DIFFERENTIAL EFFECT OF YOHIMBINE IN SEVERAL CHEMICAL MODELS OF CONVULSIONS IN MICE

K.G. Lloyd and P. Worms , Neuropharmacology Group, LERS-Synthélabo, 31 ave P.V. Couturier, 92220, Bagneux, France

Noradrenaline (NA) has been postulated to play an important role in seizure phenomena (Maynert et al, 1975) and recently, the α2-adrenoceptor antagonist yomhimbine (YOH) has been reported to block convulsions induced by handling in Quaking mice (Chermat et al, 1981). The present study investigated the effects of YOH, alone or in combination with clonidine, in 4 models of chemical convulsions in mice.

Male Swiss mice (CD_1, Charles River France) weighing 16 to 20 g were used. YOH alone, or simultaneously with clonidine, was injected intraperitoneally (ip) 30 min. before either picrotoxinin, metrazol, bicuculline or strychnine.

As shown in Table 1, YOH (3 to 12 mg/kg, ip) potentiated the clonic or tonic convulsions induced by picrotoxinin (0.75 or 1.6 mg/kg, iv), metrazol (25 mg/kg, iv) or bicuculline (0.35 mg/kg, iv). However, when higher doses of metrazol (35 mg/kg, iv) or bicuculline (0.45 mg/kg, iv), were used, a low dose of YOH (1 mg/kg, ip) exhibited a significant anticonvulsant effect. In contrast, YOH did not potentiate strychnine-induced convulsions.

Table 1. Effect of Yohimbine on convulsions in mice

Yohimbine Doses	Percent Mice Exhibiting Convulsions						
	Picrotoxinin (iv)		Metrazol (iv)		Bicuculline (iv)		Strych. (iv)
mg/kg, ip	0.75 mg/kg CC	1.6 TC	25 mg/kg CC	35 CC	0.35 mg/kg CC	0.45 CC	0.30 mg/kg TC
0	15	15	17	90	15	80	13
1	40	45	10	45**	10	40**	20
3	40	90**	15	60	15	60	20
6	65**	100**	60*	75	30	100	9
12	-	-	-	-	85**	-	2

CC : Clonic Convulsions ; TC : Tonic Convulsions

*p < 0.05 , ** p < 0.01 vs respective controls (0)

Clonidine (1 mg/kg, ip) did not block the potentiating effect of YOH (12 mg/kg, ip) on metrazol convulsions (controls = 35 %; Yohimbine = 100 %; clonidine = 5 %; combination = 80 %) or bicuculline seizures (controls = 5 %; yohimbine = 85 % ; clonidine = 15 %; combination = 85 %) ; nor did it block the antagonistic activity of YOH (1 mg/kg, ip) on metrazol convulsions (controls = 95 %; yohimbine = 50 %; clonidine = 30 % ; combination = 10 %).

The pro-convulsant effects of the higher doses of YOH in these 3 seizure models are at variance with the anticonvulsant effects reported for the same dose of YOH in the Quaking mouse model (Chermat et al, 1981). However the high level of the doses used as well as the resistance to clonidine in our study, suggest that this proconvulsant effect of YOH might be due to a blockade of post-synaptic alpha adrenoceptors, which is in agreement with the interpretation of other authors (Maynert et al, 1975).However the anticonvulsant effect of low-dose YOH suggests more complex interactions involving other actions of YOH (eg. dopamine receptor blockade, Scatton et al, 1980). Such a biphasic action on bicuculline seizures has previously been reported for neuroleptics with α-blocking properties (Worms and Lloyd, 1978).

Chermat, R. et al (1981) Naunyn-Schmiedeberg's Arch. Pharmacol., 318, 94-99
Maynert, E.W. et al (1975) In : Adv. in Neurology, vol. 13, Ed. W.J. Friedlander, New York, Raven Press, p. 79-147
Scatton, B. et al (1980) J. Pharm. Exp. Ther., 215, 494-499
Worms, P. and Lloyd, K.G. (1978) European J. Pharmacol., 51, 85-88

ANTICONVULSIVE EFFECTS OF MY-117, A DERIVATIVE OF TAURINE, IN MICE

I.-B. Lindén, A. Penttilä and H. Vapaatalo
Research Laboratories of Medica Pharmaceutical Company Ltd, Helsinki, Finland

Taurine, a major constituent of the free amino acid pool in the brain, has been suggested to be an inhibitory transmitter or alternatively a neuromodulator (for review see Oja et al 1977). Intracerebroventricularly or topically applied taurine has been shown to be anticonvulsive in many experimental animal seizure models (Huxtable 1981). However, oral and parenteral administration of taurine lack anticonvulsive effect, presumably owing to poor penetration of taurine into the brain. In contrast to taurine, MY-117, phthalimidoethanesulphon-N-isopropylamide, synthesized in our laboratories, is a lipophilic substance which readily enters the brain. This was shown by measuring the radioactivity in the brain 3 h after oral administration of ^{14}C-MY-117 and ^{14}C-taurine to rats: 5 times more radioactivity was found after ^{14}C-MY-117 than after ^{14}C-taurine.

The anticonvulsive effects of MY-117 were evaluated using various seizure tests in mice. The ED_{50} following oral administration of MY-117 was in the range of 76-322 mg/kg in the maximal electroshock, pentylenetetrazole, strychnine, thiosemicarbazide and isoniazid tests; the peak effect time was 60-120 min. The ED_{50} doses for valproate in the above mentioned tests varied from 280 to more than 600 mg/kg. MY-117 does not seem to act via the gabaergic system, since the ED_{50}values in the bicuculline and picrotoxin tests were as high as 550 mg/kg and 334 mg/kg, respectively. The rotarod test, which was used to detect minimal defects in motor coordination, showed an ED_{50} ~ 400 mg/kg. Like most antiepileptics, MY-117 induced some tolerance after prolonged treatment.

MY-117 is a new taurine derivative with a definitive anticonvulsive effect in animal tests.

References:

Huxtable, R. J. (1981) In: The role of peptides and amino acids as neurotransmitters. Eds. Lombardini, J. B. and Kenny, A. pp. 53-97.

Oja, S. S., Kontro, P. and Lähdesmäki, P. (1977) Prog. Pharmacol 1 (3), 1-119.

POSSIBLE MECHANISM OF ACTION OF SOME NOVEL ANTICONVULSANT TAURINE DERIVATIVES

Pirjo Kontro & S.S. Oja, Department of Biomedical Sciences, University of Tampere, Tampere, Finland

Taurine, 2-aminoethanesulphonic acid, strongly inhibits firing of neurons in many functionally differing areas of the central nervous system. It hyperpolarizes the neurons by increasing the chloride ion conductance of their cell membranes. Being structurally related to the well-established inhibitory neurotransmitters GABA and glycine, taurine has also been considered to act as a neurotransmitter, but a general modulatory role as a membrane stabilizer is more likely, however. Intracerebroventricularly injected taurine efficiently forestalls seizures in animal epilepsy models, but the success with oral or parenteral taurine medication on human epileptics has been only partial. The strong polar lipophobic nature of the taurine molecule prevents access from blood to brain.

Of a number of taurine derivatives, all less ionized and polarized and more lipophilic than taurine, screened in maximal electroshock seizure, pentylenetetrazole seizure threshold and strychnine seizure threshold tests, phthalimidoethanesulphon-N-alkylamides MY-103, MY-111 and MY-117 have been distinguished as potent anticonvulsants. In order to elucidate the mechanisms of action of these compounds, the possible interferences with the intracellular uptake and release of taurine in mouse cerebral slices and synaptosomal preparations and the binding of taurine to isolated synaptic membranes were analyzed. Since taurine and GABA have rather similar structures, the uptake, release and binding of GABA were similarly subjected to interference tests. The possible interaction with catecholaminergic systems was also assessed by studying effects on noradrenaline uptake.

The test compounds had no effects on the spontaneous and potassium-stimulated release of taurine or GABA from superfused brain slices. They all strongly inhibited the sodium-independent binding of taurine and GABA to synaptic membranes. Muscimol, a GABA agonist used as reference, also inhibited GABA binding but not that of taurine. Taurine and GABA uptakes by the slices were not significantly influenced by the test compounds, whereas the reference compounds L-2,4-diaminobutyric and nipecotic acids were distinctly inhibitory. The other transport processes studied were also uninfluenced by the test compounds, except that MY-117 significantly reduced taurine uptake in synaptosomes and MY-103 moderately enhanced noradrenaline uptake in slices.

The data may be interpreted to demonstrate that the efficacy of the tested anticonvulsant phthalimidoethanesulphonalkylamides results from occupancy of the postsynaptic binding sites of taurine and/or GABA rather than any modification of presynaptic events.

TOXICOLOGICAL STUDIES OF MY-117, A NEW TAURINE DERIVATIVE

S. Niemi, S. Lankinen and G. Gothoni
Research Laboratories of Medica Pharmaceutical Company Ltd, Helsinki, Finland

Taurine, a free amino acid in the brain, has anticonvulsive effects when injected intracerebroventricularly but not when administered orally. A new taurine derivative MY-117, phthalimidoethanesulphon-N-isopropylamide, has been shown to prevent various experimental convulsions even after oral administration. This study describes its toxicological and biochemical data.

In acute toxicity tests MY-117 was atoxic in male NMRI mice and in female Sprague Dawley rats; after oral administration of 2500 mg/kg to mice and 5000 mg/kg to rats all animals survived. Only mild sedation followed from these high doses.

Twenty-eight days' oral toxicity of MY-117 was studied in Sprague Dawley rats and Duncin-Hartley guinea pigs. The doses were in the range of 150-839 mg/kg; the smallest dose was approximately the anticonvulsive ED_{50}. Hematological, biochemical and histopathological studies were performed. There were no changes in hematological values. The electrolytes and protein concentrations in serum were normal, as were the biochemical parameters creatinine, urate, triglycerides, bilirubin, alkaline phosphatase and aspartate amino transferase. MY-117 had no effect on blood pressure and rectal temperature in rats. Electrocardiograms were normal in both species. No abnormal behaviour could be noticed. In rats an increase in liver weight was observed, and the liver histology showed some morphological changes at the highest dose level. Focal necroses with some acidophilic bodies mainly in the periportal area were particularly frequent in female rats. In other main organs no histological changes were found.

The effect of MY-117 on liver microsomal cytochrome P-450 concentration was regarded as an index of liver enzyme activity. In male rats oral treatment of MY-117 (150-600 mg/kg) for 28 days caused dose-dependent enzyme induction.

Ames mutagenicity test with five strains of Salmonella typhimurium was used to detect both base-pair substitutions and frameshift mutations. MY-117 showed no mutagenic activity with or without metabolic activation in the concentration range 1.0-500.0 µg/plate. It was also inactive in the other two mutagenicity tests used: induction of Sister Chromatid Exchanges (SCE) in vitro and chromosomal aberration test in vitro in mammalian cells (CHO cells).

Our results from acute and prolonged toxicity tests in mice, rats and guinea pigs indicate that the potent anticonvulsive agent MY-117 seems to be an atoxic compound, and this is supported by preliminary findings of short term studies in man.

TAURINE DERIVATIVES AND LEARNING PERFORMANCE

R. Liljequist, M.K. Paasonen & E. Solatunturi, Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10 A, 00170 Helsinki 17

Guanidinoethanesulphonic acid (GES) occurs naturally in many mammalian tissues (Guidotti & Costagli, 1970). GES and β-alanine inhibit the uptake of taurine and these agents are used as experimental tools in analyzing the actions of taurine, an inhibitory transmitter or neuromodulator. GES is able to displace taurine in some tissues to a great extent (Huxtable et al, 1979). It is not known whether GES is a taurine agonist, antagonist or neither. We have compared GES, β-alanine and a taurine homolog, homotaurine in a one-way active avoidance situation. The brain content of taurine, noradrenaline and dopamine were also measured.

Forty male Wistar-Kyoto rats at the age of three months were used. Training was given in blocks of ten trials a day with two-min intervals between the trials. The training lasted ten days for the GES and homotaurine groups. The training with rats receiving β-alanine was finished when they responded 80 % or more correct during two consecutive days.

GES, homotaurine or saline 1 mmol/kg were given i.p. one h before training to groups of eight rats. β-alanine was given in the drinking water as 1 % solution during three months (the whole life of the rats).

GES facilitated the acquisition of the task. In a two-way analysis of variance there was a significant drug effect ($P<0.001$). GES facilitated the performance during the first three days (0.05, Tukey). The homotaurine group did not differ from the controls but differed from the GES group during the first three days ($P<0.001$). The taurine concentrations in the brain and heart did not differ from the control following the GES and homotaurine treatments, but after β-alanine the brain taurine levels were slightly reduced (0.05). Brain noradrenaline and dopamine remained unchanged after all the agents.

The results confirmed our previous study that GES like taurine in small doses facilitated the acquisition of an active avoidance task. Thus GES functioned in this respect as a taurine agonist.

