of sensory nerve endings in vivo. Our paper is the first report on systemic application and effectivness of RR as an antagonist of capsaicin at sensory nerve endings.

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ACTIVE REPORTING SCHEME: AN EXAMPLE TO EVALUATE SPECIFIC ADVERSE DRUG REACTIONS

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Key words: post-marketing surveillance, NSAID.

Spontaneous reporting schemes for acquiring information on suspected Adverse Drug Reactions (ADRs) are generally well accepted as valuable alerting systems, and have proved to be of greatest benefit when signalling rare events. The increasing amount and availability of data depending on computerised international systems has stressed both the relevance of "quality" of type of information, so that it is possible to compare data originating from different countries, and the need for "denominator" data to evaluate and validate the 'rate' of estimated risk (Lawson,1988).

Since in Italy about 8% of all prescriptions are NSAIDs, and a quarter of all spontaneous reports in England concerns these drugs, we started in 1987 in Modena a program of post-marketing drug surveillance to evidence type and incidence of ADRs during NSAIDs outpatients' treatment.

We selected 12 physicians belonging to the National Health Service with care of 15000 patients in Modena. We knew all drug prescriptions made by physicians by means of the Health Service computerized system and demographic data of patients; moreover in a parallel study we evaluated the number of OTC products sold in Modena to define the possible relevance of this factor in ADRs related to these drugs. All reports were collected by the Clinical Pharmacology Dept. and every month doctors would meet to discuss the causality of ADRs reports and to verify the accuracy of the reporting and the treatment.

Within 4 months we collected 56 ADRs,33% of them "certainly" and 54% "probably" related to NSAIDs use. The quality of reports was verified comparing fully-compiled forms. In this group we noted 4 cases of oral ulcers and decided to begin a cohort study to evaluate the incidence of this unwanted effect in our population. During a two-month period we recorded 23 cases of oral ulcerations: in 10 cases they were associated with NSAID assumption. The incidence of oral ulcerations was 0.21% for DDD; ulcerations were related to Naproxen, Ketoprofen, Proglumetacine, Diclofenac and Fenprofen use. In 4 cases we did a positive rechallenge; the other 6 cases did not show any elements which could impute the oral ulceration to other causes apart from the drug. The width of the ulcerations ranged between 2 and 20 mm, and they were located on cheek (80%), gums (20%) and tongue (20%). Clinically the lesions did not differ from ulcerations unrelated to NSAID use. In all patients drug withdrawal resolved the clinical picture.

The small number of observations does not allow us to evidentiate differences between NSAIDs, but we can draw some conclusions: a) the high compliance of family doctors to this scheme spurs us to continue in this educational effort to improve spontaneous reporting; b) the active reporting scheme has shown itself to be able to evoke evidence of uncommon side effects; c) oral ulcerations seem to be a frequent, even if minor,side effect which should be taken into account in long-treatment with NSAIDs.

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NEW DIHYDROPYRIDINE COMPOUND (GYKI-46 544) WITH BRADYCARDIAC AND VASODILATORY EFFECTS

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Keywords: Antihypertensive action, lack of tachycardia

Since the early 60's great number of dihydropyridines have been investigated and introduced into the therapy of cardiovascular diseases. Almost all of them however may elicit reflexogenic tachycardia at least at the start of the treatment and have some negative inotropic effect, as well. Recently GYKI-46 544 has been selected for further investigation in our laboratory. This compound could produce marked antihypertensive effect in SH-rats, renal hypertensive and DOCA + salt hypertensive ones without causing significant tachycardia. After single administration (2.5 mg/kg p.o.) it elicited bradycardia and blood pressure decrease in SH-rats.
In the course of a 3-week oral treatment period GYKI-46 544 produced consistent decrease of both blood pressure and heart rate in the SH-rats. This new GYKI-compound was able to exert strong vasodilatory or antivasoconstrictory actions in in vivo and isolated vessel preparations. Its negative inotropic effect appeared only after 2 or 3 order of magnitude higher concentrations above the vasodilatory effective ones. GYKI-46 544 had

fourtimes stronger antianginal effect than that of nifedipine measured by the inhibition of vasopressin-induced ST-elevation in the EKG of the rats. On the base of its favourable heart rate effect and longlasting vasodilatory effect GYKI-46 544 may compete with some very new dihydropyridine compounds,too.

ELABORATION OF HIGHLY PURIFIED ANGIOHYPOTENSIN PREPARATIONS FROM MAMMALIAN LIVER

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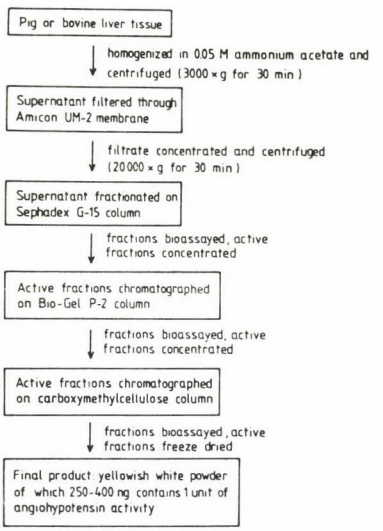
Keywords: Angiohypotensin, rabbit ear artery, gel chromatography

Angiohypotensin (AH), extracted from human serum by the aid of gel chromatography on Sephadex G-15 column, was found to inhibit neuromuscular transmission in the perfused rabbit ear artery in a dose-dependent manner, leaving the sensitivity of the vascular smooth muscle to endogenous and exogenous noradrenaline unchanged (Knoll, 1978). It was further proved that semi-purified human serum angiohypotensin inhibited with high selectivity the release of noradrenaline from the nerve terminals of vascular smooth muscle (Knoll, 1979). AH was detected in trace amounts in human and mammalian blood, but liver was found to contain significantly higher amount of the substance (Knoll, 1978).

Highly purified AH preparations from pig and bovine liver were prepared. The fractions were assayed on the central portion of the rabbit ear artery. Vasoconstriction was elicited by field stimulation (100 V) and the resulting increase in intravasal pressure was measured with an electromanometer and recorded. The content of AH in the preparation that inhibited the vasoconstriction of the rabbit ear artery to nerve stimulation was taken to contain 1 unit.

The flow sheet for the elaboration of highly purified angiohypotensin preparations from pig or bovine liver is shown below:



Using this method we succeeded to extract up to 100,000 units/kg of AH from bovine liver. The most active preparations contained 3000-3500 units AH/mg.

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LEU-ENKEPHALIN EFFECTS AT THE MOUSE NEUROMUSCULAR JUNCTION

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Key Words : Leu-enkephalin - Acetylcholine Release - Mouse End Plate

Opiate peptides have been reported to affect neurosecretion in different preparations (Haynes and Smith, 1982; Bixby and Spitzer, 1983). In particular, Met-enkephalin reduced the amount of acetylcholine (ACh) released (Bixby and Spitzer, 1983) from frog muscle by nerve stimulation. This effect suggested that enkephalin might interact with presynaptic Ca^{++} channels reducing the quantal content of transmitter release. Furthermore, a postsynaptic effect of opiate peptides related to a possible inhibition of acetylcholinesterase is also reported (Haynes and Smith, 1982). Here we study the effects of leu-enkephalin at the mouse neuromuscular junction.

The method used was previously described (Re et al, 1989) and led to the acquisition of the most significant parameters related to the spontaneous and evoked release of transmitter at the end-plate level. Stock solutions of Leu-enkephalin (acetate salt. Sigma lot. 84F-5805) dissolved in distilled water were stored at -35 °C and diluted immediately before use.

Table I shows the results obtained on five parameters related to the ACh release at the mouse diaphragm in the presence of different Leu-enkephalin concentrations. Values are expressed as

the mean of the fractional percent variations from the control values with the respective standard errors of the mean; p values, where not indicated, were always less than 0.05 by Student t test.

Table I

	Control	Leu-enkephalin (uM)		
		0	15	60
mepc frequency (Hz)	1.78±0.33	-0.16±0.08	+0.01±0.07	+0.01±0.04
mepc amplitude (nA)	0.49±0.03	+0.06±0.07	-0.04±0.11	-0.07±0.09
mepc tau (msec)	1.30±0.22	-0.11±0.07	+0.02±0.05	-0.01±0.05
epc amplitude (nA)	1.53±0.42	-0.04±0.12	-0.13±0.02	-0.27±0.04
quantal content	2.35±0.41	-0.08±0.13	-0.10±0.01	-0.21±0.03

Non statistically significant.

This results show that Leu-enkephalin reduces in a significant manner only the parameter related with the evoked ACh release. Indeed the means of the percent changes from the control values induced in all the other parameters are not significant. The decrease in the size of the evoked release averages from 13% (Leu-enkephalin 15 uM; n=5) to 27% (Leu-enkephalin 60 uM; n=4). The results obtained in this preparation do not indicate any post-synaptic effect of Leu-enkephalin. Indeed, both the amplitude and the time constant of the quantal conductance change are not modified by Leu-enkephalin treatment. These experimental data seem to exlude any interference with the postsynaptically located acetylcholinesterase which, if inhibited, would produce an increase of the rate of the ACh-receptor complex conformational change.

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IN VIVO AND IN VITRO EFFECTS OF CYTOKINES ON THE GENERATION OF NK CELL-MEDIATED ANTITUMOR ACTIVITY.

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Key words: Cytokines, Natural killer cells, Interleukin-2.

Natural reactivity mediated by Natural Killer (NK) cells is an inborn and autonomous function of bone marrow (BM) involved in resistance against infectious agents as well as in the control of tumor cell growth and dissemination. BM transplants have been used for treatments of patients with systemic or metastatic malignancies. As shown by recent studies of autologous BM transplantation, although very high doses of chemotherapy and/or lethal irradiation have resulted in complete remission, the major problem has been recurrence of tumor after a short period of time. One strategy to address this problem could be to augment the host's immunological effector mechanisms after the BM transplantation, to help to eliminate small numbers of residual tumor cells. Treatments to accellerate reconstitution of NK activity during the post-transplant period might help to eliminate metastatic tumor cells. We have evaluated the effects of various cytokines (CKs), used alone or in combination, on the in vitro generation of NK cells from BM precursors and on the in vivo reconstitution of NK cell activity and resistance to metastases from B16 melanoma in lethally irradiated (9 Gy) mice transplanted with syngeneic BM. In this regard, it is of note that NK activity is usually absent or strongly depressed after lethal irradiation and BM transplantation, returning only

after 2 weeks. In vivo treatment from day zero trough day 5 after BM transplantation with the following combinations of interleukin 2 (IL-2) and other cytokines: IL-2 + IL-1 + tumor necrosis factor (TNF) or IL-2 + IL-1 + lymphotoxin (LT) induced appreciably greater and more rapid augmentation of NK cell regeneration than IL-2 alone as evaluated 14 days after BM transplant. Generated effector cells have the characteristics of fresh NK cells in that are asialoGM1^{+}, Thy.1^{+}, CD4^{-}, CD8^{-} and lyse tumor targets. The same treatments induced significant augmentation of in vivo resistance against pulmonary metastases in C57BL/6 mice injected with B16 melanoma cells (200.000 cells/mouse iv). To analyze the mechanism(s) responsible of the above described in vivo effects we also performed in vitro experiments by culturing BM cells with IL-2 alone or in combination with other CKs. Our results show that IL-1, TNF and LT were able to augment the IL-2-dependent NK cell generation as evaluated at the end of the 7 days culture period. After this time both cytotoxic activity against NK sensitive targets and number of generated cells were significantly higher in the group of BM cells cultured with IL-2 plus IL-1, TNF or LT as compared to the group with IL-2 alone. IL-1, TNF or LT were also able to augment the mRNA expression for the α chain (p55) of IL-2 receptor (IL-2/r) in BM cells, as evaluated by the northern blot analysis, as well as the number of IL-2/r positive cells in the culture as evaluated by FACS analysis using antibodies against the α chain of the IL-2/r (clone AMT13). These data show that treatment with CKs can stimulate the generation of NK cells from BM precursors and that this effect may be of value in the control of metastatic disease.

CHRONIC EXPOSURE TO OPIOID AGONISTS AND ANTAGONISTS AFFECTS PRODYNORPHIN GENE EXPRESSION

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Prodynorphin - Gene expression - Chronic opiates - Tolerance

The neurochemical alterations related to the development of a tolerance to the effects of opiates are still far to be clearly understood.

The neuronal pathways utilizing opioid peptides have been proposed as candidate for an involvement in the modifications induced by chronic exposure to opiate drugs (Adler, 1986). Since expression of several neuropeptide genes may change in response to drug-induced alterations in neuronal function, the aim of our study was to examine the influence of chronic treatment with opioid agonists and antagonists on the gene expression of the opioid peptide prodynorphin.

It has been shown that a tolerance to antinociceptive and motor effects of dynorphin A, purported endogenous ligand for the κ opioid receptor, develops after its chronic intrathecal infusion (Spampinato and Candeletti, 1985); moreover dynorphin A is still effective in rats made tolerant to morphine, whereas it is ineffective in rats made tolerant to ethylketocyclazocine (EKC), a synthetic compound, mainly κ-opioid agonist, thus supporting the hypothesis of a specificity of dynorphin A for the κ-opioid receptor (Chavkin, 1982).

Sprague-Dawley rats were treated with morphine, EKC (10 mg/kg, i.p. twice daily for 7 days) and naloxone (3 μg/μl/h, icv infused via minipumps for 7 days); brains were rapidly removed, tissue dissected and frozen at -80°C. Total RNAs from hypothalamus and hippocampus were extracted by cesium chloride gradient and subjected to Northern analysis. Blots were hybridized with a cDNA probe, the BgBa fragment of the genomic DNA complementary to the prodynorphin mRNA (kindly provided by Drs. J.Douglass and O.Civelli, 1985), labelled by nick translation to a specific activity of 7-9 x 10^5 cpm/ng. Hybridization was performed overnight at +42°C and, after washing, blots were exposed to x-ray film for 7 days.

Under the adopted conditions, the chronic treatment with morphine reduced

prodynorphin mRNA levels in hippocampus (approximately twofold) and in hypothalamus; at the same way the chronic treatment with EKC markedly reduced prodynorphin mRNA levels in hippocampus and hypothalamus (approximately fourfold), compared with control rats.

On the contrary, a marked increase (twofold) in the levels of prodynorphin mRNA was observed in the same tissues after chronic exposure to naloxone.

Our results indicate that prodynorphin gene expression is affected by chronic treatment to both opioid agonists and antagonists, with opposite effects on the biosynthesis of the opioid neuronal system. These data are in agreement with those of other Authors (Uhl, 1988) showing a decrease of proenkephalin mRNA, after chronic morphine in rat striatum.

In conclusion the hypothesis is supported that chronic exposure to agonists and antagonists for opioid receptors (premise necessary to the development of a tolerance) induces alterations in function of neuronal systems involving opioid peptides and their gene expression.

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DIHYDROPYRIDINE CALCIUM ANTAGONISTS PREVENT COCAINE-, BUT NOT AMPHETAMINE-, INDUCED DOPAMINE RELEASE AND MOTOR ACTIVITY IN RATS.

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Keywords: cocaine, Ca^{++} antagonists, DA release

Cocaine abuse has grown rapidly during the past several years. Therefore, the search for a therapeutical control of cocaine intoxication and abuse is a major public health issue. Cocaine is thought to elicit its locomotor and rewarding effects by inhibiting dopamine (DA) reuptake in the central nervous system. By using the brain microdialysis technique it has been shown that cocaine increases DA release both in the rat striatum and in the nucleus accumbens and that, unlike amphetamine, cocaine releases DA via a calcium-dependent mechanism (Hurd and Ungerstedt, 1989; Imperato and Di Chiara, 1988). We report that dihydropyridine (DHP), L-type calcium channel antagonists, nimodipine, nifedipine and isradipine, prevent both cocaine-induced DA output in the striatum, as measured by microdialysis in awake rats, and the locomotor stimulant effects of the drug. Brain dialysis was performed as already described (Pani et al., 1990), using cyanoacrylate-methallylsulphonate membranes (220 μm o.d.) implanted transversely through the caudate (coordinates: A, 2.0; V, 6.0 from bregma) of male, 220-250g Sprague Dawley rats, using a Krebs buffer, pH 7.2, as perfusing solution. DA and metabolites were measured by HPLC-EC in 50μl/20 min fractions. Cocaine, 15 mg/kg i.p., increased the DA output in the striatum to a maximum of 360% of baseline, 90 min after treatment. Pretreatment

(120 min beforehand) with nimodipine (10 mg/kg s.c.) markedly reduced (-250%) cocaine effect on DA outflow. Isradipine (2.5 mg/kg s.c.) and nifedipine (10 mg/kg s.c.) pre-treatment produced similar inhibition of cocaine-stimulated DA release. In contrast, DHP calcium antagonists were completely ineffective against amphetamine-induced (1.5 mg/kg i,p.) DA release. DHP calcium-antagonists prevented also cocaine-induced locomotor stimulation. Motor activity was measured in individual motility cages for 3 min periods at different intervals following treatments in different groups of rats. Nimodipine and isradipine produced a dose-related inhibition of the motor response to cocaine, and completely suppressed cocaine-induced motor stimulation at the doses of 20 mg/kg and 2.5 mg/kg, respectively. However, these drugs failed to antagonize the stimulatory effect of amphetamine. In contrast, three non-DHP calcium antagonists, verapamil, diltiazem and flunarizine, had little or no effect both on DA release and on locomotor stimulation induced both by cocaine or by amphetamine. These results indicate that calcium influx through the L-type channel is essential for DA release by cocaine and suggest that DHP calcium antagonists may be clinically useful in the treatment of cocaine abuse.

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CHARACTERIZATION OF OPIOID RECEPTORS INVOLVED IN THE MODULATION OF NANC NEUROTRANSMISSION IN THE MAMMALIAN HEART

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Key words: opioid receptors, NANC neurotransmission, heart

Non-adrenergic, non-cholinergic (NANC), capsaicin-sensitive neurons are present in the mammalian heart (Saito et al., 1986a). The antidromic stimulation of these sensory nerves produces an excitatory response, mainly due to the release of calcitonin gene-related peptide (Saito et al., 1986b). We have described a simple method for the selective stimulation of NANC cardiac nerves, and have previously demonstrated that opioid peptides are able to reduce NANC transmission in isolated guinea-pig atria (Mantelli et al., 1989). In this study we have attempted to obtain a characterization of the subtype of opioid receptors involved in the inhibitory action of opioid peptides. Isolated guinea-pig atria, obtained from animals pretreated with reserpine 5 mg/Kg, and incubated in the presence of 1 μM atropine and 0.3 μM CGP 20712A (a selective $beta_1$-blocker), were electrically stimulated at 4 Hz. Transmural nerve stimulation (TNS) was applied during the refractory period by two platinum plates parallel to the preparations; the effect of TNS mainly consisted of a transient increase in cardiac contractility, as previously described (Mantelli et al., 1989).

The μ agonist [D-Ala^2,-N-Me-Phe,Gly^5-ol] enkephalin (DAGO), at concentrations ranging from 0.01 to 3 μM, dose-dependently reduced the cardiac response to TNS; also the mixed ó-μ agonist

[D-Ala^2,D-Leu^5]enkephalin (DADLE), induced a similar inhibitory effect at the same range of concentrations: the IC_{50} values for the two agonists were respectively 0.09 and 0.19 μM. On the other hand, the cardiac response to TNS was less affected by morphine and dynorphin, at concentrations ranging from 0.1 to 10 μM; the IC_{50} values for the two agonists were 0.4 and 20 μM respectively. The inhibitory effects of DAGO, DADLE and morphine were competitively antagonized by 0.01-0.1 μM naloxone. The effect of dynorphin was antagonized by 0.1 μM naloxone but was unaffected by 0.5 μM of the k receptor antagonist MR 2266. Finally, the δ-selective agonist [D-Pen^2,D-Pen^5]enkephalin (0.01-10 μM) was unable to modify the cardiac response to TNS; moreover, a δ-selective antagonist, ICI 174864 at a concentration of 0.3 μM, did not antagonize the inhibitory effect of DADLE.

These results confirm that inhibitory prejunctional opioid receptors are present on NANC cardiac sensory nerves; moreover the activities displayed by the different agonists and antagonists of opioid receptors employed in the present study suggest that these receptors belong to the μ_1 subtype.

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BETA-ENDORPHIN-INDUCED BEHAVIOURAL EFFECTS: ENDORPHIN-CATECHOLAMINES INTERACTIONS

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Key words: Behaviour - Cats - Beta-endorphin - Catecholamines

An interaction of endogenous opioid peptides and catecholamines in the genesis of some behavioural phenomena has been suggested. For instance, Stinus et al. (1980) reported that the increase in the locomotor activity evoked by infusion of beta-endorphin into the ventral tegmental area depends on the integrity of dopaminergic neurones in the n. accumbens of the rat. On the other hand, there is little information on the neurochemical mechanisms subserving the symptoms of psychomotor excitation induced by beta-endorphin in cats (Beleslin et al., 1982). Therefore, the aim of the present experiments was to study the behavioural symptoms evoked by intracerebroventricular (i.c.v.) administration of beta-endorphin in cats with destroyed catecholaminergic neurones by 6-hydroxydopamine (6-OHDA).

In an aseptic operation under pentobarbital sodium (35-40 mg/kg, i.p.) anaesthesia, an infusion cannula was implanted into the left lateral cerebral ventricle of cats of either sex (2-3.5 kg), so that i.c.v. injections of drugs could later be made without anaesthesia. On the day of testing, animals were acclimated to the test environment for 1 hour before the injection of drugs. The solutions of drugs were injected i.c.v. manually from a 1 ml syringe, in a volume of 0.1 or 0.2 ml, and washed with 0.1 ml of saline under the same conditions as the drugs. The behaviour of cats was observed continuously for 4 hours and intermittently for 24 hours. In order to avoid tolerance, each cats was used only once for these experiments.

Beta-endorphin (0.03 - 0.04 mg) injected i.c.v. in unanaesthetized cats, (n=16) induced dose-related and longlasting (up to 4 hours) psychomotor excitation (restlessness, apprehension, vacant staring, stereotyped movements of the head, impelling locomotion) accompanied with autonomic (mydriasis) and motor (tremor) phenomena. The most prominent symptoms were impelling locomotion, stereotyped movements of the head, mydriasis and tremor. In cats treated with i.c.v. beta-endorphin in a dose of 0.05 mg, impelling locomotion, stereotyped movements of the head and tremor appeared in 100 %, whereas mydriasis was observed in 50 % of these animals. The duration of these symptoms after a single i.c.v. injection of beta-endorphin (0.05 mg) in cats (n=4) is shown in the Table 1.

Another group of cats (n=7) was treated with i.c.v. 6-OHDA twice at 24 h intervals. Ten days after the last dose of 6-OHDA, these animals received beta-endorphin i.c.v. in a dose of 0.05 mg. The behavioural effects of beta-endorphin in 6-OHDA-treated cats were depressed or completely abolished, as shown on the Table 1.

Beta-endorphin, injected i.c.v., induces longlasting and dose-related psychomotor excitation. These behavioural effects are mediated via opioid receptors, since they can be blocked with opiate antagonists (Stinus et al., 1980;

Table 1.
Behavioural effect of beta-endorphin in 6-OHDA-treated cats

symptom	beta endorphin	6-OHDA + beta-endorphin	statistical significance	NaCl 0.9% 0.2 ml
	minutes	minutes	t-test	minutes
locomotion	65.0 ±13.9	14.0 ± 6.5	$p < 0.01$	0
stereotyped movement of head	111.2 ± 3.4	0	$p < 0.001$	0
mydriasis	66.2 ±39.3	4.2 ± 3.9	$p > 0.05$	0
tremor	152.5 ±35.3	44.5 ±15.2	$p < 0.05$	0

Beleslin et al., 1982). In the present experiments, the behavioural effects of beta-endorphin in cats (locomotor activity, stereotyped movements of the head and tremor) have also been significantly reduced or completely abolished in cats pretreated with 6-OHDA. Similarly, in rats, 6-OHDA inhibited the locomotor activity induced by intracerebral administration of beta- endorphin (Stinus et al., 1980). These findings suggest that intact catecholaminergic neurones are necessary for the expression of the behavioural effects of beta-endorphin. There is solid evidence that dopamine, but not noradrenaline, plays a major role in the locomotor activation and stereotyped behaviour, whereas 5-hydroxytryptamine may act as a modulator (Korsgaard et al., 1985; Samardžić et al., 1988). Moreover, opioid receptors have been found upon the dopaminergic nerve terminals (Hökfelt et al., 1980). Our results support the view that beta-endorphin induces, at least some of the behavioural effects by activating dopaminergic mechanisms in the brain. The finding that 6-OHDA completely prevents the appearance of the stereotyped movements of the head, whereas the locomotor activity and tremor are only reduced, and mydriasis remains unaffected, suggests that besides dopamine, other neurotransmitters may also be involved in the appearance of the behavioural phenomena evoked by beta-endorphin in cats.