We are greatful to Prof J. Halmekoski (Dept. of Pharmacy, University of Helsinki) for the gift of GES and homotaurine and Sigrid Juselius Foundation for support.

Guidotti, A & Costagli, P.F. (1970) Pharmacol. Res. comm. 2. 341-354
Huxtable, R.J. et al (1979) J. Pharmacol, Exp. Ther. 2., 465-471

EFFECTS OF ANTI-PARKINSON DRUGS ON PHYSOSTIGMINE-INDUCED TREMOR IN RATS

P. Gothóni, M. Lehtinen[1] & M. Fincke, Division of Pharmacology, Department of Pharmacy, University of Helsinki, Kirkkokatu 20, SF-00170 Helsinki 17 and [1]Sodankylä Geophysical Observatory, Eiscat Scientific Association, SF-99600 Sodankylä, Finland

The effect of anti-Parkinson drugs on physostigmine-induced tremor in rats was studied. Six interaction studies were performed as double-blind crossover experiments, in which all doses of the drugs were randomly administered to the rats. Tremor was measured with an electronic device as described by Gothóni et al (1981).
Anticholinergic agents atropine and biperiden antagonized physostigmine-induced tremor in a dose-related manner, and could abolish it completely. However, biperiden could not antagonize as large doses of physostigmine as atropine. Small dose of methylatropine potentiated tremor, but the potentiating effect diminished with increasing its dose and in a dose of 1.2 mg/kg it slightly inhibited tremor. As methylatropine in small doses blocks only peripheral muscarinic receptors, it seems obvious that the physostigmine-induced tremor is mediated by central muscarinic receptors.
Amantadine, which has been proposed to release dopamine from neuronal storage sites (Scatton et al 1970), reduced tremor in a dose-related manner to a certain degree. The antagonism was a parallel displacement of the log dose-response curve to the right. The dopamine agonist bromocriptine (Fuxe et al 1974) antagonized tremor induced by physostigmine, but this antagonism was not dose-related. The displacement of the log dose-response curve was not parallel.
Pimozide, which fairly selectively blocks central dopamine receptors (Pinder et al 1976), potentiated physostigmine-induced tremor in the dose of 0.2 mg/kg but not in larger doses.
When the physostigmine-induced tremor started, a small decrease in rectal temperature was observed. However, the hypothermia lasted significantly longer than the tremorigenic action. The anti-Parkinson drugs did not counteract the hypothermic effect of physostigmine although they abolished the tremor. Thus the physostigmine-induced tremor does not seem to be due to shivering.
As could be expected the anticholinergic anti-Parkinson drugs counteracted the tremor induced by physostigmine better than those anti-Parkinson drugs, which act by increasing the dopaminergic activity. However, also the latter compounds significantly reduced the tremor.

Fuxe, K. et al (1974) Medical Biology 52, 121
Gothóni, P. et al (1981) Psychopharmacology 74, 275
Pinder, R.M. et al (1976) Drugs 12, 1
Scatton, B. et al (1970) Eur.J.Pharmacol. 13, 131

THE EFFECT OF MUSCARINIC LIGANDS ON THE HIPPOCAMPAL EEG

Paul Bevan and Jeanette E.C. Wallgren, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland.

The EEG recorded from the hippocampus is characterised by the presence of rhythmic slow activity (RSA or theta waves). In rats, RSA can be divided into an atropine-resistant movement RSA and an atropine sensitive immobility RSA (Robinson, 1980). RSA can be abolished by medial septum lesions (Donovick, 1968) indicating the importance of cholinergic transmission in the generation of RSA. We have investigated the effects of some muscarinic ligands on the rat hippocampal EEG.

Rats were anaesthetised with halothane (1%) and 2 electrodes lowered into the hippocampus (A -4, L 2, V -2,5 and A -4, L 2, V -1,5 respectively, measured from bregma). The electrodes were connected to a Grass 7P511 differential amplifier. At 10 s intervals, a 5 s sample of the EEG was analysed by Fourier transformation to give the power in the bands B 1 (1-4 Hz) and B 2 (4-8 Hz). The ECG was monitored using a Grass 7P3 amplifier. Drugs, dissolved in 0,9% w/v saline, were injected through an in-dwelling femoral vein cannula. Drug effects were assessed as the percent change in the power for each band relative to its' pre-drug level.

The EEG consisted of large amplitude slow waves (typically 0,6 mV) with superimposed high frequency activity. 70% of the total EEG power was accounted for by B 1 and a further 15% by B 2. Scopolamine was tested in 11 animals. Scopolamine increased B 1 power in 4 animals, decreased the power in 2 animals but had no effect in 5 animals. In B 2 the power was increased in 2 cases, decreased in 3 cases and there was no effect in 6 cases. The effects lasted about 15 min. In the 6 animals tested, no effects of methyl-scopolamine could be seen. Arecoline was tested in 7 animals and produced a dose-related decrease in B 1 and B 2 power. The arecoline effects, which lasted up to 30 min. could be blocked by scopolamine but not methyl-scopolamine. Carbachol was tested in 10 animals. In 3 animals a decrease in B 1 and B 2 power was seen. However, the effects of carbachol were not dose-related but were coincident with the appearance of bradycardia, a phenomena not witnessed with the other agonists. The effects of carbachol on the EEG and ECG could be blocked by methyl-scopolamine as well as scopolamine.

These results show that arecoline produces scopolamine sensitive methyl-scopolamine insensitive changes in the hippocampal EEG. Carbachol, a drug which does not cross the blood brain barrier, produces scopolamine and methyl-scopolamine sensitive EEG changes. These effects are probably artefacts related to the carbachol-induced bradycardia. Thus, the hippocampal EEG in rats anaesthetised with halothane is sensitive to stimulation of central muscarinic receptors.

Donovick, P.J. (1968). J. Comp. Physiol. Psychol., 66, 569-578.
Robinson, T.E. (1980). Brain Research Reviews, 2, 69-101.

ADENOSINE RECEPTORS IN THE RAT HIPPOCAMPUS

T.V. Dunwiddie[1] & B.B. Fredholm[2], Department of Pharmacology, University of Colorado, Med. Center, Denver, Colorado[1], Department of Pharmacology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden[2].

Endogenous and exogenous adenosine inhibits evoked potentials in the CA1 field of rat hippocampal slices and interictal spikes seen after treatment with penicillin and potassium (Dunwiddie, 1980; Dunwiddie et al, 1981). Endogenous and exogenous adenosine also stimulate cyclic AMP accumulation in similar slices (Fredholm et al, 1982). Finally, specific binding sites for labelled adenosine analogues can be detected in the hippocampus (Fredholm et al, 1982). There is evidence that adenosine may exert its actions via at least two types of receptors, A1 and A2, that may be differentiated i.a. on the basis of the relative potency of agonists. On A1-type receptors N^6-cyclohexyladenosine (CHA) and N^6-phenylisopropyladenosine (PIA) are more potent than adenosine 5'-ethylcarboxamide (NECA). On A2-type receptors NECA is more potent than CHA and PIA. The aim of the present study was to characterize the receptors in rat hippocampus using these analogues. Rat hippocampus was dissected out and slices were prepared for electrophysiology (Dunwiddie, 1980) or for the determination of ^{3}H-cyclic AMP accumulation (Fredholm et al, 1982). For the study of labelled agonist binding a crude membrane fraction was prepared as described by Bruns et al. (1980). A 15 min equilibration time was used for all parameters. The major findings are shown in Table 1.

Table 1

	CHA		PIA		NECA		2-Cl-ado	
K_D-binding (nM)	17.9	(13-29)	18.5	(14-26)	60	(41-134)	-	
B_{TOT} (fmol/mg)	484	(425-543)	645	(605-690)	626	(542-710)	-	
Inhib. EPSP (nM)	25	(11-52)	17	(11-28)	200	(130-300)	660	(450-970)
(^{3}H)-cAMP (μM)	17	(5-62)	9	(6-15)	1.8	(1.1-2.8)	12	(8-21)

(mean and 95% confidence interval)

The Scatchard plot did not deviate significantly from linearity for any agonist. The number of binding sites was very similar. This suggests that the binding sites for the three agonists are identical. The potency of the agonists as inhibitors of evoked field potentials was similar to their potency in the binding assay. There was also a good correspondence between their potency as inhibitors of field EPSP and of interictal spikes. By contrast their potency as stimulators of cyclic AMP accumulation was much lower and the rank-order potency was different.

In conclusion, the results indicate that the adenosine receptors mediating inhibition of field EPSP and interictal spikes are similar to those receptors to which labelled adenosine analogues bind specifically, both as regards absolute and relative potency for three adenosine analogues. Moreover, the rank-order potency suggests that these sites should be classified as being of the A1-subtype. By contrast, the absolute and relative potency of the agonists as mediators of cyclic AMP accumulation was very different, suggesting that a different set of receptor sites are responsible. These sites may be of the A2-subtype (cf. Bruns et al, 1980; Fredholm et al, 1982).

Bruns, R.F. et al (1980) Proc. Natl. Acad. Sci. 77, 5547
Dunwiddie, T.V. (1980) Epilepsia 21, 541
Dunwiddie, T.V. et al (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316, 326
Fredholm, B.B. et al (1982) J. Neurochem. 39, 165

DISTINCT pCPA AND NALOXONE-SENSITIVE ANALGESIC MECHANISMS ARE ACTIVATED BY BRIEF AND PROLONGED FOOTSHOCK RESPECTIVELY

G.Curzon, P.H.Hutson & M.D.Tricklebank, Department of Neurochemistry, Institute of Neurology, 33 John's Mews, London U.K.

Analgesia induced by brief footshock (30 sec) is inversely related to central 5HT availability and insensitive to naloxone (Tricklebank et al, 1982). Lewis et al. (1980) and Grau et al. (1981) find separate opioid and non-opioid mechanisms are involved in footshock induced analgesia depending on duration of shock. We now present evidence for distinct 5HT mechanisms in footshock induced analgesia which also depend on shock duration.

Latencies to withdraw the tail from a water bath at 51°C were determined initially (L1), immediately after returning to home cage for 30 sec or 30 min (L2) and immediately after 30 sec or 30 min footshock (2 mA) (L3). Results are given as percentage analgesia score (PAS) = (L3/L2) x 100. The ratio (L2/L1) x 100 gives a non-shocked control value. PAS of 0.9% NaCl injected rats was increased by 30 sec footshock. The 5HT synthesis inhibitor p-chlorophenylalanine, pCPA (150 mg/kg i.p. daily X 3 with testing 24 hr later) further enhanced PAS in shocked rats, while naloxone (1 mg/kg i.p. 15 min before testing) was without effect (Table 1). However, after 30 min footshock pCPA no longer enhanced but prevented the shock induced increase of PAS as did naloxone (1 mg/kg i.p. 15 min before L2).

TABLE 1. Effect of 30 sec or 30 min footshock on percentage analgesia score.

Drug	Shock Duration	Percentage Analgesia Score: No Shock L2/L1 x 100	Shock L3/L2 x 100
Saline	30 sec	109.6 ± 24.1 (10)	199.9 ± 31.2 (10)††
pCPA	30 sec	115.8 ± 21.8 (9)	351.9 ± 125.1 (9)**
Saline	30 min	102.9 ± 18.3 (8)	152.3 ± 16.9 (8)††
pCPA	30 min	104.1 ± 17.1 (8)	111.9 ± 38.5 (8)*
Saline	30 sec	106.8 ± 17.9 (9)	243.1 ± 32.8 (9)††
Naloxone	30 sec	99.2 ± 21.9 (9)	249.1 ± 65.6 (7)††
Saline	30 min	108.8 ± 23.8 (8)	149.1 ± 19.3 (8)†
Naloxone	30 min	105.9 ± 11.9 (8)	115.8 ± 19.3 (8)**

Values are mean ± SD, no. of animals in brackets. *$p < 0.02$; **$p < 0.01$ vs saline injected shocked controls. †$p < 0.01$; ††$p < 0.001$ vs non-shock controls.

When latency measures were taken repeatedly after various periods of footshock, pCPA increased the PAS significantly from (mean ± SD) 266 ± 50 to 406 ± 135 ($p < 0.02$) after 30 sec but by 20 min exposure, values were similar.

Results suggest two mechanisms of footshock induced analgesia. After brief footshock it is increased by pCPA and insensitive to naloxone; after prolonged footshock it is prevented by both drugs.

Grau et al. (1981). Science, 213, 1409-1411.
Lewis et al. (1980). Science, 208, 623-625.
Tricklebank et al. (1982). Neuropharm., 21, 51-56.

INTERACTION BETWEEN SELECTIVE MONOAMINE OXIDASE INHIBITORS OR 5HT UPTAKE INHIBITORS AND NARCOTIC ANALGESICS

Regina Botting, Department of Biological Sciences, North East Surrey College of Technology, Ewell, Surrey, U.K.

The non selective monoamine oxidase inhibitor (MAOI), phenelzine, modifies the actions of morphine and pethidine in different ways (Botting et al, 1978). It increases the toxicity of pethidine but not morphine and potentiates pethidine analgesia and hypothermia, while reducing that due to morphine. This interaction was investigated further using clorgyline and deprenyl, selective inhibitors of MAO A and MAO B, and alaproclate and zimelidine, inhibitors of 5HT uptake (Ögren and Holm, 1980).

Toxicity and locomotor activity were measured in mice (25-40g) using a doppler actograph for recording activity. Analgesia and rectal temperatures were recorded in mice (20-28g) with the hot plate test (56°C) and electric thermometer, and in Sprague Dawley rats (150-210g) with a paw pressure analgesimeter. Pretreatments were given s.c. 4h (toxicity), 30 min (locomotor activity) and 1h (analgesia and hypothermia) before i.p. morphine and pethidine, except for alaproclate, at the same time and zimelidine, 90 min before analgesic.

Acute toxicity. Low doses (2.5 mg/kg) of clorgyline and deprenyl injected together increased the lethality of pethidine (LD50 = 75(65-87)mg/kg). Separate large doses (20 mg/kg) of clorgyline or deprenyl had no effect (LD50 = 135(118-154 mg/kg).

Locomotor activity. Pretreatment with clorgyline and deprenyl (10 mg/kg of each) raised activity due to 20 mg/kg morphine (43±25 to 129±37 counts/2 min). Pretreatment with 50 mg/kg phenelzine, but not 20 mg/kg clorgyline or deprenyl had a similar effect.

Analgesia and hypothermia. Morphine, 5 and 10 mg/kg, analgesia was increased by pretreatment with deprenyl 10 mg/kg and alaproclate 20 mg/kg, reduced by clorgyline 5 mg/kg and unaltered by clorgyline + deprenyl (5 mg/kg of each). Pethidine, 10 mg and 20 mg/kg, analgesia was potentiated by all the treatments. Hypothermia due to the analgesics was also increased by all the treatments. Zimelidine 10 mg/kg potentiated morphine 10 mg/kg hypothermia only. All results were significant at $P<0.05$. In the rat paw pressure test, 10 mg/kg clorgyline significantly reduced analgesia due to morphine, 5 and 10 mg/kg and pethidine, 10 and 20 mg/kg.

Squires and Buus Lassen (1975) and Jounela (1977) showed that a maximum rise in brain 5HT levels, maximum pethidine hyperthermia and maximum increase in l-tryptophan induced hypermotility occurred only if MAO A and MAO B were both inhibited by clorgyline and deprenyl given together. Here also, pethidine toxicity and morphine locomotor activity could only be increased by this combination of MAOIs. This supports involvement of 5HT in both interactions. Potentiation by alaproclate of pethidine and morphine analgesia and hypothermia indicates a supraspinal action involving 5HT of both drugs. Clorgyline inhibition of morphine and pethidine analgesia suggests a second 5HT receptor mediating increase in nociceptive responses. This $5HT_2$ receptor may be in the spinal cord, as increased nociception was more evident in the rat paw pressure than hot plate test (Tyers, 1980).

Thanks are expressed to Professor J. Knoll for deprenyl and Dr. S.O. Ögren for alaproclate and zimelidine.

Botting, R. et al (1978) J.Pharm.Pharmac. 30, 36
Jounela, A.J. et al (1977) Biochem.Pharmac. 26, 806
Ögren, S.O. and A,-C Holm (1980) J.Neural Transmission 47, 253
Squires, R.F. and J. Buus Lassen (1975) Psychopharmacologia 41, 145
Tyers, M.B. (1980) Br.J.Pharmac. 69, 503

CHANGES IN NORADRENALINE IN DIFFERENT BRAIN AREAS OF RATS WITHDRAWN FROM 60 DAYS' MORPHINE TREATMENT

L.M.J. Attila & L. Ahtee, Division of Pharmacology, Department of Pharmacy, University of Helsinki, Kirkkokatu 20, SF-00170 Helsinki 17, Finland

The effect of withdrawal after chronic morphine administration on the α-methyl-p-tyrosine (αMPT)-induced cerebral dopamine depletion in rats has been reported in many experiments (Ahtee & Attila, 1981; Gunne & Jonsson, 1969). The studies concerning cerebral noradrenaline (NA) in rats treated chronically with morphine are less conclusive, probably because of different experimental designs.

Male Wistar rats were injected s.c. twice daily at 08 h and 18 h for 60 days with saline or morphine increasing the daily morphine dose gradually from 20 to 135 mg/kg. After a withdrawal period of 1, 2 or 4 days the rats were given a challenge ('test') injection of either saline or morphine (10 mg/kg) 2.5 h and αMPT (200 mg/kg, i.p.) 2 h before decapitation. NA concentrations were measured by LCEC modifying slightly the method described by Keller et al (1976).

Chronic morphine treatment did not alter NA concentration significantly in hemispheres of rats withdrawn from morphine for 1-4 days, but tended to increase (by 6-15%) NA concentrations in diencephalon and lower brain stem of these rats. In these three brain parts of control rats treated chronically with saline, αMPT decreased the NA concentration by 34-45%. In rats withdrawn from chronic morphine treatment the αMPT-induced NA depletion was significantly accelerated at 2 days' withdrawal in hemispheres (35→52%, $P<0.01$), in diencephalon (36→43%, $P<0.05$), and in lower brain stem (35→47%, $P<0.001$) and also at 4 days' withdrawal in lower brain stem (34→44%, $P<0.05$).

The test dose of morphine retarded similarly the αMPT-induced NA depletion in hemispheres of control rats (45→22%, $P<0.001$; 35→22%, NS; 40→16%, $P<0.001$) and in those of rats withdrawn from chronic morphine treatment for 1, 2 or 4 days (50→24%, $P<0.01$; 53→28%, $P<0.001$; 45→32%, $P<0.01$, respectively). The test dose of morphine did not change significantly the NA depletion in diencephalon and lower brain stem of control rats. The test dose of morphine slightly retarded (5-7%, NS) NA depletion in diencephalon of rats withdrawn from morphine, whereas it most clearly retarded NA depletion in lower brain stem of rats after shortest withdrawal from morphine (1 day: 44→32%, $P<0.01$; 2 days: 47→39%, $P<0.05$; 4 days: 44→38%, NS).

The acceleration of αMPT-induced NA depletion in rats withdrawn for 2 days from 60 days' chronic morphine treatment coincides with the most pronounced withdrawal signs (weight loss, irritability etc.) in our rats. Only in lower brain stem, which contains the NA cell bodies in locus coeruleus, this acceleration outlasts the somatic and behavioural withdrawal signs. Acute morphine administration (the test dose) retarded NA depletion similarly in hemispheres and did not alter that in diencephalon of control rats or rats withdrawn from morphine. However, the test dose did not change the NA depletion in lower brain stem of control rats, but retarded it in that of rats withdrawn from morphine when the withdrawal signs were most prominent.

Ahtee, L. & Attila, L.M.J. (1981) Br.J.Pharmac. 74, 900P
Gunne, L.-M. & Jonsson, J. (1969) J.Eur.Pharmac. 5, 338
Keller, R. et al (1976) Life Sci. 19, 995

EFFECTS OF OPIATE ANTAGONISTS ON THE HYPERDIPSIA INDUCED BY CHLORDIAZEPOXIDE AND PHENOBARBITONE IN THE THIRSTY RAT

S.J. Cooper, Department of Psychology, University of Birmingham, Birmingham B15 2TT, England.

Diazepam stimulates increased feeding in hungry and satiated animals, and this hyperphagic effect can be suppressed by low doses of naloxone, an opiate antagonist (e.g. Birk & Noble, 1981; Britton et al, 1981; Stapleton et al, 1979). Benzodiazepines also increase water consumption in thirsty rats (Cooper, 1982), and the aim of the first experiment was to determine if chlordiazepoxide (CDP)-induced hyperdipsia could be antagonized by either naloxone or naltrexone. The possible effects of morphine on CDP-induced hyperdipsia were also examined.

Subjects were 48 male hooded rats (General strain, 300-400g) from our colony. They were housed individually with free access to food (Diet 41B), and had been adapted over several weeks to a daily 22h water-deprivation schedule. They were kept on a 12h light: 12h dark cycle, at a room temp. 21°C. Following drug or vehicle injection, the rats were run in a 30 min drinking test, when water intake (ml) was measured. The rats were assigned to 3 groups, and tested with naloxone, naltrexone or morphine, respectively. Each animal was tested at several doses (0.01-10 kg/mg, s.c.), order of testing being counterbalanced across rats. Within each group, half were tested with CDP (10 mg/kg, i.p.), half with CDP vehicle. Statistical analysis was by analysis of variance, and t-test.

CDP injected 30 min before the test significantly increased water intake ($p < 0.001$) by a mean 5.5 ml above control level. Administration of either naloxone or naltrexone (0.01 - 10 mg/kg in each case), 15 min before the test, significantly reduced water intake in a dose-related manner ($p < 0.001$ for main drug effects in both cases). Naloxone at 1 mg/kg and naltrexone at 0.1 mg/kg completely blocked CDP-induced drinking. Morphine (0.01 - 3 mg/kg, s.c.), injected 25 min before the test, had no effect on baseline drinking or CDP-induced drinking. At 10 mg/kg, morphine non-specifically suppressed drinking.

In a related experiment (using 60 male rats, same age and strain), phenobarbitone sodium (33 mg/kg, i.p.) injected 30 min before testing, also significantly enhanced water intake ($p < 0.001$) by a mean of 5.0 ml in 22 h water-deprived animals. Naloxone (0.1 - 10 mg/kg, s.c.) injected 15 min before testing, dose-dependently reduced water intake ($p < 0.001$), but did not attenuate the barbiturate-induced drinking.

Thus, CDP-induced hyperdipsia was completely blocked by opiate antagonists, indicating that benzodiazepine facilitation of ingestive behaviour (both feeding and drinking) is reversible using these antagonists. Morphine, however, did not enhance CDP-induced hyperdipsia. Barbiturate-induced hyperdipsia was not similarly blocked by naloxone. Hence, the benzodiazepine and barbiturate effects on drinking were distinguishable.

Naloxone and naltrexone were generously provided by Endo Laboratories, and CDP by Roche Products Ltd.

Birk, J. & Noble, R.C. (1981) Life Sci. 29, 1125-1131
Britton, D.R. et al (1981) Life Sci. 29, 1297-1302
Cooper, S.J. (1982) Neuropharmacology (in press)
Stapleton, J.M. et al (1979) Life Sci. 24, 2421-2426

CONDITIONED TASTE AVERSIONS (CTA) PRODUCED BY NICOTINE IN RATS

J. A. Pratt & I. P. Stolerman, Department of Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Rats can learn to reject a distinctively flavoured solution when its consumption reliably precedes and thus signals injection of a psychoactive drug. Many different agents can produce such conditioned taste aversions (Stolerman & D'Mello, 1981). The ability of nicotine to suppress behaviour in this way has now been examined with the procedures of Booth et al. (1977), and some pharmacological characteristics of the effect have been examined. Water-deprived rats were allowed access to flavoured solutions for 15 min and immediately afterwards, were injected with either a dose of nicotine or saline. The flavoured solutions were sodium saccharin (0.1%) or sodium chloride (0.9%). All injections were given subcutaneously and all doses are expressed as those of the bases. Etscorn (1980) has reported on CTA produced by nicotine (2 mg/kg) in mice.

(-)-Nicotine bitartrate produced clear CTA which was directly related to the dose over the range 0.025-0.8 mg/kg. For example, after three conditioning trials with nicotine (0.08 mg/kg), intake of the nicotine-paired flavoured solutions was 9.3 $\pm$ 1.7 ml as compared with 17.3 + 2.2 ml for the control solutions (means $\pm$ S.E.M., n = 8). The nicotine-paired and the control solutions were then presented simultaneously in a more sensitive two-stimulus test; the intakes were 2.1 $\pm$ 0.8 ml and 17.4 $\pm$ 2.2 ml for the nicotine-paired and the control solutions respectively. The amount of nicotine needed to produce a half-maximal degree of CTA in the two-stimulus tests decreased from 0.66 mg/kg (95% of confidence limits: 0.46-1.54 mg/kg) after one conditioning trial to 0.15 (0.07-0.29) mg/kg after two trials, and to 0.046 (0.022-0.075) mg/kg after four trials. Thus, the sensitivity of the procedure was directly related to the number of conditioning trials. For further studies, the two-trial procedure was considered to maintain reasonable sensitivity without being excessively time-consuming.