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EFFECTS OF NEUROHYPOPHYSEAL HORMONES ON COCAINE-INDUCED BEHAVIORAL SENSITISATION

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Key words: cocaine, behavioral sensitisation, oxytocin, vasopressin

Introduction

Subchronic administration of cocaine induces sensitisation to the locomotor response of a challenge dose of the drug upon cessation of treatment (Post et al. 1981). The biological basis of the sensitisation to cocaine is an adaptive change in the dopaminergic neurotransmission within the central nervous system (Dackis and Gold 1985). Earlier data suggest that the neurohypophyseal hormones oxytocin (OXT) and arginine-vasopressin (AVP) play an important regulatory role in the adaptive processes induced by narcotic drugs via the dopaminergic mechanisms (Kovács and Telegdy 1987). The aim of the present experiment was to study the effects of OXT and AVP on the development of cocaine-induced behavioral sensitisation in mice.

Methods

Male albino CFLP mice weighing 20-30 g were used. Locomotor activity was measured by open-field methods. Mice were treated subchronically with either saline or cocaine (7.5 mg/kg s.c.) twice a day for 5 days. The animals were tested for locomotor activity with a challenge dose of cocaine 7.5 mg/kg s.c.) 3 days after cessation of the subchronic treatment.

OXT and AVP were administered 1 hour prior to cocaine or saline injections, except on the test day. The data were subjected to analysis of variance and post hoc Dunnett tests.

Results and Discussion

Table 1. Effects of neurohypophyseal hormones on behavioral sensitisation to cocaine

TREATMENT	No. of animals	LOCOMOTOR ACTIVITY	SIGN.
Sal+Sal	42	159.0 + 10.9	
Sal+Coc	48	218.4 + 11.4	+
0.005 µg OXT+Coc	10	232.4 + 33.8	NS
0.05 µg OXT+Coc	20	236.1 + 14.8	NS
0.5 µg OXT+Coc	11	290.4 + 40.4	++
0.005 µg AVP+Coc	17	138.3 + 16.8	++
0.05 µg AVP+Coc	16	160.8 + 18.8	++
0.5 µg AVP+Coc	16	152.6 + 19.3	++

The locomotor activity is the effect of the challenge dose of cocaine on mice treated subchronically with cocaine or saline and pretreated with OXT and AVP, this effect being expressed as a percentage of the basal activity level of naive mice. +: $P < 0.05$ vs. Sal+Sal; ++: $p < 0.05$ vs. Sal+Coc

Neither OXT nor AVP treatment alone interfered with the effect of the challenge dose of cocaine (data not shown). The data (Table 1) indicated that cocaine-induced sensitisation was potentiated by the highest dose of OXT. In contrast, all doses of AVP used inhibited the development of cocaine-induced behavioral sensitisation. The results suggest that the behavioral sensitisation induced by cocaine can be modulated in the opposite direction by neurohypophyseal hormones.

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INOTROPIC RESPONSE OF "STUNNED" MYOCARDIUM INDUCED BY DOPAMINE IN DOGS

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Key words: inotropic response, stunned myocardium, dopamine, dog

Transient coronary occlusion (15 minutes) does not result in irreversible myocardial injury but is associated with a depression of contractile function sustained for several hours to days ("stunned myocardium"). The defect in the contractile process responsible for this phenomenon has been suggested to be causally releated to a reduced energetic state, altered excitation or excitation-contraction coupling, or damaged contractile filaments (Mercier et al., 1982; Ellis et al., 1984; Heusch et al., 1988).

The purpose of our study was to evaluate the contractile reserve of postischemic myocardium early after reperfusion in open chest dogs. Global and segmental contractile function was measured as positive LVdP/dtmax and contractile force (CF), respectively using strain gauges in the basal (nonischemic) and apical (ischemic) region of the left ventricle. We compared inotropic responses 15 minutes before and 20,40 and 60 minutes after ischemic insult (15 minutes LAD ligation). Inotropic response was induced by 50 µg/kg dopamine intravenously.

TABLE I

Inotropic responses induced by dopamine

Parameters of contractility	Preischemia	Postischemia 20 min.	40 min.	60 min.
+dP/dt:	166.3+7.2	171.3+24.8	166.0+62.0	142.8+58.7
CF:(base)	52.0+21.9	57.7+29.0	51.7+20.3	55.8+11.7
CF:(apex)	100.3+41.0	95.2+28.9	89.8+34.3	105.3+45.9

Comment: n:6, mean+SD, values are % of change (max. increase of contractility)

There was no significant difference between inotropic responses before and after transient ischemic insult, although CF of the postischemic segment was significantly depressed, about 50% of preischemic value. Dopamine could transiently improve segmental contractile function and it seems that efficiency increases with the duration of reperfusion. These findings imply that the "stunned" myocardium can generate ATP and suggest that postischemic dysfunction in humans may be effectively reversed by inotropic therapy.

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BIOCHEMICAL BACKGROUND OF CIMETIDINE-INDUCED GASTRIC MUCOSAL PREVENTION IN RATS TREATED WITH HCL (ACTIONS OF CYTOPROTECTIVE AND ANTISECRETORY DOSES).

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Key-words:cimetidine, gastric mucosal biochemistry,SOD,energy systems;

The H_2-blocking compounds are able to prevent the development of gastric mucosal damage by their antisecretory and cytoprotective properties. The phenomenon of "gastric cytoprotection" differs from the inhibition of gastric acid secretion (Robert et al.,1979). The dose of 2.5 mg/kg was found to be cytoprotective agent in case of cimetidine (Morón et al.,1983).

The aim of this study was to compare the changes in the gastric mucosal biochemical parameters produced by cytoprotective and antisecretory doses of cimetidine.

The observations were carried out in Sprague-Dawley (LATI,Gödöllő, Hungary)-strain rats,weighing 180-210g.The gastric mucosal lesions were produced by the intragastric administration of 0.6 M HCl (1 ml), and the animals were sacrificed at 1 hour after administration of 0.6 M HCl. Cimetidine (used in cytoprotective and antisecretory doses) was given ip. at 30 min before administration of necrotizing agent. After sacrifice of animals the number and severity of gastric mucosal lesions was noted. Different biochemical compounds such as ATP,ADP,AMP enzymatically,cAMP by RIA were measured, from the total homogenate of gastric mucosa. From the same homogenate, the superoxide dismutase activity was measured (Misra & Fridovich,1972).

It has been found that: 1.Cimetidine dose-dependently prevented the development of 0.6 M HCl-induced gastric mucosal damage; 2.The extent of ATP-ADP transformation was increased by cimetidine administration, while the transformations of ATP-cAMP and cAMP-AMP decreased in that time; 3.The SOD activity returned back to its normal value by administration of cimetidine.

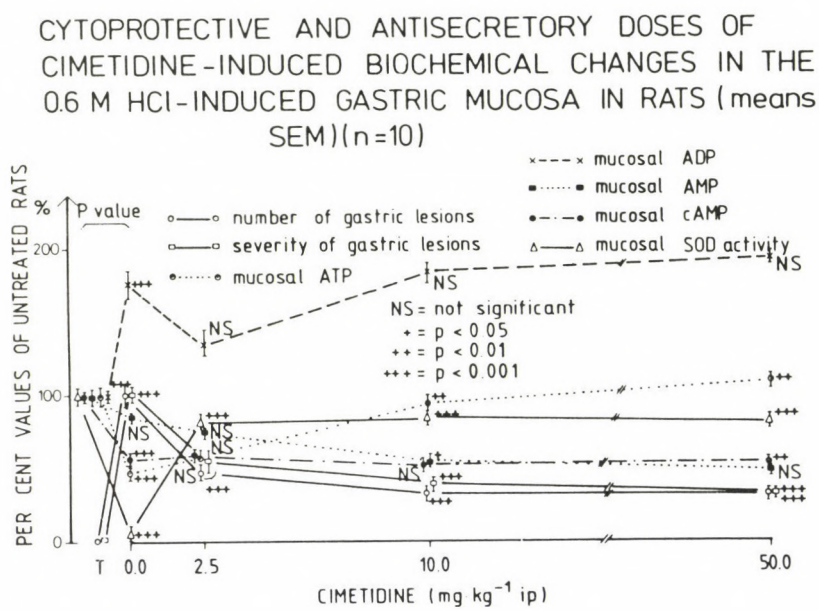

Fig.1.Cimetidine-induced biochemical changes in the rat gastric mucosa. The results were expressed as means ± SEM.Abbreviations: T,saline-treated groups (normal control animals); NS,not significant;+, $P < 0,05$;++, $P < 0.01$ and +++, $P < 0.001$.Cimetidine was given in cytoprotective (2.5 mg/kg ip.) and antisecretory (50 mg/kg ip.) doses at 30 min before administration of 0.6 M HCl.

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EFFECT OF CH-103, A BETA ADRENERGIC RECEPTOR ANTAGONIST ON THE ACTIVITY OF Ca^{2+},Mg^{2+}-ATPase IN RAT CARDIAC SARCOLEMMA

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Key words: sarcolemmal Ca^{2+},Mg^{2+}-ATPase, beta adrenergic antagonists, membrane perturbation

CH-103 (4-cyclohexylamino/-1-/1-naphtolenyloxy/-2-butanol maleate), a newly developed beta antagonist with quinidine-like membrane stabilizing property has produced a marked inhibitory effect on sarcolemmal Na^+,K^+-ATPase in rat myocardium (SZABÓ et al., 1988). Because this inhibition presumably is not the only manifestation of membrane perturbation caused by this compound, the effect on Ca^{2+},Mg^{2+}-ATPase activity was also studied in comparison with propranolol and practolol.

Pooled rat heart ventricles were homogenizated in 10 volumes of 20 mM Tris-HCl containing 1 mM ethylene-diamine-N,N,N'N'-tetraacetic acid and 0.1 mM phenylmethanesulfonyl fluoride (pH 7.0). Isolation of cardiac ventricular sarcolemma was performed at 4^oC as described (VELEMA and ZAAGSMA, 1981). Protein was determined by the method of LOWRY et al. (1951). The sarcolemmal ATPase activity was determined at 37^oC according to RUDINGER et al. (1984) by measuring the inorganic phosphate produced during the ATPase reaction by the method of TAUSSKY and SHORR (1953). To characterize the physicochemical property of CH-103 and other beta adrenergic antagonists the octanol:water partition coefficient was measured.

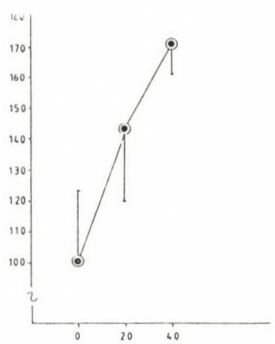

Fig.1. Effect of exogenous calmodulin on sarcolemmal Ca^{2+}-ATPase (ordinate: ATPase activity in percent of control±SE; abscisse: ng calmodulin/ug membrane protein)

Sarcolemmal Ca^{2+},Mg^{2+}-ATPase activity was sig-

Table I. Effect of beta antagonists on Ca^{2+},Mg^{2+}-ATPase in rat cardiac sarcolemma

Drugs $3x10^{-3}$M	N	ATPase activity* umol P_i/mg protein/hour			% distribution of ATPase	
		basic	total	Ca^{2+}-stimulated	basic	Ca^{2+}-stimulated
control	5	20.2±2.4	23.0±3.4	2.8±0.6	88	12
CH-103	4	3.1±0.1^{x}	5.8±0.3^{x}	2.7±0.3	53	47
propranolol	4	7.9±0.5^{x}	9.9±0.5^{x}	2.0±0.3	80	20
practolol	4	30.2±3.8	33.4±4.2	3.2±0.5	90	10

Each value is a mean±SE. ^{x}significantly different from control ($p < 0.05$), *in the presence of 40 ng calmodulin/ug membrane protein

nificantly stimulated by exogenous calmodulin (Fig.1). CH-103 and propranolol have drastically decreased the basic and total Ca^{2+},Mg^{2+}-ATPase activities; the Ca^{2+}-stimulated portion, however - remained nearly unchanged. At the same time practolol has increased both basic and total ATPases (Table I). The octanol:water partition coefficients were as follow: propranolol: 26.03, CH-103: 65.09, practolol: 0.0107.

Conclusion: CH-103, - similarly to other membrane active beta blockers, e.g. propranolol, - possesses some specific membrane activity which may be related to the physicochemical nature of the drug.

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EFFECTS OF INHIBITION OF CARDIAC POTASSIUM AND CALCIUM CHANNELS ON THE DIGOXIN-INDUCED CARDIOTONIC ARRHYTHMOGENIC AND TOXIC ACTIONS

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Key words: digoxin, 4-aminopyridine, verapamil, cardiotonic action, ventricular arrhythmias

Although digitalis has a benefical action in treatment of congestive heart failure, its arrhythmogenic action causes many problems for clinicians. In the present experiments the influence of inhibitors of cardiac potassium channels (4-aminopyridine: 4-AP) and of slow calcium channels (verapamil) was investigated on the digoxin-induced ventricular arrhythmias (infusion rate of digoxin: 17 ug/kg/min) and toxicity in anaesthetized (1.5 g/kg urethane i.p.) guinea pigs. In vitro experiments were made to analyse the positive inotropic action in isolated electrically stimulated (3 Hz, 1 ms voltage twice of threshold) left atrial myocardium of guinea pigs.

Table 1: Effects of 4-aminopyridine and verapamil on the digoxin-induced ventricular arrhythmias (A) and cardiotonic action (B)

A	Dose of digoxin (ug/kg) inducing			
Treatment	Ventricular extrasystole	Ventricular tachycardia	Ventricular fibrillation	Electric standstill
	in anaesthetized guinea pigs			
Control (n=6)	430±46.4	676±73.3	790±95.6	818±19.9
4-AP (n=6) (4 mg/kg)	317±24.7 n.s.	390±29.2 $p < 0.05$	430±33.9 $p < 0.01$	517±40.1 $p < 0.01$
Verapamil (1 mg/kg) (n=6)	500±106.1 n.s.	713±85.1 n.s.	1063±51.5 $p < 0.05$	1042±56.9 $p < 0.05$

B	Increase in contractile force of atrial myocardium		
Treatment	in the absence of 0.3 umol/l digoxin	in the presence of 0.3 umol/l digoxin	p
4-AP (n=5) (40 umol/l)	2.1±0.27 mN	4.5±0.68 mN	< 0.01
Verapamil (1 umol/l) (n=5)	3.4±0.51 mN	1.3±0.25 mN	< 0.01

In in vivo experiments on guinea pigs, both the dose requiring the precipitation of different ventricular arrhythmias and the lethal dose of digoxin decreased after addition of 4 mg/kg 4-AP (Table 1, Part A). In the case of verapamil (1 mg/kg) the reverse was true (Table 1, Part A).

Digoxin (0.3 umol/l) increased the contractile tension of myocardium by $53.6 \pm 4.46\%$ (n=10). In the presence of 40 umol/l 4-AP the cardiotonic action increased by 114%, whereas after addition of 1 umol/l verapamil this effect reduced by 62% (Table 1, Part B).

The findings obtained with verapamil are in good correlation with the clinical observations, namely, digitalis toxicity may be precipitated by elevated calcium levels (GUBNER and KALLMANN, 1957) it could be reduced by using chelating agents.

On the other part, it is well known that cardiac manifestations of toxicity occur when intracellular potassium concentration is reduced (LOWN and LEVINE, 1958). Paradoxycally, inhibition of potassium channels by 4-AP did not prevent, even enhance, the toxic reactions of digoxin. It is supposed that voltage-dependent opening of slow calcium channels in the presence of 4-AP could increase the influx of calcium to the intracellular space, therefore synergetic interaction of calcium and digitalis might be manifested. In addition, an indirect action mediated by releasing various transmitters from nerve varicosities could also be proposed.

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CONCENTRATION-DEPENDENT ACTION OF 4-AMINOPYRIDINE ON THE ADENOSINE-INDUCED SINUS SLOWING AND VASORELAXATION

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Key words: adenosine, 4-aminopyridine, pulmonary artery, sinus node

The cellular action of adenosine has been generally thought to be connected with the modification of transmembrane Ca^{2+} and K^+ fluxes. 4-aminopyridine (4-AP), a K^+ channel blocker, has been recently shown to have a dose-dependent biphasic action on the adenosine-induced coronary vasodilatation in anaesthetized dogs (KÉKESI et al., 1985; JUHÁSZ-NAGY et al., 1989). In the present experiments the action of different concentrations of 4-AP was studied on the suppressing effect of adenosine in the sinu-atrial node (tissue containing A1 adenosine receptors) and vascular smooth muscle (tissue containing A2 adenosine receptors). In spontaneously beating right atria of guinea pigs (technique: extracellular recording by bipolar platinum electrodes from the nodal area), adenosine (1-300 μmol/l) exerted sinus slowing, which was significantly potentiated in the presence of low concentration (2 μmol/l) of 4-AP (Fig 1A). Higher concentrations of 4-AP (1-10 mmol/l), however, antagonized the adenosine-induced reduction in the sinu-atrial pacemaker activity (Fig 1A).

Similar findings have been obtained from experiments on isolated pulmonary arterial strips of rabbits (technique: vessels precontracted with 0.3 μmol/l noradrenaline; recording by isometric mechano-electric transducer). The relaxing effect of adenosine was enhanced in the presence of 2 μmol/l 4-AP, but it was antagonized by exposure to 50 μmol/l 4-AP (Fig 1B and 1C).

These results are in accord with previous findings in anaesthetized dogs

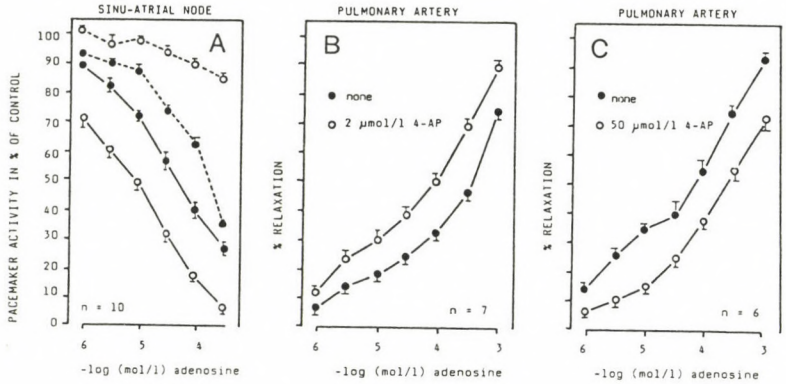

Fig.1: Modification of the adenosine-induced suppression in sinu-atrial pacemaker activity in the presence of 2 µmol/l (o——o), 1 mmol/l (●----●) and 10 mmol/l (o----o) 4-aminopyridine (Fig. 1A; ●——● : before exposure to 4-aminopyridine). Effect of 4-aminopyridine on the arterial vasorelaxation elicited by adenosine (Fig. 1B and 1C). Vasorelaxation (% Relaxation) is expressed as percent of relaxing action of 100 µmol/l papaverine in vessels precontracted by 0.3 µmol/l noradrenaline. Results are expressed as means±S.E.M.

(KÉKESI et al., 1985; JUHÁSZ-NAGY et al., 1989), in isolated dog coronary arteries (JUHÁSZ-NAGY et al., 1989) and in isolated, electrically paced atrial myocardium of guinea pigs (SZENTMIKLÓSI et al., 1983). It is supposed that 4-AP low concentrations might inhibit the adenosine turnover(i.e. dipyridamole-like action: inhibition of cellular adenosine uptake or coformycin-like effect: inhibition of intracellularly localized enzyme adenosine deaminase). The antagonistic effect of 4-AP could probably be due to its K^+ channel blocking property.

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EFFECT OF CAPSAICIN, RESINIFERATOXIN AND PIPERINE ON ETHANOL-INDUCED GASTRIC ULCER OF THE RAT

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Key words: capsaicin, piperine, resiniferatoxin, gastric ulcer

Introduction

Impaired defense mechanism to experimental ulcer formation after systemic pretreatment of rats with high, desensitizing doses of capsaicin has been demonstrated (Szolcsànyi, Barthò 1981). On the other hand intragastric capsaicin in lower dose range elicited local mucosal protection (Szolcsànyi, Barthò, 1981, Holzer, Lippe 1988, Holzer et al. 1989). These effects are not due to cytoprotection or direct mucosal action (Szolcsànyi, Mòzsik 1984, Holzer et al. 1989). A hypothesis has been put forward (Szolcsànyi, Barthò 1981), that capsaicin enhances mucosal microcirculation by releasing vasodilator mediators from local sensory receptors. This protective mechanism operates also in untreated animals and an impaired defense ensues when the nerve endings are blocked in capsaicin desensitized rats. In the light of these considerations other stimulants of capsaicin-sensitive receptors could also protect against experimental gastric ulcer. Furthermore, gastric application of capsaicin in desensitizing concentrations should aggravate ulcer formation. In the present paper piperine (PIP), the pungent ingredient of black pepper and resiniferatoxin (RTX), an ultrapotent capsaicin type agent (Szàllàsi et al. 1989) were used besides capsaicin (CAP) in the ethanol-induced ulcer model.

Methods

Female Wistar rats (250-310g) deprived of food for 24 h before the experiment, received through a stomach tube 50% ethanol in water v/v in 3 ml/kg volume for gastric ulcer production. The rats were killed 1 h later, and the number and area of mucosal lesions were determined under an operating microscope. Mann-Whitney U-test and X^2 statistics were used.

Results and discussion

The experiments with different pungent agents support our hypothesis outlined above. Table 1 shows that 1 h after intragastric application the incidence of lesions is significantly decreased when piperine, and low concentration of capsaicin or RTX were used. Piperine is a weak local stimulant and desensitizing agent (Szolcsányi, Barthó 1981). Desensitization with high local concentrations of capsaicin aggravated the ulcer formation 24 h after the treatment.

Table 1

Pretreatment agent	conc g/ml	time h	n	Incidence of lesions %	Ulcer index (mean mm^2)
Solvent	--	1	14	93	21.6
CAP	10^{-4}	1	10	80	14.1
CAP	10^{-6}	1	11	45***	9.5
PIP	10^{-2}	1	11	55**	7.4*
RTX	10^{-8}	1	9	56*	15.1
Solvent	--	24	11	82	22.5
CAP	10^{-3}	24	11	73	59.4*
CAP	10^{-4}	24	11	100	61.5*
PIP	10^{-2}	24	7	86	27.8
RTX	10^{-6}	24	11	64	15.5

*<0.05; **<0.03; ***<0.01

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INHIBITION OF LIPID PEROXIDATION BY MONOAMINE OXIDASE ENZYME INHIBITORS

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Key words: lipid peroxidation, MAO inhibitors

The peroxidation of unsaturated lipid components of biomembranes is a degradative free radical process. It has been considered to be involved in basic deteriorative mechanisms which are responsible for various pathological and toxicological states. Free radicals can be produced by enzymatic as well as non-enzymatic reactions in the organism. Monoamine oxidase enzyme (MAO) is also capable to generate hydrogen peroxide, one of the reactive oxygen species that may be involved in the initiation of lipid peroxidation.