The stereoisomer (+)-nicotine also produced dose-related CTA. The dose of (+)-nicotine needed to produce half-maximal CTA in the two-trial procedure was 0.66 (0.25-1.27)mg/kg; (+)-nicotine was therefore about four times less potent than (-)-nicotine in this procedure. The effects of ganglion-blocking drugs were examined by administering them 30 min before (-)-nicotine (0.4 mg/kg). In subsequent two-stimulus tests, rats that had received mecamylamine hydrochloride (2 mg/kg) consumed 9.8 $\pm$ 2.3 ml of the nicotine-paired flavoured solution and 9.5 $\pm$ 1.8 ml of the control solutions (n = 8). The equivalent scores for rats receiving saline instead of mecamylamine were 2.7 $\pm$ 0.4 ml and 13.1 $\pm$ 0.9 ml (n = 14). Thus, mecamylamine completely blocked the CTA produced by nicotine. In contrast, hexamethonium hydrobromide had no such effect in doses up to 10 mg/kg. Other effects of nicotine on conditioned behaviour show similar degrees of stereospecificity and susceptibility to the ganglion-blocking drugs (Meltzer et al., 1980; Spealman et al., 1981), suggesting that the pharmacological mechanisms involved may be similar.

We thank Dr. J. M. Littleton for supplying (+)-nicotine and the Medical Research Council for financial support.

Booth, D.A. et al (1977) Br. J. Pharmac. 61, 669-677
Etscorn, F. (1980) Bull. Psychon. Soc. 15, 54-56
Meltzer, L.T. et al (1980) Psychopharmacology 68, 283-286
Spealman, R.D. et al (1981) J. Pharmac. exp. Ther. 216, 484-491
Stolerman, I.P. & D'Mello, G.D. (1981) Adv. Behav. Pharmac. 3, 169-214

HALOPERIDOL RESTORES EXPLORATION BUT NOT LOCOMOTION FOLLOWING AN AUTORECEPTOR DOSE OF APOMORPHINE

L. Ståhle & U. Ungerstedt , Department of Pharmacology, Karolinska Institutet , S-104 01 Stockholm, Sweden

In an automatic holeboard apparatus (see Ljungberg and Ungerstedt 1978) the behaviour of rats put in this test-box for the first time was recorded for ten min. Activity, locomotion, rearing and two hole-variables (hole count defined as the number of holes investigated and hole time defined as the accumulated time spent in the holes) were recorded. Apomorphine (APO) in low doses (0.1 mg/kg and less) is known to suppress spontaneous behaviour while high doses (0.2 mg/kg and more) produce stereotyped behavioural patterns. In this study, we found a highly significant inhibition of exploratory behaviour following a low dose of APO (0.05 mg/kg sc, n=11, injected 15 min before testing). All holeboard parameters were significantly decreased compared to saline treated controls (n=13, injected 15 min before testing) and no stereotypies were observed. Haloperidol (HALO) pretreatment 30 min before APO (n=6 at all doses utilized) did not change locomotion but the hole variables and rearing were restored approximatly to the level of saline controls (n=6) pretreated with HALO (see fig).

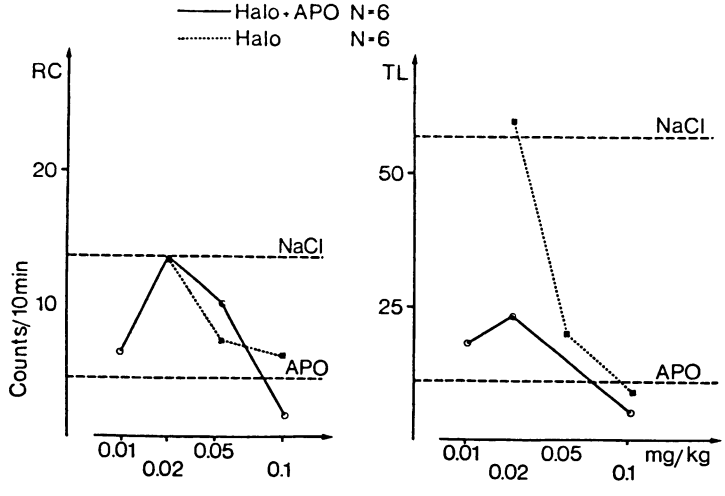


Figure 1 HALO antagonizes APO induced suppression of rearing (RC) but not locomotion (TL). The difference from controls is significant at 0.02 ($p<0.01$) and 0.05 ($p<0.05$) mg/kg HALO (Mann-Whitney U-test). HALO alone inhibits both behavioural components at higher doses.

Thus, HALO antagonizes the APO effect on exploratory behavioural components while locomotion remain unaffected. This effect may be due to a blockade of dopamine autoreceptors by HALO thus inhibiting the stimulation of these receptors by APO. However, since locomotion was not restored by HALO some dopaminergic autoreceptors appears to be less sensitive to this drug e.g. those in limbic areas, such as the nucleus accumbens, where dopamine neurotransmission is thought to be involved in locomotor behaviour.

Ljungberg, T. & Ungerstedt, U. (1978) Pharmacol. Biochem. Behav. 8, 483-489

A POSSIBLE IN VIVO BINDING TECHNIQUE USING BRAIN DIALYSIS

C Forster and U Ungerstedt, Dept of Pharmacology, Karolinska institutet, S-104 01 STOCKHOLM, Sweden.

A technique of brain dialysis has recently been described (Ungerstedt et al, 1981) where it has been possible to recover endogenously released dopamine and amino acids in awake as well as in anaesthetised rats. In this report only anaesthetised animals have been utilised and used to compare the features of radioactive labelling with either ^{3}H-dopamine or ^{3}H-haloperidol.

Male rats (<200g) were held in a stereotaxic frame under halothane anaesthesia. Holes were drilled bilaterally in the temporal bones in order to insert a dialysis tube. This tube was passed through the brain inducing minimal damage. The tube had been previously prepared so as to leave a 3.0 mm portion open which will correspond to the position of the right caudate nucleus.The viability of the tube was checked by perfusing with Ringer solution at a speed of 2 μl.min $^{-1}$. ^{3}H-dopamine or ^{3}H-haloperidol was perfused for 60, 90 or 180 min in order to achieve steady state conditions. Samples of the perfusate were collected at 5 or 10 min intervals and were analysed in 5 ul volumes in a liquid scintillaton counter. At the end point the rats were killed by cervical dislocation, the brain were rapidly removed and frozen. The forebrain (minus olfactory bulbs) was sliced (1 mm) sagitally on a freezing microtone, samples were weighed and solubilised with Protosol in scintillation vials. Following this procedure 10 mls of scintillant was added and each sample was counted. Results were expressed as cpm.mg^{-1} tissue or (for perfusates)) % of a similar volume of standard. Three further experiments were conducted. Firstly, labelling with isotope for 60 min then replaced by Ringer for 60 min, secondly, labelled with isotope for 60 min then replaced by cold drug for 60 min, finally, labelling for 120 min during which cold drug was administered during the latter 60 min.

Perfusion with ^{3}H-dopamine reached a steady state situation at a more rapid rate than ^{3}H-haloperidol. Similarly the washout curve for these two isotopes differed, as the curve for ^{3}H-haloperidol declined at a slower rate. Steady state for ^{3}H-dopamine was approximately 80% of the input radioactivity whereas with ^{3}H-haloperidol steady state was almost 100%. Finally, the simultaneous perfusion of ^{3}H-dopamine and cold dopamine and that of ^{3}H-haloperidol and cold haloperidol differed in that with haloperidol there was a siginificant increase in the amount of radioactivity appearing in the perfusate whereas with dopamine there was a significant decrease in the perfusate level in the radioactivity.

These differences may be explained in the following manner, firstly, dopamine is subjected to uptake phenomenon. If this is the case further stimulation of dopamine receptors, particularly autoreceptors, will inhibit the release of ^{3}H-dopamine and hence there will be a decline in the radioactivity in the perfusate. Haloperidol does not appear to be taken up into the neuron and therefore the resulting increase in radioactivity may simply be due to displacement of the radiolabelled drug by the cold drug. In conclusion the above technique may provide a method for studying in vivo receptor binding and experiments are progressing in these laboratories.

References

Ungerstedt, U et al (1981). Satellite symposium on Dopamine. Okayama. Japan. 8th International Congress of Pharmacology.

IN VIVO MEASUREMENT OF DOPAMINE AND ITS METABOLITES BY INTRACEREBRAL DIALYSIS: CHANGES AFTER d-AMPHETAMINE

G.W. Bennett, C.A. Marsden, T. Sharp, U. Ungerstedt* and Tyra Zetterström*. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Department of Pharmacology, Karolinska Institutet, 10401 Stockholm 60, Sweden.

The behavioural effects produced by peripheral administration of d-amphetamine to rats have been associated with its complex action on central catecholaminergic neurones. In addition to increasing release of dopamine and preventing its reuptake (Carlsson, 1970) amphetamine also reduces striatal levels of dihydroxyphenylacetic acid (DOPAC), a major dopamine metabolite, possibly by monoamine oxidase inhibition (Miller et al, 1980). Currently few techniques are available which allow measurement of drug-induced neurochemical events in specific brain regions in the freely moving animal. Here we report the use of intracerebral dialysis to monitor changes in endogenous dopamine and its metabolites; DOPAC and homovanillic acid (HVA) together with 5-hydroxyindoleacetic acid (5HIAA) in the striatum and nucleus accumbens following peripheral administration of amphetamine to awake rats.

A dialysis tube (0.25 mm in diameter) was folded into a loop and stereotaxically implanted into the striatum or nucleus accumbens during halothane anaesthesia. Following surgery animals were allowed to recover from anaesthesia and the dialysis tube was perfused with physiological salt solution at a rate of 2 µl/min via polythene tubing attached to a liquid swivel. Samples were collected at 20 min intervals and at least 3 control samples were taken before administration of d-amphetamine. The 40 µl samples were assayed for dopamine or metabolites using high performance liquid chromatography with electrochemical detection (carbon paste working electrode set at +0.7 V or +0.65 V respectively). Dopamine was separated by cation exchange (Vydac CX 30-44 µm, 0.05 M acetate-citrate buffer pH 5.2) and the metabolites by reverse phase chromatography (Spherisorb ODS 5 µm, 0.1 M acetate-citrate buffer pH 4.1 containing 10% methanol).

With the dialysis tube implanted into the striatum it was possible to measure basal levels of the metabolites (uncorrected for recovery) DOPAC (77.9 ± 15.3 pmols/sample n=5), HVA (52.0 ± 7.1 pmols/sample n=5) and 5HIAA (19.2 ± 0.9 pmols/sample n=4). The detectable amount of DOPAC was always at least 100 times greater than dopamine. D-amphetamine (2 mg/kg) within 100 min of injection increased the amount of dopamine measured in the striatal perfusates by a maximum of 800% while the levels of DOPAC and HVA decreased by 86% and 67% respectively. The levels of dopamine, DOPAC and HVA returned to pre-injection values within 4 hours of drug administration. There was no consistent change in 5HIAA. The increase in dopamine after d-amphetamine is dose-related (0.5-5 mg/kg s.c.). Further preliminary experiments have shown that d-amphetamine produces similar, but less marked, changes in DOPAC and HVA in the nucleus accumbens.

The results of these experiments confirm that d-amphetamine markedly reduces dopamine metabolism and provide evidence that the extracellular levels of the monoamine metabolites are high relative to their parent amine. These findings support other results using in vivo voltammetry (Gonon et al, 1981). In addition they emphasize the usefulness of the brain dialysis technique for monitoring drug-induced changes in neurotransmitter release and metabolism.

TS is an SERC CASE award student in collaboration with Reckitt and Colman Ltd.

Carlsson, A. (1970) Amphetamine and Related Compounds, Eds. E. Costa and S. Garattini, Raven Press, N. York, p 289
Gonon, F. et al (1981) Brain Research 223, 69-80
Miller, H.H. et al (1980) Biochem.Pharmacol. 29, 1347-1354

L-TRYPTOPHAN REVERSES THE BIOCHEMICAL EFFECTS OF LOW TRYPTOPHAN BUT NOT THE REDUCTION IN 5-METHOXYTRYPTAMINE-INDUCED BEHAVIOUR

Jane Irons, I.A. Macdonald & C.A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

Feeding rats diets low in tryptophan, such as a maize based diet, results in reduced brain tryptophan and 5-hydroxytryptamine (5HT) turnover (Zambotti et al, 1976). Several studies have used these diets to investigate the role of 5HT in behaviour such as aggression (Gibbons et al, 1979), pain (Lytle et al, 1975) and sleep (Clancy et al, 1978). In the present study, we have investigated the effects of a low tryptophan diet and the same diet supplemented with 0.2% tryptophan on mouse brain indoleamine metabolism, release, 5HT receptor responsiveness and metabolic rate.