We studied the effect of two irreversible MAO inhibitors in vitro on lipid peroxidation in rat brain homogenate. The two compounds were l-deprenyl, a selective inhibitor of MAO type B and J-512 (/1,2,3,4-tetrahydro-alpha-naphthyl/-methyl-propargylamine), a non-selective inhibitor of MAO enzyme. Lipid peroxidation was induced by NADPH or ascorbic acid in the presence of ADP and $FeCl_3$. Thiobarbituric acid (TBA) test was performed by the method of Buege and Aust (1978). In both systems l-deprenyl and J-512 inhibited lipid peroxidation in a concentration-dependent manner as shown in Figure 1. The concentrations of l-deprenyl producing 50% inhibition were 680 μmol/l (inducer: NADPH) and 310 μmol/l (inducer: ascorbic acid) and those of J-512 were 120 μmol/l and 160 μmol/l, respectively. Both in the abscence and presence of the inhibitors the formation of TBA-reactive products was time-dependent: over an experimental period of 90 min the amounts of these metabolites were continuously increased with time; nevertheless their produc-

tion remained suppressed (as compared with control) by l-deprenyl or J-512 throughout the experiment. The inhibitory effect was tested in the concentration range of 0.5-1 mmol/l for the former and 0.05-0.5 mmol/l for the latter substance.

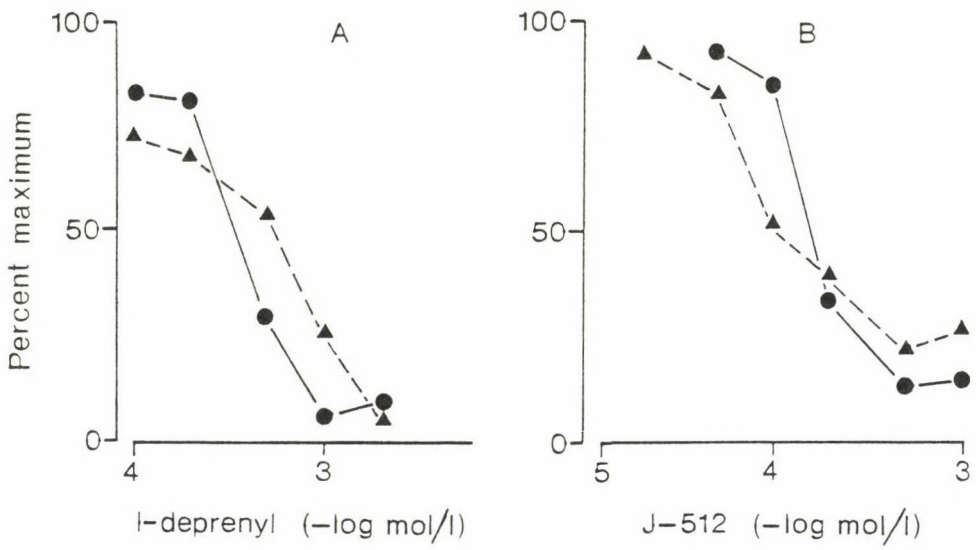

Figure 1 Effect of l-deprenyl A/ and J-512 B/ on NADPH (--▲--) and ascorbic acid (—●—) stimulated lipid peroxidation in rat brain homogenate. TBA-reactive products were tested after 45 minutes incubation. Results are presented as percent of control. Each point represents the mean of three determinations.

The regional distribution of TBA-reactive products was also estimated after a four-week l-deprenyl treatment of rats (daily dose: 0.25 mg/kg s.c.) Significant changes were observed in two of seven brain areas. The level of lipid peroxides was decreased in striatum, whereas it was elevated in the white matter.

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ROLE OF BIOCHEMICAL CHANGES IN THE DEVELOPMENT OF EARLY ISCHEMIC AND REPERFUSION ARRHYTHMIAS

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Key words: ischemia,reperfusion,arrhythmias,prostaglandins

One of the most serious consequences of the interruption of blood flow to the myocardium is the development of ventricular arrhythmias. Ventricular ectopic activity appears within minutes of the onset of myocardial ischemia in experimental animals and probably also in the clinical situation of an acute coronary attack (Parratt and Coker, 1985). There seems to be a close relationship between the development of these arrhythmias and the biochemical consequences of ischemia (Coker and Parratt, 1984). The purpose of our work is to study this problem.

In our previous experiments 15 min occlusion and 60 min reperfusion were induced by ligature of the left anterior descending coronary artery (LAD) in anesthetized open-chest dogs. The injury of the myocardium was investigated by measuring the hemodynamic, global and segmental function and electrophysiological parameters of the heart (Takács et al., 1988).

In the present study we determined thromboxane (TxB_2) and prostacycline (6-keto $PGF_{1\alpha}$) release and free-radical production (MDA) of the heart during the occlusion and reperfusion period.

The normal TxB_2/6-keto $PGF_{1\alpha}$ ratio (n=6 mean ± SE): 0.69±0.13 increased to 2.0±0.7 during occlusion when ectopic activity was high or ventricular fibrillation occured.

We found that treatment with an inhibitor of thromboxane synthesis and several scavenger drugs exerted a protective effect against ventricular ectopic activity.

We suppose that the increase in the ratio of thromboxane/prostacycline and in the production of free radicals, in addition to other biochemical factors, play an important role in the development of the arrhythmias resulting from both myocardial ischemia and subsequent reperfusion.

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ANTI - AMNESIC EFFECT OF A THIAZOLIDINE COMPOUND, GYKI - 20 238

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Keywords: GYKI-20 238, anti-amnesic effect, muscarinic agonist

It was shown earlier that a thiazolidine derivative, 2-methylimino - 3 - (2,6-dichloro-phenyl)-4-methyl-thiazolidine (GYKI - 20 238) facilitated the learning performance of mice in a simple one-trial passive avoidance test (Borsy et al., 1971).

Recently the growing interest in the possible application of cholinergic agents for the treatment of Alzheimer's disease has prompted us to find out whether the memory improving ability of GYKI - 20 238 is related to the central cholinergic system.

In this study we employed in vitro radioligand binding assay using 3H-quinuclidinyl benzylate (3H-QNB) (Yamamura and Snyder, 1974). Specific 3H-QNB binding in rat cortical membranes was inhibited by GYKI-20 238 with an IC_{50} value of 0.4 μM, showing that the compound has affinity to central muscarinic receptors.

In an attempt to prove a possible central muscarinic agonistic effect, we used the potentiation of oxotremorine evoked tremor and salivation in mice (Ögren et al., 1985). It was found that the compound (1 mg/kg ip. or 3 mg/kg p.o.) enhanced the tremorigenic response of oxotremorine without exerting any effect on peripheral cholinergic signs, such as salivation.

In order to study the anti-amnesic effect of GYKI - 20 238 we applied two rat behavioural models (Cumin et al., 1983).

We used the scopolamine - induced memory deficit in a passive avoidance test. The amnesic agent scopolamine (0.5 mg/kg ip.) and GYKI-20 238 were administered simultaneously after the last passive avoidance training. The other behavioural model we employed was the electroconvulsive shock - induced amnesic test. Electroshock (ESC; 45 mA, 2s) or sham ECS was applied after the avoidance acquisition. The training method and the administration of GYKI-20 238 were similar to those used in the scopolamine behavioural test.

Our results show (Fig. 1.) that GYKI-20 238 at a dose of 0.5 mg/kg p.o. significantly antagonized the memory deficit induced by both scopolamine and an electroconvulsive shock, giving an

inverted U-shaped dose response function.

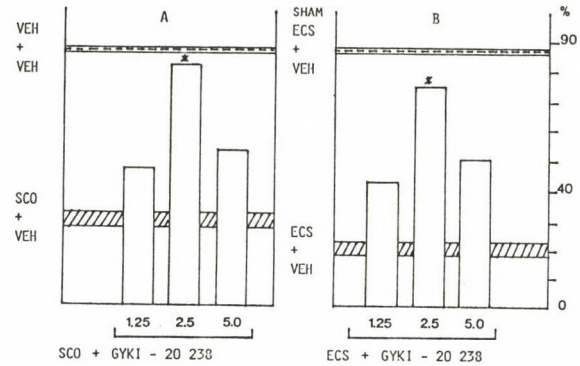

Fig. 1. Anti-amnesic effect of GYKI - 20 238 (1.25, 2.5 and 5 mg/kg p.o.) on memory deficit induced by scopolamine (A) or an electroconvulsive shock (B) in rats (N = 12). SCO = scopolamine hydrobromide (0.5 mg/kg ip.) VEH + VEH = vehicle ip. (distilled water) and vehicle p.o. (0.1 % carboxymethylcellulose in distilled water). Sham ECS + VEH = sham electroconvulsive shock and vehicle p.o. Ordinate = per cent of rats remaining on the platform for 60s in the retention test. * = $p < 0.05$ vs SCO + VEH or ECS + VEH controls, χ^2 test.

For studying the putative selectivity to M_1 muscarinic receptors, the assessement of purposeless chewing behaviour was carried out in rats (Stewart et al., 1989). Administration of GYKI-20 238 (1-4 mg/kg ip.) didn't produce a marked increase in purposeless chewing behaviour mediated via M_2 muscarinic receptors.

In summary, the results indicate that this compound has a marked anti-amnesic effect through the central muscarinic receptors of M_1 type.

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PRESYNAPTIC EFFECT OF DEXMEDETOMIDINE ON CHOLINERGIC CHEMICAL TRANSMISSION

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Key words: cholinergic system, dexmedetomidine

Alpha-2 adrenoceptor agonists (xylazine, clonidine) have been used in clinical practice, in a variety of medical treatments, including those of hypertension, opiate, alcohol and tobacco withdrawal, chronic pain. Very recently dexmedetomidine (4(5)-(1-(2,3-dimethylphenyl)ethyl)-imidazole) , a novel alpha-2 adrenoceptor agonist has been shown to reduce anesthetic requirements during surgery (Doze et. al., 1989). Alpha-2 adrenoceptor agonists prolong barbiturate sleep time (Hársing et al., 1989). The site of action of these effects is supposed to be on the peripheral and central noradrenergic system. Since the work of Paton and Vizi (1969) it is also known that cholinergic axon terminals are equipped with presynaptic inhibitory alpha-2 adrenoceptors (Beani et al., 1978 , Vizi and Pásztor, 1981) whose stimulation results in an inhibition of ACh release. In order to study the effect of l-dexmedetomidine HCl (MPV-1441,Farmos,Finland) on cholinergic transmission, on the intestinal motility ,isolated longitudinal muscle strip with Auerbach plexus attached was set up in Krebs solution and stimulated with 0.1Hz (1 msec).The contractions were

isometrically recorded (1.24±0.08 mN, n=16).

Dexmedetomidine (1 uM) reduced the force of contraction by 23%. CH-38083, a selective alpha-2 adrenoceptor antagonist, completely prevented the inhibitory effect of dexmedetomidine (Table 1.)

Table 1. Effect of dexmedetomidine on the contraction of guinea-pig ileal longitudinal muscle strip evoked by stimulation (0.1 Hz, 1 msec) and by neurotensin (0.03 uM)

	Contraction (%) evoked by stimulation	neurotensin
control	100±6,1	100±16
dexmedetomidine (1uM)	71,3±4,4	36,2±3,4
CH-38083 (0.8uM) +dexmedetomidine (1uM)	-	92,8±1.8

The contraction produced by neurotensin was completely reduced by dexmedetomidine.

It is suggested that dexmedetomidine inhibits ACh release from the myenteric plexus, reduces gastrointestinal motility.

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REFLEX INHIBITORY ACTION OF A NON-NMDA TYPE EXCITATORY AMINO ACID ANTAGONIST, GYKI 52466

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key words: spinal reflexes, excitatory amino acids

Excitatory amino acids glutamate and aspartate are regarded as major excitatory transmitters all over the mammalian central nervous system. Receptors mediating their actions are subdivided into NMDA and non-NMDA (kainate and quisqualate) receptors. Under physiological conditions, the role of NMDA receptors in mediation of fast synaptic events is a question of debate. They definitely play a role, however, in some so called plastic phenomena in the central nervous system. Although glutamate is the main transmitter candidate which mediates segmental spinal reflexes, only the polysynaptic component of them seems to be affected by NMDA antagonists (Davies and Watkins, 1983). The role of non-NMDA receptors in these processes has been difficult to investigate because of the lack of any in vivo effective specific antagonist.

GYKI 52466 is a 2,3-benzodiazepine compound which shows muscle relaxant and anticonvulsant properties in several animal models (Berzsenyi et al., 1988; Tarnawa et al., 1989). Recently it has been shown to selectively inhibit neuronal depolarization evoked by iontophoretic application of quisqualate and kainate in rat neocortex slices (Tarnawa et al., 1990). Our present data demonstrate the role of non-NMDA type excitatory amino acid receptors in both poly- and monosynaptic spinal reflexes.

Flexor reflex was investigated in intact, chloralose anesthetized cats. The hind paw was stimulated and the response was recorded from the anterior tibial muscle using a quantitative electromyographic method (Farkas et al., 1988). GYKI 52466 (1.0 mg/kg i.v.) inhibited the flexor reflex to about 50%. Patellar reflex was studied in chloralose anesthetized cats. ED_{50} values of GYKI 52466 were about 1.5 mg/kg i.v. both in intact and in C1 spinal cats. With

intraduodenal application, 4-8 mg/kg caused a 50% inhibition which lasted for several hours. After i.v. application maximum effect developed quickly. A two phase kinetics was observed in the recovery: a fast but only partial decrease in the drug effect was followed by a second phase of slow, gradual recovery. Spinal root reflexes evoked by stimulation of the tibial nerve were examined by recording reflex potentials directly from the L6, L7 and S1 spinal roots in spinal, unanesthetized cats. GYKI 52466 (2 mg/kg i.v.) inhibited both the mono- and the polysynaptic ventral root reflexes by about 50%. In contrast to the classical benzodiazepines like midazolam or diazepam, GYKI 52466 did not enhanced the dorsal root reflex and the dorsal root potential.

The fast onset and the characteristics of the recovery correlate with pharmacokinetic findings: 2 minutes after an i.v. dose of 4 mg/kg ^{14}C-GYKI 52466, a 20-30 µM concentration of the drug could be detected in the rat brain. The concentration quickly decreased down to about 5 µM, but a level of a few µM was still detectable even after several hours.

The fact that GYKI 52466 at the concentration of 10-50 µM considerably attenuated quisqualate responses in vitro suggets that inhibition of the non-NMDA type receptor mediated excitatory amino acid transmission is responsible for spinal reflex inhibition. Our results support the suggestions on the involvement of glutamate or other excitatory amino acids in mediation of the spinal monosynaptic reflex.

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COMPARISON OF THE EFFECTS OF (-)DEPRENYL, 1-PHENYL-2-PROPYL-AMINOPENTANE (MK-306) AND AMPHETAMINE ON THE STRIATAL AND LIMBIC DOPAMINERGIC SYSTEM

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Key words: MK-306, (-)deprenyl, striatal DA, mesolimbic DA

The stimulation of the nigrostriatal and the mesolimbic dopamine (DA)-ergic systems is known to have different behavioral consequences. The DA agonists-provoked stereotyped behavior is connected mainly to the nigrostriatal machinery, whereas hypermotility and rearing are related to the mesolimbic DA-ergic system (Costall et al., 1977). (-)Deprenyl, up to now the only selective monoamine-oxidase (MAO) B-type inhibitor used in clinical practice, facilitates the activity of the nigrostriatal dopaminergic neurons with peculiar selectivity (Knoll, 1987). It strongly potentiates the phenylethylamine-induced stereotyped behavior without inducing hypermotility or rearing (Timár et al., 1988).

(-)Deprenyl is known to inhibit the reuptake of catecholamine (CA) which makes this drug unique in beeing free from the cheese effect. On the base of this observation a series of deprenyl-derived compound were developed which inhibited the DA-reuptake, but have neither MAO-inhibitory nor noradrenaline releasing action. One of the most characteristic representatives of these substances is MK-306 (Knoll B. et al., 1988).

In this study the action of MK-306 on DA-stimulated activity was analysed in comparison to that of (-)deprenyl and amphet-

amine. The locomotion (LM), the rearing and the stereotyped head movements (STHMV) were simultaneously measured in a 30 min observation period. (-)Deprenyl following either single administration of as high dose as 20 mg/kg sc, or following a repeated administration of the selective MAO-B inhibitory dose (0.25 mg/kg sc) failed to induce any type of hyperactivity. MK-306 increased the activity of the rats, however, as Table 1. shows it, stereotypy was induced only with a higher dose.

Table 1. MK-306-and amphetamine-induced hyperactivity in rats

	Dose mg/kg	Time (min) spend with LM	rearing	STHMV
Saline		2.82	3.32	0.48
MK-306	1.0	4.94	3.71	0.77
	5.0	5.48[x]	6.70[x]	0.78
	20.0	2.51	12.12[x]	0.93
	25.0	6.53[x]	5.53	5.01[x]
Amphetamine				
	0.5	4.48	4.24	1.29[x]
	5.0	7.12[x]	6.85	3.09[x]

[x] mathematically significant compared to saline

MK-306, influences learning behavior in smaller doses (1-2 mg/kg, sc) (see Knoll B. et al., in this volume), which speaks in favor of the assumption that this compound has a stronger effect on the mesolimbic system than on the striatal DA-ergic neurons.

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SODIUM-AZIDE-EVOKED NORADRENALINE RELEASE FROM PERIPHERAL NERVES OF RABBIT PULMONARY ARTERY

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Key Words: tritiated noradrenaline release, sodium-pump, sodium-azide

The spontaneous release of /^{3}H/noradrenaline (/^{3}H/NA) has been measured from the isolated main pulmonary artery of the rabbit in the presence of uptake blockers (cocaine, 30/uM; corticosterone, 50/uM). The tone of the smooth muscle was simultaneously recorded. The Na^+-pump inhibitor sodium-azide (NaN_3, 2mM) moderately increased the /^{3}H/NA release and relaxed the smooth muscle. K^+-removal from the Krebs solution increased the release of /^{3}H/NA and the tone of pulmonary artery. NaN_3 further increased the transmitter release in "K-free" solution, but relaxed the muscle. External K^+-readmission was effective in inhibiting the transmitter releasing action of NaN_3. In another series of experiments the transmitter releasing action of ouabain (10^{-4}M) was also measured. Ouabain further increased the /^{3}H/NA release in the absence of external K^+ and in the presence of azide, but failed to produce post-synaptic response. In conclusion, we found that in peripheral sympathetic nerves sodium-azide also inhibited the Na^+-pump in a similar manner, like in motor nerve terminals (Elmqvist and Feldman, 1965). The elevated /^{3}H/NA release, which was a consequence of Na^+-pump inhibition was abolished during reactivation of Na^+-pump by readmission of K^+ to Na^+-loaded cells.

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DIFFERENT SENSITIVITY OF CHOLINERGIC AND DOPAMINERGIC NEURONS TO SULPIRIDE

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Cholinergic and dopaminergic neurotransmission are strongly interrelated in the striatum. The release of acetylcholine (ACh) is modulated by presynaptic muscarinic autoreceptors (Szerb, 1977) and by dopaminergic heteroceptors of the D-2 subtype (Vizi et al., 1977). The release of dopamine (DA) is inhibited by dopaminergic D-2 autoreceptors (Groves et al., 1975). However, the physiological significance of these interactions is still debated. The existence of a presynaptic receptor does not necessarily indicate that the presynaptic modulation operates under physiological conditions. If the biophase concentration of the neurotransmitter is not high enough in the vicinity of the axon terminal, presynaptic receptors are not functioning.

The aim of this study was to determine, whether or not presynaptic DA-2 receptors, which inhibit neurotransmitter release from cholinergic and dopaminergic neurons in the striatum are tonically stimulated by endogenous DA in an in vitro slice preparation.

Rat striatal slices (450 um thickness) were loaded with ^{3}H-choline or ^{3}H-DA, superfused with Krebs solution and stimulated with electrical field stimulation (20 V/cm, 2 Hz 1 ms, 240 pulses). ^{3}H-ACh or ^{3}H-DA release into the superfusate was determined with scintillation spectrometry. Alteration of neurotransmitter release was characterized by the S_2/S_1 ratio.

Sulpiride, a selective antagonist of DA-2 receptors, increased the release of ^{3}H-DA in a concentration-dependent mannner, probably by interrupting the negative feed-back inhibition of endogenous DA. On the other hand sulpiride had no effect on the release of ^{3}H-ACh. Evidence was presented, that cholinergic terminals were equipped with presynaptic DA-2 receptors as well as dopaminergic terminals, since PPHT, a selective DA-2 receptor agonist, was able to decrease ^{3}H-ACh release, an effect, which could be antagonized with sulpiride.

Transmitter	Sulpiride conc.	S_2/S_1	n	Significance
^{3}H-DA	-	0.98 $\pm$ 0.06	3	
^{3}H-DA	1 uM	1.10 $\pm$ 0.05	3	$p > 0.05$
^{3}H-DA	10 uM	1.33 $\pm$ 0.05	3	$p < 0.05$
^{3}H-DA	100 uM	2.03 $\pm$ 0.07	4	$p < 0.001$
^{3}H-ACh	-	1.02 $\pm$ 0.06	4	
^{3}H-ACh	10 uM	0.95 $\pm$ 0.04	4	$p > 0.05$

Our results suggest, that although in the striatum cholinergic nerve terminals are equipped with presynaptic DA-2 receptors, the biophase concentration of endogenous DA at the cholinergic terminals is not high enough to inhibit ACh release. An other possibility is, that due to different intracellular signalling pathways, presynaptic DA-2 receptors of cholinergic and dopaminergic neurons are not similarly sensitive to endogenous DA.

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IMMUNMODULATORY ACTIVITY OF D-Met^2– Pro^5–ENKEPHALINAMIDE

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KEY WORDS: D-Met^2-Pro^5-enkephalinamide, immunmodulator

D-Met^2-Pro^5-enkephalinamide (EA) is an opioid peptide analogue having analgesic activity in animals more potent than morphine after intravenous administration. Clinical studies revealed that EA has analgesic effect in human, as well.

Recently it has become clear that, in addition to their role in nervous system, opioid peptides have pleiotropic effect on several body system, including the immune system.

Inspired by these findings we studied the effects of EA on various immune functions.

Materials and methods

Animals: CBA mice and Long Evans rats were used throughout the experiment.

Mouse spleen cell mitogenesis

Spleen cells were stimulated in vitro with Phytohemagglutinin and Concanavalin A in the absence and presence of the test compound. After 48 h incubation at 37 ^{0}C in 5 % CO_2 atmosphere, cultures were pulsed with ^{3}H-thymidine for further 16 hours. Cells were harvested on Whatman GF/c filter and the radioactivity incorporated to the cells was counted in a liquid scintillation spectrophotometer.

Macrophage phagocytosis

Macrophages were obtained by peritoneal lavage and allowed to adhere to culture plate. IgG coated sheep erythrocytes were used as the indicator of phagocytic activity. The ratio of phagocytic cells was quantitated microscopically.

Antibody formation

Mice were immunized with sheep red blood cells (SRBC) and treated with the test compound or vehicle. At different times after immunization mice were bled and the levels of lysing antibodies against SRBC were tested.

Delayed type hypersensitivity (DTH) reaction

Mice were immunized intravenously with 10^6_8 SRBC. Five days later animals were restimulated with 10^8 SRBC intraplantarly. Footpad swelling was measured 24 h later.

Rat adjuvant arthritis

Adjuvant arthritis was induced by CFA. The progress of the disease was monitored by measuring the volume of both hind feet by a plethysmograph.
The effect on the primary inflammation (0-10 days) and on the immune-arthritis phase (10-21 days) was tested separately. The test compound was applied sc. in 0.1-1.0-10 mg/kg/day doses.

Results

EA treatment slightly modified spleen cell mitogenesis. It had no effect on in vivo antibody formation. Macrophage phagocytosis was inhibited by 25-30 % at the 10-30 mg/kg dose range when 1 day ip. pretreatment was applied.
EA inhibited the DTH reaction by 25 % in 5x100 mg/kg daily doses if the treatment was applied after immunization.
EA resulted in more severe inflammation both on the immunized (+70 %) and on the other foot (+40 %), when the compound was applied in the first and second phase of the adjuvant arthritis, respectively.