Mice (BK. TO males) were maintained on a normal powdered diet (41B modified, tryptophan 0.21%), tryptophan deficient (BP Nutrition-based on cooked maize meal and containing tryptophan 0.07%) or the tryptophan deficient diet with added tryptophan (0.2%) for 14 days. Weight gain was monitored, brain tryptophan measured fluorimetrically and brain 5HT and its metabolite 5-hydroxyindole acetic acid (5HIAA) determined by HPLC with electrochemical detection (carbon paste working electrode set at +0.65 V). The indoleamines were separated on a spherisorb reverse phase column (5 μm) using 0.1 M acetate-citrate buffer pH 4.1 containing 10% methanol. 5HT release was measured *in vitro* using whole brain slices and HPLC with electrochemical detection (Bennett et al, 1980). 5HT receptor responsiveness was determined by counting the number of head twitches induced by 5-methoxytryptamine (2.5 mg/kg) administered 30 mins after tranylcypromine (10 mg/kg). Head twitches were counted for 1 min every 10 min until 40 min after the 5-methoxytryptamine injection. Metabolic rate (kJ/kg/min) was determined by measurement of oxygen uptake using a ventilated cage.

Mice on the low tryptophan diet compared with the control diet had lower body weight, reduced brain tryptophan (-56% n=8), 5HT (-28% n=8) and 5HIAA (-56% n=8), decreased 5HT release (-52% n=6) while metabolic rate was increased from 3.56 ± 0.66 (6) to 4.28 ± 0.95 (6) kJ/kg/min. The number of head twitches induced by 5-methoxytryptamine was significantly decreased from the control value of 21 ± 5 (16) to 8 ± 2 (16). The addition of tryptophan (0.2%) to the low tryptophan diet reversed the effects on body weight, brain tryptophan (+11% n=8 compared to control value) 5HT (+3% n=8), 5HIAA (+11% n=8) and restored 5HT release to a level above that of the control slices (+31% n=6). Metabolic rate also tended to revert towards the normal value (3.90 ± 0.72 n=6). In contrast, supplementation of the low tryptophan diet with tryptophan failed to reverse the decrease in head twitch response seen in the animals on the low tryptophan diet (low tryptophan 8 ± 2 n=16, added tryptophan 7 ± 4 n=16).

The results suggest that receptor changes observed following tryptophan deficiency may not simply reflect a lack of tryptophan but also other nutritional factors associated with a maize based diet. This possibility should be taken into account when assessing the importance of behavioural studies using such diets.

Bennett, G.W. et al (1980) In: Central Neurotransmitter Turnover Eds. C.J. Pycock and P.V. Taberner. Croom-Helm, London, pp 183-189
Clancy, J.J. et al (1978) Brain Res.Bull. 3, 83-87
Gibbons, J.L. et al (1979) Brain Res. 169, 139-153
Lytle, L.D. et al (1975) Science 190, 692-694
Zambotti et al (1975) Life Sci. 17, 1663-1670

THE EFFECT OF ZIMELIDINE ON THE 24 HOUR VARIATION OF 5HT AND TRYPTOPHAN CONCENTRATIONS IN THE RAT BRAIN

K.F. Martin & P.H. Redfern, Pharmacology Group, School of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY.

Although there is considerable circumstantial evidence linking depressive illness with disturbed circadian rhythms (for instance see Gerner et al, 1979) little is known of the differential effects of antidepressant drugs at different times of day. We have previously shown that in the rat clomipramine decreased brain 5HT and tryptophan (TRY) concentrations more during the day than during the night, effectively abolishing normal 24 hour rhythms (Martin & Redfern, 1982). The effects of Zimelidine, an antidepressant drug claimed to have a more selective action on 5HT uptake, have therefore been investigated.

Groups of 6 male Wistar rats (University of Bath strain), 150-200g were housed under a 12:12h L:D lighting regime (lights on 0600h) for 14 days before Zimelidine HCl, 100 or 200mg/ℓ^{-1} was administered in the drinking water. After 2 or 14 days drug treatment, groups of animals were killed at 0100, 0700, 1300 and 1900h. The brains were quickly removed and frozen in liquid nitrogen. Concentrations of 5HT and TRY were determined by HPLC assay (Pleece et al, 1982).

The effect of Zimelidine treatment was similar after 2 and 14 days (Table 1); concentrations of 5HT showed little consistent change while TRY levels were reduced in a dose-dependent manner. The reduction in TRY was, however, significantly greater during the light period, so that the effect of the drug was to remove the normal 24hr variation in brain TRY concentration.

A fall in TRY concentrations associated with relatively stable 5HT levels might indicate decreased availability of TRY to the brain. At the same time, although from the data presented here it is not possible to say what effect, if any Zimelidine had on 5HT turnover, it is interesting to note that the more pronounced change in TRY levels occurs during the light phase, when 5HT turnover is normally greatest.

TABLE 1

TIME			0100h		0700h		1300h		1900h	
TREATMENT			TRY	5HT	TRY	5HT	TRY	5HT	TRY	5HT
CONTROL			2.01±0.19	0.50±0.06	2.25±0.12	0.74±0.08	2.39±0.09	0.74±0.06	3.12±0.31	0.73±0.04
ZIMELIDINE	100mg/ℓ	2/7	1.98±0.15	0.52±0.03	1.93±0.12	0.59±0.04	1.56±0.11	0.51±0.04	1.49±0.28	0.43±0.01
- " -	100mg/ℓ	14/7	2.19±0.26	0.44±0.08	2.29±0.14	0.67±0.07	1.55±0.13	0.58±0.04	1.48±0.08	0.47±0.04
- " -	200mg/ℓ	2/7	0.67±0.05	0.68±0.07	0.97±0.08	1.12±0.01	0.54±0.09	0.87±0.13	0.64±0.08	0.86±0.09
- " -	200mg/ℓ	14/7	0.48±0.07	0.52±0.05	0.91±0.09	0.93±0.08	0.63±0.09	0.75±0.07	0.60±0.06	0.90±0.01

CONCENTRATION OF TRY AND 5HT ARE EXPRESSED AS μg/g WET WEIGHT (MEAN ± SEM, n=4.5 or 6)

K.F.M. is a S.E.R.C. student. We thank Astra Labs for a gift of Zimelidine HCl.

Gerner, R. et al, (1979), J. Psychiat. Res. 15, 21.
Martin, K.F. & Redfern, P.H., (1982) Br. J. Pharmac. (in press).
Pleece et al (1982), Analyst (in press).

REDUCTION OF SPECIFIC (^{3}H)-CIS-FLUPENTHIXOL BINDING SITES IN RAT STRIATUM FOLLOWING DECORTICATION

S.W. Davies, W.W. Templeton and G.N. Woodruff, Department of Physiology and Pharmacology, University of Southampton, U.K.

Kainic-acid induced lesions of rat striatum cause an 85% reduction of striatal dopamine-sensitive adenylate cyclase (Schwarz et al, 1978). Kainic acid also reduces the binding of dopaminergic ligands to striatal tissue. Thus a 50-60% loss of [^{3}H]-spiroperidol binding occurs following striatal injection of kainate, suggesting that spiroperidol binding sites on striatal cell bodies are associated with adenylate cyclase whereas the remaining sites, not destroyed by kainate, are located on cortico-striatal afferents. In support of this, cortical ablation produces a 30-50% loss of striatal butyrophenone binding (Schwarz et al, 1978). In contrast to [^{3}H]-spiroperidol binding, it has been suggested that [^{3}H]-cis-flupenthixol binding sites are located almost exclusively on striatal cell bodies, associated with adenylate cyclase, and destroyed by kainic acid (Cross & Waddington, 1981). One might therefore expect little or no loss of [^{3}H]-cis-flupenthixol binding following cortical ablation. To investigate this, we have studied the binding of [^{3}H]-cis-flupenthixol to striatal membranes from rats which had undergone unilateral cortical ablation 2-28 days previously.

Male Wistar rats (180-200 g) were used throughout. Rats were anaesthetised with pentobarbitone sodium (60 mg/kg). The skull overlying the right frontal and parietal cortex was removed and the underlying cortex removed by suction to the level of the corpus callosum. Bleeding was controlled by implantation of Sterispon (Allen & Hanbury), the wound closed, and the animal allowed to recover. Each rat received a prophylactic injection of ampicillin/cloxacillin (250 mg/ml, 0.2 ml, I.M.). After the required number of days, animals were decapitated and striatal membranes prepared as described previously (Freedman et al, 1981). [^{3}H]-cis-flupenthixol binding was assayed by conventional binding techniques (Freedman et al, 1982) and using (+)-butaclamol to define specific binding. To check that any changes in binding observed were not due to striatal damage, activities of glutamic acid decarboxylase (GAD) choline acetyltransferase (CAT) and adenylate cyclase (as cAMP formation) were measured.

In striatal membranes from the control hemisphere, the number of specific flupenthixol binding sites (Bmax) was 1007 fmol/mg protein and the dissociation constant (Kd) was 2.65 nM. These values were not significantly different from values obtained using membranes from unoperated rats. Ablation of the frontal and parietal cortex, 2, 6 and 28 days previously reduced the Bmax for [^{3}H]-cis-flupenthixol to 59.5%, 54.9% and 58.4% of control levels, respectively. The Kd values were not altered. Striatal CAT, GAD and adenylate cyclase activities were unchanged. This data suggests that, in addition to binding to sites on striatal cell bodies, [^{3}H]-cis-flupenthixol also binds to sites on the cortico-striatal glutamate afferents. Thus cis-flupenthixol shares the same distribution of binding sites as [^{3}H]-sulpiride (Freedman et al, 1981) and [^{3}H]-spiroperidol (Cross and Waddington, 1981). Furthermore, since [^{3}H]-cis-flupenthixol binding was reduced under conditions not effecting the activity of dopamine-sensitive adenylate cyclase, the linkage of these two particular measures of dopamine function is not absolute.

We thank Dr J. Hyttel for ^{3}H-cis-flupenthixol. WWT is a Postdoctoral Fellow of the Parkinson's Disease Society, SWD is supported by Duphar Laboratories.

Cross, A.J. and Waddington, J.L. (1981) Eur. J. Pharmac., 71, 327-332.
Freedman, S.B. et al. (1982) Br. J. Pharmac., 75 Proc. Suppl., 39P.
Freedman, S.B. et al. (1981) Neuropharmac., 20, 1151-1156.
Schwarz, R. et al. (1978) Nature, 271, 766-768.

INFLUENCE OF CALCIUM ON THE AUTORECEPTOR-MEDIATED INHIBITION OF (^{3}H)-DOPAMINE RELEASE FROM THE RABBIT CAUDATE NUCLEUS

S. Arbilla , S.Z. Langer and J.Z. Nowak, Biology Department, Laboratoire d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris - FRANCE.

The presynaptic inhibitory dopamine (DA) autoreceptors in the rabbit caudate nucleus modulate the calcium-dependent release of ^{3}H-DA elicited by electrical stimulation but not the calcium-independent release elicited by tyramine or amphetamine (Kamal et al., 1981). We examined the influence of calcium on the effects of DA, apomorphine and (-) adrenaline on the electrically evoked release of the labelled transmitter from slices of rabbit caudate as described by Kamal et al., 1981. Two periods of electrical stimulation (S_1 and S_2, during 2 min., 0.3 Hz, 16 mA) were applied with an interval of 44 min. Drugs were added 20 min before S_2. Nomifensine 10 µM was present throughout the superfusion in order to inhibit neuronal uptake of DA.

Table 1 : Effects of apomorphine, dopamine and (-) adrenaline on dopaminergic neurotransmission in the rabbit caudate nucleus.

	µM	S_2/S_1 1.3 mM Ca^{2+}	0.6 mM Ca^{2+}
CONTROL		0.79 ± 0.02 (17)	0.81 ± 0.02 (22)
APOMORPHINE	0.05	0.73 ± 0.02 (4)*	0.41 ± 0.05 (4)**
	0.1	0.47 ± 0.03 (4)**	0.18 ± 0.03 (4)**
DOPAMINE	0.1	0.69 ± 0.07 (4)	0.67 ± 0.04 (6)*
	0.3	0.69 ± 0.07 (6)	0.50 ± 0.04 (9)**
(-) ADRENALINE	1.0	0.52 ± 0.03 (21)**	0.56 ± 0.08 (5)**
	10.0	0.37 ± 0.03 (11)**	0.50 ± 0.06 (10)*

** $p < 0.001$; * $p < 0.05$; ( ) = number of experiments

The percentage of total tissue radioactivity released during S_1 in the presence of 1.3 mM Ca^{2+} or 0.6 mM Ca^{2+} was 2.74±0.25 % (n=17) and 1.52±0.10% (n=22) respectively. The ratio S_2/S_1 in control experiments at both calcium concentrations are given in Table 1. The inhibitory effect of apomorphine on the overflow of ^{3}H-DA was more pronounced at 0.6 mM than at 1.3 mM calcium (Table 1). DA failed to inhibit ^{3}H-DA release at 1.3 mM calcium but significantly reduced the overflow of ^{3}H-DA at 0.6 mM calcium (Table 1). (-) Adrenaline inhibited significantly the overflow of ^{3}H-DA but this action was independent of the calcium concentration (Table 1). In rabbit hypothalamic slices (-) adrenaline is about 100 times more potent to inhibit ^{3}H-Noradrenaline overflow through stimulation of α_2-adrenoceptors (Galzin et al., 1982) when compared with the present results on ^{3}H-DA overflow. The inhibition of ^{3}H-DA overflow by apomorphine and DA were antagonized by haloperidol, while the inhibition elicited by (-) adrenaline, although it was stereoselective, was not antagonized by propranolol, phentolamine, prazosin or sulpiride. The inhibition of ^{3}H-DA overflow by (-) adrenaline does not seem to be receptor-mediated and it is unlikely to be of physiological significance.

Thus, the present results indicate that the inhibition of DA release through presynaptic DA autoreceptors is highly dependent on the calcium concentration in the medium and this effect of calcium would be restricted to receptor mediated inhibitory mechanisms.

Galzin A.M. et al. (1982) J. Pharmacol. Exp. Ther. (in press).
Kamal L. et al. (1981) J. Pharmac. Exp. Ther. 216, 592-598.

COMPARISON OF DOPAMINE OVERFLOW FROM STIMULATED SLICES OF THE NEOSTRIATUM AND MEDIAN EMINENCE 'IN VITRO'

G.W. Arbuthnott, A. Horn & V. Kapoor, M.R.C. Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh, EH8 9JZ, Scotland

Because of the greater clinical accessibility of the hormones which they control, the dopamine containing neurones of the median eminence are often used as a model system through which to examine the functional state of central dopaminergic systems in general. The purpose of the present experiments was to compare, in as direct a manner as possible, the release of transmitter from the dopamine containing terminals of the neostriatum with that from the median eminence terminals.

Both areas were dissected from cooled rat brain and preincubated for 20 min. in Krebs bicarbonate buffer at 37°C. Tissue pieces from both areas were then transferred to small perspex chambers and superfused with the same Krebs buffer. The release of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) was assayed in the superfusate collected at two minute intervals from the chambers. The samples were analysed by high performance liquid chromatography on a 5μ ODS-Hypersil column (0.1M phosphate-citrate buffer, pH 4.0, with 20mg/l Sodium Octyl Sulphate, 10% Methanol and 0.1mM EDTA), and the dopamine and DOPAC in the samples measured by electrochemical detection on a carbon paste electrode (+0.65 V). The retention times of dopamine and DOPAC were 7 min. and 12 min. respectively. (Kapoor and Arbuthnott, 1982).

Median eminence samples from four rats weigh only about 1.5mg but the dopamine output in response to electrical stimulation at 20Hz (2msec biphasic pulses), for 30sec at 10mAmps, is easily measureable and is about 17fmoles/mg protein/impulse, which compares well with the 7fmoles/mg protein/impulse from the striatum.

Several differences in the characteristics of release are worthy of note. The most striking is the very small amount of DOPAC coming from the median eminence slices. Whereas DOPAC is a major component of the output from striatal slices, the resting output of DOPAC from the eminence slices is very small, usually below the detection limits of the assay even during stimulation. Umezu & Moore (1979) also report low levels of DOPAC in tissue from the median eminence. The smaller volume of tissue involved is reflected in the fact that pooled tissue from several rats is required for the median eminence samples, while ample dopamine is released from single slices of neostriatum from one side of one rat brain in the experiments on that area. The effect of the uptake inhibitor, Nomifensine, is clear on the stimulated release from the striatum where at 10^{-6}M it causes a three fold increase of dopamine overflow. However Nomifensine (10^{-6}M) has a very much smaller effect on the median eminence slices, increasing the overflow of dopamine by only about 50%. These results may be explained by the suggestion that the median eminence neurones lack a high affinity dopamine uptake system (Demarest and Moore, 1979), although it has been shown (Sarkar et al., 1981) that Nomifensine does block the uptake of ^{3}H dopamine into similar slices of the median eminence.

These experiments suggest that the control of dopamine release and metabolism in the median eminence is different from that in the striatum and thus counsel caution in the use of neuroendocrine markers to monitor the functional activity of dopamine in areas of the brain other than the median eminence.

References:

Demarest K.T., Moore K.E. (1979) Brain Res. 171: 545-551
Kapoor V., Arbuthnott G.W. (1982) Brit. J. Pharm. (in press)
Sarkar D.K., Horn A., Dow R.C., Cuello A.C. & Fink G. (1981) Procs. of the Endocrine Society, 1981.
Umezu K., Moore, K.E. (1979) J. Pharmacol. exp. ther. 165: 78-86.

α_2 RECEPTORS ARE NOT INVOLVED IN THE REGULATION OF STRIATAL DOPAMINERGIC TRANSMISSION

J. Dedek, B. Scatton and B. Zivkovic. Biochemical Pharmacology Group Synthélabo-L.E.R.S., 31 Avenue Paul Vaillant Couturier, 92220 Bagneux, France.

The acceleration of brain dopamine (DA) metabolism by the α_2 antagonists yohimbine (Anden and Grabowska, 1976) and SKF 64139 (Rabey et al, 1981) has been given as evidence for the involvement of α_2 adrenoceptors in the regulation of cerebral dopaminergic transmission. This view has however been challenged by the demonstration that the yohimbine-induced enhancement of cerebral DA turnover is related to a blockade of DA receptors (Scatton et al, 1980). Moreover, other potent α-adrenoceptor blocking agents e.g. tolazoline, phentolamine or prazosin fail to modify cerebral DA turnover (Scatton et al, 1980). In order to get a further insight into this problem we have studied the influence of other α_2 adrenoceptor blocking agents e.g. RX 781094 (Dettmar et al, 1981), rauwolscine (a diastereoisomer of yohimbine) and piperoxane on the metabolism of striatal DA and cerebral noradrenaline (NA) in the rat.

Experiments were carried out on male Sprague Dawley rats (140-160g). Homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy,4-hydroxyphenylethyleneglycol (MOPEG)-SO_4 were measured by high pressure liquid chromatography with electrochemical detection (Semerdjian-Rouquier et al, 1981) and acetylcholine was assayed by a radioenzymatic technique (Scatton and Worms, 1979). The turnover of DA and NA was estimated by measuring the disappearance of the amines after administration of α-methyl-p-tyrosine (αMT) (250 mg/kg, ip).

RX 781094 (1-10 mg/kg, ip) and rauwolscine (1-10 mg/kg, ip) both caused a marked enhancement of NA turnover in the rat hypothalamus or whole brain with ED_{50}'s of 0.9 and 1.6 mg/kg, respectively. These compounds also increased brain MOPEG-SO_4 levels with ED_{50}'s of 0.8 and 3.6 mg/kg, respectively. However, while rauwolscine also increased striatal HVA and DOPAC levels (ED_{50} 3.6 mg/kg, ip), RX 781094 (1-20 mg/kg) even in doses that maximally increase cerebral NA turnover failed to affect the striatal DA metabolite levels (as measured at 2 h postinjection). At the single dose (60 mg/kg, ip) tested, piperoxane markedly increased both hypothalamic NA turnover (+40%) and striatal HVA levels (+156%). These results suggest that rauwolscine and piperoxane block striatal DA receptor but that RX 781094 is ineffective in this respect. This view is supported by the fact that RX 781094 (10 mg/kg) fails to affect, whereas piperoxane (60 mg/kg) and rauwolscine decrease striatal ACh levels. Moreover, in vitro, RX 781094 (10 µM) fails to modify but piperoxane (10 µM) and rauwolscine (1 µM) antagonize the DA (10 µM)-induced inhibition of the potassium-evoked release of ^{3}H-ACh from striatal slices.

In conclusion, the present results indicate that like yohimbine, rauwolscine (α-yohimbine) and piperoxane increase striatal DA turnover, an effect which may be ascribed to their DA receptor blocking properties. In contrast, similarly to tolazoline and phentolamine, RX 781094 fails to modify DA turnover. The differential effects of the various α_2 antagonists investigated on striatal DA turnover strongly argues against the involvement of α_2 adrenoceptors in the regulation of striatal DA transmission.

Anden, N.E. and Grabowska, M. (1976) European J. Pharmacol. 39, 275-282.
Dettmar, P.W. et al (1981) Br. J. Pharmac. 74, 843P.
Rabey, J.M. et al (1981) Brain Res. 230, 422-426.
Scatton, B. et al (1980) J. Pharmacol. Exp. Ther. 215, 494-499.
Scatton, B. and Worms, P. (1979) J. Pharm. Pharmacol. 31, 861-863.
Semerdjian-Rouquier, L. et al (1981) J. Chromatog. 218, 663-670.

EVIDENCE THAT THE RAT STRIATONIGRAL AND STRIATOPALLIDAL EFFERENTS ARE CONTROLLED BY DIFFERENT DOPAMINE RECEPTORS

M Herrera-Marschitz and U Ungerstedt, Department of Pharmacology, Karolinska institutet, S-104 01 STOCKHOLM, Sweden.

ABSTRACT

The dopamine agonists apomorphine and pergolide induce dose-dependent contralateral rotation in 6-OH-DA-denervated animals. The characteristics of their pattern of rotation have suggested different receptor mechanisms, which is supported by the fact that pergolide and apomorphine responses can be blocked by DA antagonists believed to have selective affinity on D1 or D2/D4 type of receptors. Apomorphine responses are blocked by the D1/D2 antagonist, cis-flupenthixol, but are inhibited to a lesser extent by high doses of the selective D2/D4 antagonist, sulpiride. Moreover, pergolide responses are blocked by antagonists showing high affinity for D2/D4 receptors (Ungerstedt & Herrera-Marschitz, 1981).

Different classes of dopamine receptors may be differentiated not only by their binding characteristics but also by their localisation on different pre- or postsynaptic structures in the striatum. We have studied the effect of drugs interfering with GABAergic and cholinergic transmission on the rotation elicited by pergolide and apomorphine. We have found that the muscarinic antagonist scopolamine enhances the apomorphine rotation, whilst it inhibits the pergolide rotation. Furthermore, the GABA antagonist picrotoxin potentiates the pergolide responses, but rather inhibits the apomorphine responses (Ungerstedt et al, 1981).

We now show that the contralateral rotation induced by apomorphine is dramatically changed by lesioning the striatonigral efferent with kainic acid injection (2ug/ml dissolved in a Ringer solution) into the substantia nigra reticulata (SNR), however, the dose-dependent contralateral rotation induced by pergolide, although inhibited, remains qualitatively intact.

The striatopallidal pathway has been analyzed using a combination of local and systemic approaches. The GABA antagonist picrotoxin induces a dose dependent contralateral rotation when injected unilaterally into the globus pallidum (GP), whilst the GABA agonist muscimol induces a dose-dependent contralateral turning when injected in the SNR. Apomorphine (0.5 mg/kg s.c.) changes the contralateral rotation induced by the GP injection of picrotoxin (1 ug/ul) to an ipsilateral rotation, but pergolide 0.1 mg/kg s.c. does not significantly change the picrotoxin-induced rotation. Moreover, we are now studing the effect of apomorphine and pergolide on the contralateral rotation induced by intra-SNR injection of muscimol (10 ng/0.5 ul).

The above experiments support the idea that apomorphine induces rotation by preferentially stimulating a ACh-GABAergic striatonigral pathway controlled by a D1 type of receptor. Pergolide induces rotation by preferentially stimulating a GABAergic striato-pallidal pathway controlled by a D2/D4 type of receptor.

References

Ungerstedt, U and Herrera-Marschitz, M (1981). Proceedings of the III World Congress of Biological Psychiatry, Stockholm (in press).

Ungerstedt, U et al (1982). Proceedings of International Dopamine Symposium, Japan, Pergamon Press, N Y.

POTENT LIPOPHILIC SUBSTITUTED BENZAMIDE DRUGS ARE NOT SELECTIVE D-1 DOPAMINE RECEPTOR ANTAGONISTS IN THE RAT

S. Fleminger, P. Jenner, C.D. Marsden, C. Reavill, N.M.J. Rupniak, B. Testa[1] & H. van de Waterbeemd[1], University Department of Neurology, Institute of Psychiatry and the Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5, UK & [1]School of Pharmacy, University of Lausanne, CH-1005, Lausanne, Switzerland

Sulpiride is considered a selective D-2 adenylate cyclase independent dopamine receptor antagonist (Jenner & Marsden, 1981). Recently, YM 09151-2 (cis-N-[1-benzyl-2-methylpyrrolidin-3-yl]-5chloro-2-methoxy-4-methylaminobenzamide) was shown to inhibit dopamine-stimulated adenylate cyclase (D-1 receptors) in canine caudate preparations (Usuda et al, 1981). This potent lipophilic drug was suggested to be a selective D-1 antagonist supporting the hypothesis that the poor lipophilicity of sulpiride might explain the failure to inhibit adenylate cyclase (Woodruff et al, 1980). We now compare in vitro activity at D-1 and D-2 receptors in rat striatum, inhibition of apomorphine-induced stereotyped behaviour and lipid solubility of a range of substituted benzamide drugs with that for cis- and trans-flupenthixol.