These findings indicate that D-Met^2-Pro^5-enkephalinamide in high doses has immunmodulatory property.

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INTERNAL SODIUM-DEPENDENT /^{3}H/NORADRENALINE RELEASING ACTION OF MANGANESE FROM THE ISOLATED MAIN PULMONARY ARTERY OF THE RABBIT

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key words: rabbit pulmonary artery, /^{3}H/NA-release, Mn^{2+}, Na^+-pump, Na^+/Ca^{2+}-exchange

Spontaneous /^{3}H/noradrenaline (/^{3}H/NA) release from the isolated main pulmonary artery of the rabbit has been measured in the presence of uptake blockers (cocaine, $3x10^{-5}$M; corticosterone, $5x10^{-5}$M) and after blocking the MAO enzyme (pargyline, $1.2x10^{-4}$M).

The Na^+-pump was inhibited by removal of K^+ from the external medium (Na^+-loading) in the absence of external Ca^{2+} (+ 1 mM EGTA). In K^+-, and Ca^{2+}-free medium manganese (Mn^{2+}, 2 mM) increased the release of /^{3}H/NA from the arteries and the rate of release was directly proportional to Na^+-loading being bigger if longer exposure time was used to K^+-free solution. Without Na^+-loading (Ca^{2+}-free, EGTA alone), Mn^{2+} failed to produce transmitter release. Using constant Na^+-loading (120 min K^+-free perfusion) the Mn^{2+}-evoked /^{3}H/NA-release was also directly proportional to the concentration of Mn^{2+} used (1.2, 1.6, 2.0 mM). When most of the external Na^+ (113 mM) was substituted for Li^+ during Na^+-loading, Mn^{2+} was ineffective in producing transmitter release. Mn^{2+} was also ineffective in releasing neurotransmitter if the Na^+-pump

was previously reactivated by readmission of K^+ to Na^+-loaded nerves.

It is concluded that under reversed Ca^{2+}-gradient, the transition metal Mn^{2+}, the well known blocker of Ca-channel and Na^+/Ca^{2+}-exchange, releases neurotransmitter by an internal Na^+-dependent manner from peripheral sympathetic nerves.

AN ALGORITHM FOR EVALUATING THE ABSORPTION RATE CONSTANT FROM URINARY EXCRETION DATA.

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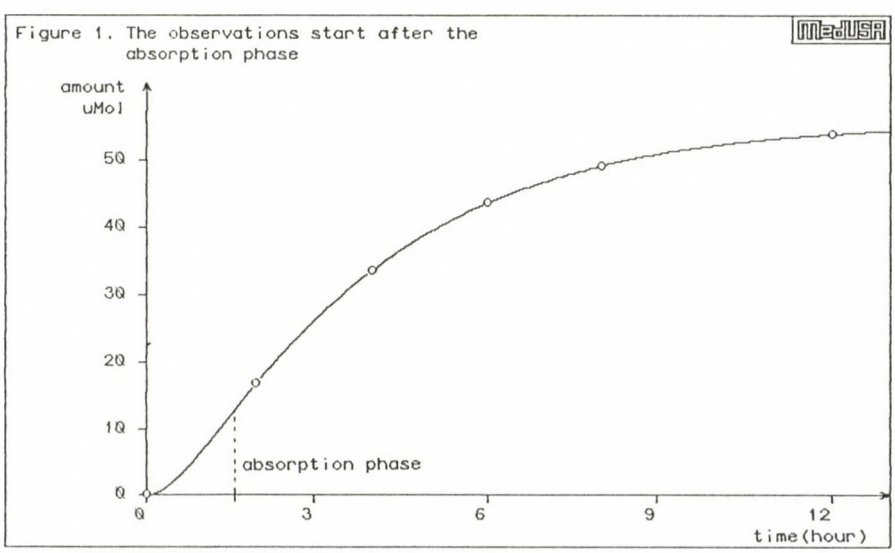
Simple calculations providing the parameter estimates of the Linear Compartment Open Models with first order absorption, from cumulative urinary excretion data are described in this paper. The estimation of the parameter characterizing the absorption can be very difficult from urinary excretion data because the frequently applied methods of measurement give the first relevant data at several hours after administration, thus the first region of the expected curve directly related to the absorption is lost (Fig. 1). The suggested algorithm is able estimate the absorption constant and the other parameters of the models in the case of the above conditions. It is based on the sigma minus plot with linear regression or "stripping technique" accomplished with some



simple calculations. Of course a more reliable solution can be obtained by a complicated computer program for nonlinear estimation, but even so, the algorithm can provide a good set of preliminary estimates.

METHOD

The equation of the cumulative amount excreted to the urine in the case of the general model containing n compartments is as follows:

$$X(t) = X_\infty - Ke^{-k_a t} - \sum_{i=1}^{n} A_i e^{-\alpha_i t} \qquad (1)$$

where t - time
$X(t)$ - amount of drug excreted into urine until time t
X_∞ - total amount excreted
k_a - absorption rate constant
K - coefficient of the exponential term of the absorption
A_i - coefficient of the i-th exponential term of the elimination
α_i - apparent elimination rate constant of the i-th elimination region

From Equation 1 and the corresponding plasma equation the following can be obtained:

$$k_a = \frac{\sum_{i=1}^{n} A_i \alpha_i}{\sum_{i=1}^{n} A_i - X_\infty}$$

To obtain k_a, A_i-s, α_i-s and X_∞ have to be provided. For the One and the Two Compartment Model these parameters can be obtained by employing the sigma minus plot.

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EFFECT OF NITROGLYCERIN AND A NITROGLYCERIN-LIKE SUBSTANCE IN ISOPRENALINE (ISO) NECROSIS AND HYPOXIA

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(cardioprotection, hypoxia, nitroglycerin, molsidomine, SIN-1)

Molsidomine is new antianginal agent with effects on hemodynamics, Fiedler and Scholthols (1978). The drug has been shown to be effective in the treatment of angina pectoris by a nitroglycerin-like mode of action but of substantially longer duration, Majid (1980).A number of sympathomimetic amines can cause cardiac damage. The cardiotoxic effect of ISO, which induced an infarct-like necrosis, has been of particular interest since standard dosages consistently produce myocardial lesions of reproducible severity. ISO induced cardiac necrosis has been widely used for studying the potential protective effect of pharmacological interventions on the morphological evaluation of the size of myocardial infarction.

We tested the relative cardioprotective potencies of nitroglycerin, molsidomine and SIN-1 in the rat ISO model according to Körmöczy et al (1987).ISO was injected in 5 mg/kg i.p. doses, daily for 5 consecutive days. We evaluated the developed hypoxia (HP) and necrosis (NC) quantitatively by histological methods.To follow the time course of cardioprotection the drugs were injected daily i.p. 60, 120 and 240 min before ISO.Cardioprotection was defined as the relative reduction of percentually expressed necrotic (NC) and hypoxic (HP) areas, respectively from corresponding values in the orally treated physiological saline and ISO control group.The comparative cardioprotective effect of nitroglycerin, molsidomine and SIN-1:

hour	nitroglycerin 1 mg/kg i.p.		molsidomine 1 mg/kg i.p.		SIN-1 0.25 mg/kg i.v.	
	NC%	HP%	NP%	HP%	NC%	HP%
1	70.3^{xx}	68.4^{xx}	58.2^{xx}	65.5^{xx}	95.1^{xx}	95.4^{xx}
2	59.9^{xx}	59.4^{xx}	69.1^{xx}	77.7^{xx}		
3	37.7^{x}	39.2^{x}	50.1^{xx}	57.4^{xx}		

Values represent percentage difference in hypoxic and necrotic areas compared to control: x = $p < 0.01$, xx = $p < 0.001$

The effect of molsidomine is more prolonged than that of nitroglycerin.

The pretreatment with SIN-1 prevents cardiac necrosis and hypoxia.

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BIOCHEMICAL BACKGROUND OF β-CAROTENE-INDUCED GASTRIC CYTOPROTECTION IN RATS TREATED WITH HCl.

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Key-words:gastric cytoprotection,carotene,mucosal biochemistry.

Vitamin A and other carotenoids prevent the development of gastric mucosal damage produced by intragastric administration of different necrotizing agents (such as 96% ethanol, 0.6 M HCl) without presence of any inhibition of gastric acid secretion (Jávor et al.,1983).

The aim of this study was to compare the β-carotene-induced changes in the gastric mucosal biochemistry,during the development of gastric cytoprotection produced by its different doses.

The observations were carried out on Sprague-Dawley(LATI,Gödöllő, Hungary)-strain rats,weighing 180-210 g. The animals were fasted for 24 hours before the experiments. The gastric mucosal damage was produced by intrgastric administration of 0.6 M HCl. The animals were treated with β-carotene at 30 min before administration of HCl.The different doses of β-carotene were dissolved in sunflower oil and given intragastrically. The animals were sacrificed at 1 hour after the administration of HCl, when the number and severity of gastric mucosal lesions was noted.The tissue levels of ATP,ADP,AMP and lactate were enzymatically measured, while cAMP by RIA.The values of ATP/ADP,adenylate pool (ATP+ADP+AMP) and "energy charge " [(ATP+0.5 ADP)/(ATP+ADP+AMP)] were calculated. All biochemical parameters were calculated in relation to 1.0 mg mucosal protein, and their results expressed as mean ± SEM.

It has been found that: 1.The extent of ATP-ADP transformation decreased in association with the increased extent of ATP-cAMP transformations and of cAMP-AMP transformations; 2.The changes in membrane-bound ATP-dependent energy systems depended on doses of β-carotene; 3.The value of energy charge was unchanged.

BIOCHEMICAL BACKGROUND OF GASTRIC MUCOSAL PROTECTION OF 0.6M HCl-INDUCED GASTRIC MUCOSAL LESIONS BY DIFFERENT CYTOPROTECTIVE DOSES OF β-CAROTENE (means±SEM)(n=10)

(in comparison with results of damaged mucosa)

doses of β-carotene (mg·kg^{-1} ig.)

	0.01	0.1	1.0	10.0
Ulcer number	0±0 NS	-6.0±0.5 +++	-6.5±0.5 +++	-9.0±0.6 +++
Ulcer severity	0±0 NS	-10.0±1.0 ++	-15.0±0.6 +++	20.0±0.6 +++
ATP	-2.0±0.7 NS	-0.0±0.0 NS	+2.0±0.5 NS	+2.1±0.5 NS
ADP	0±0 NS	-1.7±0.8 NS	-4.0±0.5 +++	-5.0±0.6 +++
ATP·ADP^{-1}	-0.09±0.01 NS	-0.09±0.02 NS	+0.22±0.02 +++	+0.24±0.03 +++
AMP	+2.0±0.8 NS	+2.8±0.7 NS	+4.2±0.7 ++	+8.0±0.1 +++
cAMP	+0.3±0.01 NS	+0.5±0.04 NS	+1.0±0.02 +++	+1.1±0.02 +++
Adenylate pool	+1.0±0.9 NS	+1.0±0.8 NS	+1.2±0.7 NS	+2.0±0.9 NS
„energy charge"	-0.07±0.01 NS	-0.08±0.01 NS	-0.09±0.01 NS	-0.10±0.01 NS
Lactate	-30±10 NS	-20±10 NS	-15±15 NS	-20±10 NS

NS = not significant ; ++ = $P < 0.01$; +++ = $P < 0.001$

Fig.1.Changes in the gastric mucosal biochemistry,during the development of gastric cytoprotection, produced by different doses of β-carotene. The results were calculated as differences between the results obtained without and with application of β-carotene.

It has been concluded that: 1.A modification,between the different membrane-bound ATP-dependent energy systems,could be produced by application of β-carotene in the ulcerated gastric mucosa of rats treated with HCl; 2. The extent of phosphorylation and/or dephosphorylation of cells, in both cases of without and with treatment of β-carotene, was unchanged; 3. The extents of biochemical changes depended on the dosage of β-carotene.