Striatal dopamine (100 µM)-stimulated adenylate cyclase activity was weakly inhibited by YM 09151-2 (IC50 20 µM) and clebopride (IC50 19 µM) but not by the other substituted benzamide drugs (IC50 > 100 µM). YM 09151-2 (IC50 22,000 nM) and clebopride (IC50 100,000 nM) also weakly displaced ^{3}H-piflutixol (0.3 nM) from its binding site on striatal membranes. Again, the other substituted benzamide drugs were ineffective (IC50 > 100,000 nM). The isomers of flupenthixol potently inhibited both dopamine stimulated adenylate cyclase (IC50: trans-0.85 µM: cis-0.024 µM) and ^{3}H-piflutixol binding (IC50: trans- 89 nM: cis- 3.2 nM).

All drugs displaced ^{3}H-spiperone (0.2 nM) from striatal membranes but YM 09151-2, clebopride and cis-flupenthixol were most potent in this respect. Each of the substituted benzamide drugs examined, except sulpiride, inhibited apomorphine (0.5 mg/kg sc 15 min previously)-induced stereotyped behaviour. Again, YM 09151-2 and clebopride were the most potent compounds examined. Stereotyped behaviour was inhibited by cis- but not trans-flupenthixol.

Determination of log P' (n-octanol/buffer apparent partition coefficient at pH 7.4) showed YM 09151-2 (log P'3.51) and clebopride (log P' 2.99) to be highly lipophilic in comparison to the other substituted benzamide drugs examined (log P' range -1.1 to +1.2). The isomers of flupenthixol possessed idential lipophilicity, which was equivalent to that of YM 09151-2.

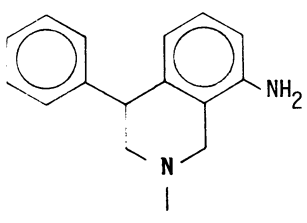
The potent lipophilic substituted benzamide drugs examined, like the less potent and less lipophilic members of this series, do not appear to selectively interact with D-1 receptors, but are selective D-2 receptor antagonists. Lipophilicity alone does not explain this specificity of action, which suggests steric factors also are involved.

Jenner, P. & Marsden, C.D. (1981) Neuropharmacology 20, 1285
Usuda, S. et al (1981) Psychopharmacology 73, 103
Woodruff, G.N. et al (1980) J.Pharm.Pharmacol. 32, 802

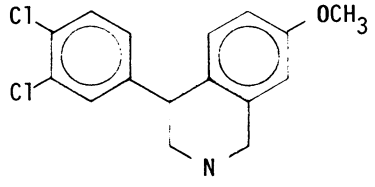
CONFORMATION ON AMINE UPTAKE SITE: RESULTS WITH Ro 8-4650

Jouko Tuomisto & Raija Voutilainen, Department of Pharmacology and Toxicology, University of Kuopio, P.O.B. 138, SF-70101 Kuopio 10, Finland

Nomifensine is one of the few compounds which potently inhibit dopamine uptake by brain synaptosomes (Hunt et al, 1974). This compound can be visualized as having the same basic structure as the tricyclic antidepressants with two differences: there is no ethylene bridge between the two phenyl rings, and the side chain nitrogen is rigidly bound to a ring structure. We have previously suggested that these features are essential for affinity to the dopamine uptake site (Tuomisto, 1977, 1978). On the other hand, Koe et al (1976) suggested that the 8-amino function of nomifensine rather than the ring-1 nitrogen is the crucial binding group. To solve this we studied an experimental analogue of nomifensine, Ro 8-4650 which lacks the 8-amino group.



Nomifensine



Ro 8-4650

Dopamine, noradrenaline and 5-HT uptake were studied in rat brain synaptosomes in Krebs-Henseleit bicarbonate buffer (Tuomisto et al, 1974), and the IC_{50} values were assayed. Monoamine uptake was also studied ex vivo in rat plasma samples after giving 1-10 mg of Ro 8-4650 to rats i.p. The synaptosomes were obtained from untreated animals (method: Tuomisto et al, 1980).

Ro 8-4650 was very potent inhibitor of dopamine uptake in vitro: the IC_{50} values were 1.8×10^{-8}M (dopamine), 1.2×10^{-7}M (5-HT) and 4.3×10^{-7}M (noradrenaline). Ro 8-4650 inhibited noradrenaline uptake by intact synaptosomes in plasma samples of rats administered the compound 1 h previously by 30-60 %. The inhibition of other uptake systems was trivial.

The present results indicate that Ro-8-4650 is a potent inhibitor of amine uptake in vitro; as a dopamine uptake inhibitor it is probably the most potent compound as yet found. This demonstrates that the 8-amino group of nomifensine is not necessary for its activity. Hence the results also support the hypothesis that nomifensine might bind to the uptake site through its ring nitrogen and the two phenyl rings (Tuomisto, 1977, 1978). The differences between the in vitro and ex vivo results remain to be clarified, but this could be due to formation of active metabolites with a spectrum different from that of the parent compound.

Hunt, P. et al (1974) J. Pharm. Pharmacol. 26, 370
Koe, B.K. et al (1976) J. Pharmacol. exp. Therap. 199, 649
Tuomisto, J. (1977) Europ. J. Pharmacol. 42, 101
Tuomisto, J. (1978) Adv. Pharmacol. Therap., vol. 2, 231, Pergamon Press
Tuomisto, J. et al (1980) Psychopharmacol. 69, 137

TISSUE DISTRIBUTION AND RETINAL UPTAKE OF ^{14}C-6-MeO-THBC AFTER INTRAVASCULAR AND INTRAVITREAL INJECTIONS IN RATS AND MICE

M. Leino, M.M. Airaksinen, R. Antikainen & I. Kari, Department of Pharmacology & Toxicology, University of Kuopio, SF-70101, Finland

6-Methoxy-1,2,3,4-tetrahydro-ß-carboline (6-MeO-THBC) is a condensation product of 5-methoxytryptamine and formaldehyde. 6-MeO-THBC possesses diverse biochemical and pharmacological properties (Airaksinen & Kari, 1981b) and has been suggested to act as a neuromodulator. 6-MeO-THBC has been shown to occur in various tissues (Airaksinen & Kari, 1981a) including the retina in animals and human beings (Kari et al., 1982). Our present results show that the mouse and rat retina and other tissues uptake radioactively labelled 6-MeO-THBC injected intravascularly or intravitreally.

Wistar albino rats and BALB/C albino mice were kept in 10-14 hours dark-light cycle in standardized laboratory conditions. Radioactive 6-MeO-THBC in saline (6 µl) was injected intravitreally into aether-anesthetized rats and intravenously (250 µl) into the tail vein of the mice. The rats and mice were sacrificed by decapitation, the rats after 5 and 30 minutes, 2, 24 and 48 hours following intravitreal injections, the mice after 5 and 30 minutes, 2, 8 and 24 hours following intravascular injections. The anterior parts and the vitreous gel were removed and the retina was gently teased from the pigment epithelium. Numerous other organs of the mice were also removed. The samples were placed in vials, weighed and a solvent and a scintillation liquid were added. Radioactivity was measured on a Wallac beta counter using the preset ^{14}C-counting window. Counts were corrected for quenching and background.

The retinal concentrations showed a prompt rise after the injections and high levels were maintained after two days. The highest level of uptake in rats was after two hours but a considerable uptake level was also seen at all other time-stages. The retinal tissue of the mice showed the highest radioactivity after 30 minutes and likewise considerable levels of radioactivity were seen through all time-stages.

In other tissues high levels of radioactivity were seen in the liver, kidneys, lungs, spleen and heart. Somewhat lower levelswere seen in the brain, adrenal glands, muscle, testes and in the mesenterial membranes. In all tissues the highest levels of radioactivity were at the 30-minute stage.

We have suggested that the site of biosynthesis of 6-MeO-THBC is in the pineal gland. The present study shows an active uptake of 6-MeO-THBC or its radioactive metabolites into the retina. Furthermore,the occurrence of hydroxyindole-O-methyltransferase activity in the retina (Wainwright, 1979) makes even a retinal synthesis possible.

This study was supported by the Sigrid Jusélius Foundation, the Academy of Finland and the Lääke Oy Research Foundation.

Airaksinen, M.M. & Kari, I. (1981a) Med. Biol. 59, 21
Airaksinen, M.M. & Kari, I. (1981b) Med. Biol. 59, 190
Gern, W & Ralph, C.L. (1979) Science 204, 183
Kari, I. et al. (1982) This meeting
Wainwright, S.D. (1979) J. Neurochem. 32, 1099

6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN THE RETINA

I. Kari[1], M. Leino[2], M.M. Airaksinen[3] and J. Gynther[1], Departments of Pharmacy[1], Ophthalmology[2] and Pharmacology & Toxicology[3], University of Kuopio, SF-70101 Kuopio 10, Finland

Up to now several β-carbolines have been demonstrated in tissues of man and some animals (Airaksinen & Kari, 1981a). 6-Methoxy-tetrahydro-β-carboline (6-MeO-THBC) has been reported in rat brain and adrenals (Barker et al, 1981) and in the pineal gland of male fawls (Kari, 1981) and man (Kari et al, 1981). By using gas chromatography mass fragmentography we now measured 6-MeO-THBC in the retinal tissue of pigmented pigs (Yorkshire) and albino rabbits (New Zealand white).

The animals were sacrificed at daytime (the rabbits with mebumal and the pigs with electricity), the eyes were enucleated immediately, the anterior portions and the viterous gel were removed and the retinas were teased from the pigment epithelium using microinstruments. The retinas were homogenized in 0.1 M HCl containing pure 3,3,4,4-d_4-6-MeO-THBC as internal standard. The extraction and derivatization were as described (Kari et al, 1980). In all steps of analytical procedure semicarbazide was used for binding of free aldehydes. The identification of heptafluoro-derivative of 6-MeO-THBC was performed by monitoring the ions m/e 398, 229, 201, 185 and 173 and gas chromatographic retention, which was identical to that of internal standard. The peak height ratio of the molecular ions of 6-MeO-THBC and internal standard (m/e 398/402) was used in the quantification.

6-MeO-THBC was found to be a normal constituent in the retinal tissue of pigs and rabbits, concentrations being 320.6 ± 111.5 pg/retina (pigs) and 27.0 ± 4.5 pg/mg of retina (rabbits). We have preliminary determined 6-MeO-THBC also in two human retinae obtained from enucleations and its concentration seemed to be similar to those in pig retina. The concentrations of 6-MeO-THBC in the retina are, however, considerably lower than in pineal gland and adrenals of the previously mentioned species but in the same range as in the rat brain. The origin of retinal 6-MeO-THBC remains to be shown, but the occurence of hydroxyindole-O-methyltransferase activity in the retina (Gern & Ralph, 1979) makes its local synthesis possible. On the other hand, our studies suggest considerable retinal uptake of circulating 6-MeO-THBC (Leino et al, 1982). The effects of β-carbolines are numerous, they act e.g. on the receptors and metabolism of several neurotransmitters (Airaksinen & Kari, 1982b) and 6-MeO-THBC have been shown to inhibit retinal uptake of 5-HT and promote the release of this transmitter. However, the significance of 6-MeO-THBC for the functions of retina are unknown at present.

This study was supported by grants from the Sigrid Jusélius Foundation, the Academy of Finland and the Lääke Oy Research Foundation.

Airaksinen, M.M. & Kari, I. (1981a) Med.Biol. 59, 21
Airaksinen, M.M. & Kari, I. (1981b) Med.Biol. 59, 190
Barker, S.A. et al (1981) Biochem.Pharmacol. 30, 9
Gern, W. & Ralph, C.L. (1979) Science 204, 183
Kari, I. (1981) FEBS Lett. 127, 277
Kari, I. et al (1980) Biomed.Mass Spectrom. 7, 549
Kari, I. et al (1981) Proceeding of 8th international symposium on mass spectrometry in biochemistry, medicine and environmental research, in press
Leino, M. et al (1982) This meeting

(^{3}H)-SPIPERONE CAN BE USED TO LABEL EITHER A SINGLE OR MULTIPLE DOPAMINERGIC SITES IN RABBIT RETINA AND STRIATUM

Margarita L. Dubocovich & Nancy R. Zahniser*, Department of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262, U.S.A.

The presence of dopamine (DA) receptors of the D-2 subtype (Kebabian & Calne, 1979) has been difficult to demonstrate in the mammalian retina using radioligand binding (Watling & Iversen, 1981). Recent evidence indicates, however, that the stimulation-evoked release of DA from the rabbit retina is regulated by activation of presynaptic inhibitory DA autoreceptors of the D-2 subtype (Dubocovich & Weiner, 1982). ^{3}H-Spiperone binds predominantly to D-2 receptors in the striatum, which are distinct from both D-1 sites linked to adenylate cyclase (Marchais & Bockaert, 1980) and D-3 sites labeled by DA receptor agonists (Hamblin & Creese, 1982). In view of these findings we have studied D-2 receptors in the albino rabbit retina and striatum using the specific binding of ^{3}H-spiperone defined by the isomers of the selective D-2 receptor antagonist sulpiride as well as the nonselective antagonists flupenthixol and butaclamol. Homogenates of membranes (~60 µg protein) from retina and striatum were washed in Krebs' solution (pH 7.6, no ascorbic acid) and incubated with ^{3}H-spiperone and competing drugs for 20 min at 37° C. Binding was terminated by the addition of ice-cold buffered saline and rapid filtration through glass fiber filters. Specific binding was defined as the difference in ^{3}H-spiperone bound in the presence of either (R)- and (S)- sulpiride, (β)- and (α)- flupenthixol or (-)- and (+)- butaclamol.