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MICROELECTROPHARMACOLOGICAL STUDY OF PRAJMALINE IN RABBIT PAPILLARY MUSCLE

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Keywords: prajmaline, papillary muscle, transmembrane potential, use--dependence.

Little information is available on the microelectropharmacological properties of prajmaline, a widely used Class 1 antiarrhythmic drug (Späth, 1983). A systematic study to subclassify this agent has not yet been carried out. The present experiments were therefore performed to gain more insight into the mechanism of action of prajmaline at the cellular level.

Right ventricular papillary muscles were prepared from hearts of young rabbits and placed in an organ bath containing modified Locke's solution. Transmembrane potentials were recorded by utilizing a microelectrode technique, as described earlier (Freedberg et al., 1970; Szekeres and Papp, 1986; Virág et al., 1987). The preparations were exposed to increasing concentrations of prajmaline; each concentration was allowed to act for 60 min.

The results have been summarized in Table I. Prajmaline (0.1, 0.2 and 0.5 mg/l) diminished the maximum rate of depolarization ($\dot{V}_{max}$). The action potential amplitude (APA) was also depressed considerably (at 0.5 mg/l), while the other characteristic parameters of the transmembrane potential remained unaltered, i.e. the action potential duration (APD) at 50 or 90 % repolarizations was not prolonged or shortened to any significant extent, and the resting potential (RP) and the effective ref-ractory period (ERP) did not change either.

In the presence of prajmaline (0.5 mg/l) the blockade of $\dot{V}_{max}$ occurred in a use-dependent manner, but a considerable resting block was also observed. The drug-induced use-dependent block was fitted by using the following formula:

$$dV/dt_{max} = P(1) + P(2)\ \exp(P(3)t)$$

where P(1) is the steady-state level of $\dot{V}_{max}$ block, - P(2) is the use-dependent block, P(3) is the kinetic constant. At an interstimulus interval of 300 ms, exposure to 0.5 mg/l prajmaline resulted in (mean ± S.D.)

- 64.3 ± 2.7 V/s for P(1), 34.3 ± 3.7 V/s for P(2) and

- 0.051 ± 0.012 s^{-1} for P(3).

Table I

Changes in action potential parameters in the presence of prajmaline

Drug	RP (mV)	APA (mV)	APD_{50} (ms)	APD_{90} (ms)	$\dot{V}_{max}$ (V/s)	ERP (ms)
Control (n=10)	-78.5 ±2.0	104.3 ±3.8	152.3 ±20.4	200.8 ±23.9	129.3 ±19.3	198.2 ±18.0
Prajmaline						
0.1 mg/l (n=3)	-78.3 ±0.4	102.5 ±4.2	157.0 ±25.1	202.2 ±25.5	110.7 ±17.5	211.8 ±21.6
0.2 mg/l (n=3)	-78.2 ±2.6	102.8 ±4.3	140.4 ±20.3	194.0 ±21.9	99.5^{+} ±13.8	189.2 ±21.3
0.5 mg/l (n=4)	-77.3 ±1.1	93.7^{+} ±3.5	159.9 ±17.0	210.4 ±20.4	57.3^{+} ±18.8	220.0 ±18.4

Means ± S.D. n = number of preparations. $^{+}$ $P<0.05$.
See text for abbreviations.

In conclusion, prajmaline appears to belong to that subgroup of Class 1 antiarrhythmics which is characterized by a marked blockade of $\dot{V}_{max}$, ineffectiveness on the APD or ERP and also by a slow rate of onset of $\dot{V}_{max}$ inhibition.

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HETEROGENEITY OF PRESYNAPTIC ALPHA-2-ADRENOCEPTORS

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Key words:alpha-2-adrenoceptors, presynaptic modulation

Presynaptic alpha-2-adrenoceptors, which modulate transmitter release from different nerve terminals have been found in numerous animal tissues as well as in various human blood vessels. Recently, we have shown that presynaptic alpha-2-adrenoceptors are also present in the human cystic artery (Kapocsi et al., 1987a). Also recently, studies performed by a number of laboratories have indicated the possibility of the heterogeneity of alpha-2-adrenoceptors. Heterogeneity of alpha2-adrenergic receptors has been noted in many laboratories both on the basis of functional and radioligand binding studies. More recently this heterogeneity has been interpreted to result from pharmacological subtypes of the receptor.

Isolated vas deferens of the rat and longitudinal muscle strip of guinea-pig ileum were used to study the presynaptic effect of alpha-2-adrenoceptor agonists and antagonists on chemical neurotransmission (for methods see Kapocsi et al., 1987b). In addition the effect of different alpha-2-antagonists on the release of 3H-noradrenaline from cerebral cortex was also measured. There was a significant difference in the presynaptic inhibitory activity (IC50 in uM) of l-NA and Xylazine on rat vas

deferens and long. m. strip of guinea pig of ileum (Table 1). CH-38083, a selective alpha-2-adrenoceptor antagonist with alloberbane structure (Vizi et al., 1986) enhanced the release of 3H-NE from the cerebral cortex slice. However the stereoisomers of CH-38083 antagonized the inhibitory effect of exogenous NE with different potencies: K_B values for (-)-isomer and for (+)-isomer were 14.3 and 97. 2 nmol/l. In addition, difference was found between receptors located in the CNS and in the periphery.

Table 1. Presynaptic inhibitory effect of l-noradrenaline and xylazine (IC_{50} in uM) on chemical neurotransmission in rat vas deferens and guinea-pig ileal longitudinal muscle strip attached with Auerbach plexus 0.1 HZ stimulation (1 msec).

	rat vas def(a)	Auerbach pl.(b)	b/a
l-noradrenaline	1.67	2.5	1.5
xylazine	0.05	3.7	74.0

Our findings do not support the unified hypothesis of feedback modulation of NE release. It is concluded that two different subtypes of presynaptic alpha-2-adrenoceptors are involved in the modulation of NE release in the CNS and in the periphery.

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COMPARISON OF THE DEPENDENCE CAPACITY OF AMPHETAMINE, MK-306 and (-)DEPRENYL

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Key words: (-)deprenyl, Mk-306, amphetamine, dependence capacity

(-)Deprenyl is a methamphetamine derivative, free of catecholamine releasing properties. It was the first described selective inhibitor of B-type MAO, is still the only one used in clinical practice, being free of the cheese effect. The safeness of the drug is due to its ability to inhibit the uptake of indirectly acting amines. This spectrum of the drug initiated the development of (-)deprenyl-derived compounds which are devoid of MAO inhibitory potency but are more potent than the parent drug in inhibiting the uptake of tyramine. MK-306 is one of these derivatives possessing a peculiar psychostimulant effect without having either MAO inhibitory or catecholamine releasing properties (Knoll, B. et al., 1988).

We analyzed the physiological dependence capacity of (-)deprenyl and MK-306 in comparison to amphetamine in rats using the method of Nickel and Aledter (1987). Rats were treated either with drug or saline daily for 8 weeks and treatment was withdrawn on every seventh day. Body weight was measured daily four hours after the administration of the drug or saline. As a sign of physical dependence in amphetamine-treated rats body weight was significantly increased on the day of drug with-drawal and to end of the experiment the amphetamine-

treated rats has a significantly lower body weight than their saline treated peers. No sign of physical dependence was observed in (-)deprenyl treated rats (4 mg/kg/day, orally).

MK-306, which was as potent as amphetamine in facilitating learning in the shuttle box, was in a series of experiments completely free of showing signs of physical dependence. Even in the series of experiments in which a high dose of MK-306 (20 mg/kg/day) was given, inconsistent sign of withdrawal only were observed, without any loss of body weight compared to the saline-treated controls. In the parallel group treated with 5 mg/kg/day amphetamine physical dependence developed uniquivocally and the loss of body weight was highly significant.

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THE EFFECT OF DINH LANG AND (-)DEPRENYL ON THE SURVIVAL RATE OF MALE RATS

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Key words: sexual activity, motility, Dinh lang, deprenyl, longevity.

Dinh lang root extract (DLRE) was found to exert an aphrodisiac effect (Yen, 1981). It was known from previous experiments that (-)deprenyl, the first selective inhibitor of MAO-B and still the only one in clinical practice, facilitates both sexual activity and learning performance in rats, because it facilitates with high selectivity the activity of the striatal dopaminergic system (Knoll, 1987). Long term treatment with (-)deprenyl extended lifespan of the rats well over the known maximum age of rats (Knoll, 1988, Knoll et al., 1989).

We studied the effect of a combination of (-)deprenyl and DLRE on male rats' behavioral performance and survival.

195 aged sexually inexperienced Wistar male rats (24 months old) which did not display ejaculation were tested. 66 rats were given saline, 65 were treated with DLRE (10 mg/kg, per os) and 64 with the combination of DLRE (10 mg/kg) and (-)deprenyl (0.25 mg/kg, s.c.) three times a week.

Sexual activity was tested once a week, motility once a month and body weight was measured twice in a mounth.

DLRE and the combination of DLRE and (-)deprenyl restored full scale of sexual activity (around 96% of rats in both

groups ejaculated in at least one test during the experimental period). The age-related motor function is known to be decreased with advanced age. But the treatment of DLRE and (-)deprenyl slowed down this phenomenon. The mean activity in open field behavior in drug-treated groups was significantly higher than that of controls. The body weight loss of aged rats was commonly observed. The control body weight loss was about 40% but there were only 15-30% in the drug treated groups.

The changes of behavioral activities may have a possible correlation with the survival time in rats. The longest living rat in control group lived 164 weeks. The average lifespan of the group was 147.05+1.56 weeks. The average of surviving time of DLRE and its combination of (-)deprenyl treated groups were 179.98+1.87 weeks and 206.33+3.40 weeks, respectively. The longest living animal in the DLRE treated group lived 194 weeks, whereas in the group treated with DLRE + deprenyl the last animal completed 239 weeks.

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THE EFFECT OF ANGIOTENSIN I AND III ON THE ARACHIDONATE CASCADE OF BRAIN MICROVESSELS

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Key words: angiotensin I and III, arachidonate cascade brain microvessels

Introduction

Experimental evidence supports the hypothesis that prostaglandins and lipoxygenase metabolites have physiological and/or pathological role in central blood pressure control. The cerebral cortical microvessels have been studied specifically, because arachidonate interactions might occur with other cells (circulating blood cell, platelets or neural elements) and peptides - angiotensin I and angiotensin III - that come into contact with the endothelial lining of the microvessels. The locally synthesized and released arachidonate metabolites might modify the endothelial cell functions, resulting in an altered cerebral microcirculation and blood-brain-barrier function, with consequences. Endothelial cells are rich in angiotensin -converting enzyme, and it therefore seemed worthwhile to determine whether angiotensin I and/or angiotensin III have any effect on the arachidonate cascade of brain microvessels.

Methods

Blood-free cerebral cortex microvessels were isolated from male rats of CFY strain, weighing 160-180 g, by a micrometod of Hwang et al. (1980) with modifications.

The isolated microvessels were preincubated in TC Medium 199 at 37 ^{o}C for 10 min and then angiotensin I or angiotensin III (10^{-9}M - to 10^{-5}M) was given to the incubation mixture, ten minutes later the enzyme reaction was started by the addition of ^{14}C-arachidonic acid (3.7 kBq) as tracer substrate to the incubation mixture. Radioactive arachidonate metabolites were separated and quantitatively determined in a Beckman LS liquid scintillation counter.

Results and Discussion

Angiotensin I (10^{-9} M to 10^{-6} M) significantly inhibited the release of the total arachidonate metabolites from brain microvessels. The cyclooxygenase pathway was attenuated by the the only concentration (10^{-9} M) of angiotensin I. The peptide was more effective on the lipoxygenase pathway resulting in an inhibition (10^{-9} M to 10^{-6} M). This effect might be indirect, because the endothelial cells are rich sources of angiotensin converting enzyme (Johnson and Erdős 1979). Angiotensin III was less effective on the arachidonate cascade of brain microvessels than angiotensin I. The release of total arachidonate products was inhibited by two concentrations of angiotensin III (10^{-9} M and 10^{-8} M). The ratio of lipoxygenase and cyclooxygenase products was not modified by any concentration of angiotensin III.

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