The pharmacological profiles of the sites labeled by ^{3}H-spiperone in both the retina and striatum were similar (affinity: (+)-butaclamol = domperidone = (α)-flupenthixol > (S)-sulpiride > (β)-flupenthixol = (-)-butaclamol = N,N-di-n-propyldopamine > (R)-sulpiride) and like that previously published for ^{3}H-spiperone binding in the bovine retina (Watling & Iversen, 1981). Concentrations as high as 0.3 µM of the selective serotonergic S-2 receptor antagonist R41-468 did not inhibit the binding in either tissue. The most striking finding from the competition studies was that only S-sulpiride, of all the active antagonist isomers tested, had a Hill coefficient = 1.0; this indicates a simple interaction with a single receptor subtype. From Scatchard analysis in both retinal and striatal membranes, it was apparent that ^{3}H-spiperone (0.05 - 0.75 nM) labeled more sites (35% retina; 25% striatum) when specific binding was defined with the isomers of flupenthixol or butaclamol than with the isomers of sulpiride. When sulpiride was used to define specific binding, the number of binding sites equaled 200 fmol/mg protein in the retina and 570 fmol/mg protein in the striatum. Preliminary results in the striatum indicated that the number of binding sites detected by direct ^{3}H-S-sulpiride binding, regardless of whether spiperone or the isomers of flupenthixol or butaclamol were used to define specific binding, was identical to that detected by ^{3}H-spiperone binding defined with the isomers of sulpiride.

These results indicate that sites with the characteristics of the D-2 receptor subtype can be measured in both the rabbit retina and striatum. The number of D-2 sites in the striatum is 2-3 fold higher than that in the retina; thus, the retina contains only a small population of D-2 receptors. In addition to labeling D-2 sites, ^{3}H-spiperone also labels an additional site defined by flupenthixol or butaclamol, possibly a D-1 site. In contrast, ^{3}H-spiperone binding defined with the isomers of sulpiride or direct ^{3}H-S-sulpiride binding can be used to measure a single dopamine receptor subtype.

This work supported by USPHS (NS 09199).

Dubocovich, M.L. & Weiner, N. (1981) J. Pharm. Exp. Ther. 219,701.
Hamblin, M.W. & Creese, I. (1982) Mol. Pharmacol. 21,52.
Kebabian, J.W. & Calne, D.B. (1979) Nature (Lond.) 227,93.
Marchais, D. & Bockaert, J. (1980) Biochem. Pharmacol. 29,1331.
Watling, K.J. & Iversen, L.L. (1981) J. Neurochem. 37,1130.

THE EFFECT OF HIGH PRESSURE ON (3H)GABA RELEASE FROM FROG ISOLATED SPINAL CORD

A.R. Bichard & H.J. Little, University Department of Pharmacology, South Parks Road, Oxford, OX1 3QT.

The behavioural effects of general anaesthetics and of high pressure show a reciprocal antagonism. The high pressure neurological syndrome (HPNS) is observed as an increased excitability, consisting of tremor (40-50 atmospheres of helium) and convulsions (80-90 atm). The early stages are a problem for deep sea divers, but the mechanism is unknown. We have shown that drugs which facilitate GABA transmission can protect against HPNS convulsions (Bichard and Little, 1982a, b). We have also shown in vitro that post-synaptic responses to applied GABA can remain unaffected at pressure (Little, 1981). We have now investigated the effects of helium pressure on the release of 3H GABA in vitro.

The method of Collins (1974) was adapted for the pressure chamber. An isolated hemisected frog spinal cord was incubated in 0.5 μ Ci 3H GABA for 1 hour, with 14 C urea as a marker for non-specific release. The tissue was then superfused with Ringer solution (containing 1 mM amino-oxyacetic acid to inhibit GABA breakdown) at 0.15 ml/minute and samples collected every 10 minutes. Wash-out was for 60 minutes before transfer to a pressure chamber, which was flushed with 95% O_2/5% CO_2 for 1 minute. Helium pressure was applied at 5 atmospheres/min. In control experiments 1 atm of helium was added to simulate temperature changes (maintained at 21°C ± 1°C). The tissue was stimulated rostrally (50 Hz, 4 mA, 2 min) at 10 and at 80 minutes after pressurisation. Spontaneous release over a 2 hour period was determined separately. The concentrations of 3H GABA in the fractions were measured by liquid scintillation counting. Results are expressed as sample d.p.m. as a percentage of tissue d.p.m.

No change was seen in spontaneous release of 3H GABA at 50 or 100 atmospheres, or in the percentage increase of stimulated GABA release 10 minutes after pressurisation to 50 atmospheres (Table 1). A significant increase in spontaneous GABA release was seen for 30 minutes, only after stimulation. At 100 atmospheres, there was a large increase in release on stimulation; this remained elevated for 30 minutes. By 80 minutes there was no elevation of release at 50 or 100 atm (although release at 1 atm helium showed a decrease at this time). The pattern of urea release did not show these increases.

Table 1 — 3H GABA release (Mean % ± s.e.m.), 10 min periods

Helium Pressure (atm)	n	Basal Release	1st Stimulation	10 min After	2nd Stimulation	10 min After
1	(5)	0.37±0.07	1.27±0.17	1.04±0.10	0.87±0.09	0.63±0.06
50	(5)	0.41±0.11	1.19±0.20	1.57±0.30*	0.86±0.18	0.98±0.13*
100	(5)	0.35±0.05	2.87±0.50**	2.10±0.50*	1.01±0.18	1.10±0.16*

** $p < 0.02$ vs control and 50 atm, * $p < 0.05$ vs control.

The changes seen may be due to a general hyperexcitability of neurones caused by pressure. Pressure may also be affecting other neuronal properties such as feedback mechanisms.

A.R.B. is an M.R.C. scholar.

Bichard, A.R. & Little, H.J. (1982a). Brit. J. Pharmac. (in press).
Bichard, A.R. & Little, H.J. (1982b). Neuropharmacology (in press).
Collins, G.G.S. (1974). Brain Res. 66, 121-137.
Little, H.J. (1981). Brit. J. Pharmac. 74, 880P.

POTENCY AND DURATION OF THE EFFECT OF SOME LIPID-SOLUBLE BARBITURATES

H.G. Bolander & G. Wahlström, Department of Pharmacology, University of Umeå, S-901 87 Umeå, Sweden

The rate of penetration of barbiturates to the brain and other tissues is assumed to depend mainly on their lipid-solubility. Short-acting barbiturates are very lipid-soluble, which means a rapid penetration to the brain and a fast redistribution. In the present investigation differencies between barbiturates have been found that cannot be explained by differences in lipid-solubility.

The effects of thiopental, hexobarbital and pentobarbital were studied in male rats with an EEG-threshold method (Wahlström, 1966). The barbiturates as sodium salts were infused with a constant rate through a tail vein. When a specific EEG criterion, the first period of burst-suppression of one second or longer ("the silent second") appears, the infusion is stopped and the amount of drug infused to reach the criterion is calculated. After the infusion is stopped the anesthesia time is recorded. Repeated determinations in the same rat can be done with weekly intervals.

Slow infusion rates allow larger amounts of barbiturate to be redistributed and the dose for "silent second" is high. With increasing dose rates one passes an optimal dose rate where the amount of drug to reach the criterion is the lowest and above this there is an approximately linear increase in threshold dose. This increase is in part explained by the increasing amount of drug on its way from the tail to the head.

Table 1 Results of threshold tests with three barbiturates

	Optimal dose rate $mg \cdot kg^{-1} \cdot min^{-1}$	Dose for "silent second" $mg \cdot kg^{-1}$	Anesthesia time min
Pentobarbital	5	56.1 ± 1.6	109.8 ± 6.7
Hexobarbital	15	63.0 ± 3.2	14.3 ± 1.4
Thiopental	10	50.7 ± 1.2	104.2 ± 6.6

Among these three compounds pentobarbital is the least lipid-soluble and thiopental the most, according to Bush (1963).

Table 1 shows that thiopental has a considerably longer anesthesia time than hexobarbital in spite of a lower dose infused to reach the criterion. The infusion time is approximately the same with both barbiturates. Since thiopental is very lipid-soluble it is clear, from these data, that this property is not always found together with rapid redistribution. The optimal dose rate is dependent on the pharmacologic characteristics of the barbiturate used and a higher optimal dose rate could indicate a faster penetration and redistribution. Support for the redistribution part is found in the hexobarbital data above.

Bush, M.T. (1963) Physiol.Pharmacol. Vol. 1, The Nervous System - Part A, 185
Wahlström, G. (1966) Acta pharmacol.toxicol. 24, 404

EVIDENCE FOR CENTRAL HYPOTENSIVE ACTION OF 4(5)-(2,6-DIMETHYL-BENZYL)IMIDAZOLE (MPV-207)

S.Kaipiainen, J. Puurunen, H. Ruskoaho & N.T. Kärki, Department of Pharmacology, University of Oulu, SF-90220 Oulu 22, Finland

Several new 4(5)-substituted arylalkyl imidazole derivatives have shown hypotensive activity in our screening experiments, one of the most effective being 4(5)-(2,6-dimethylbenzyl)imidazole (MPV-207). The aim of the present study was to investigate the possible involvement of a central mechanism in the hypotensive action of MPV-207.

The action of intravenous (i.v.) and intracerebroventricular (i.c.v.) injections of MPV-207 on mean arterial pressure (MAP) and heart rate (HR) and the effect of i.c.v. administered yohimbine pre-treatment were investigated in urethane-anaesthetised male Wistar rats. Administrations of MPV-207 and yohimbine into the lateral cerebral ventricle of the rat were performed using the stereotaxic method described by Paakkari (1980). The possible central site of action of MPV-207 was further evaluated by investigating the cardiovascular activity of the compound upon administration into the vertebral artery of chloralose-anaesthetised cats (van Zwieten 1975).

I.v. injections of 3-30 μg/kg of MPV-207 into the rat caused dose-dependent falls in MAP and HR, the maximal decreases at 30 μg/kg being about 37 and 27 % respectively. Doses of 30 μg/kg or greater induced a transient, initial rise in MAP, which was followed by hypotension, whereas HR fell immediately. Administration of 5 μg/kg of MPV-207 i.c.v. lowered MAP and HR by an average of 24 and 8 % respectively. The hypotensive effect of 5 μg/kg of MPV-207, injected i.v., was attenuated by 100 μg/kg of yohimbine administered i.c.v. 10 min beforehand.

In the cats an i.v. injection of 30 μg/kg of MPV-207 had a biphasic effect on MAP, causing an initial rise of about 14 %, lasting about 5 min, followed by a fall to a maximal decrease of 23 % observed 20 min after the administration. HR fell immediately after the administration. When the same dose was injected into the vertebral artery, MAP fell immediately, without the initial hypertensive phase. Although the hypotensive phase developed more rapidly up to 5 min after the injection into the vertebral artery, the maximal hypotensive response was about the same magnitude with both routes of administration.

In summary, MPV-207, when injected into anaesthetised rats and cats has cardiovascular actions resembling those of clonidine, a centrally acting antihypertensive drug. MPV-207 lowered MAP and HR upon i.c.v. injection, and its hypotensive effect after i.v. administration was attenuated by an i.c.v. administration of yohimbine, an α_2-adrenoceptor antagonist. The central component in the hypotensive effect of MPV-207 was further suggested by a more rapidly developing hypotensive response upon administration into the vertebral artery of the cat than upon i.v. injection. It is concluded that the hypotensive effect of MPV-207 is at least in part due to the stimulation of central α-adrenoceptors.

Paakkari, I. (1980) Experientia 36, 887-888
Zwieten, P.A. van (1975) Progress in Pharmacol.1, 9-11

NEW POTENT ANTIHYPERTENSIVE AGENTS, OR H3088 AND OR N0676

H. Karppanen, I. Paakkari, Pirkko Paakkari, E. Honkanen, Aino Pippuri, P. Kairisalo & P. Nore, Department of Pharmacology, University of Helsinki, SF-00170 Helsinki 17, and Orion Pharmaceutical Co., Research Laboratories, SF-00101 Helsinki 10, Finland

The cardiovascular effects of the newly synthesized compounds OR H3088, 2-[4-(1-azacyclooctylcarbonyl)piperidin-1-yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride, and OR N0676, 2-[4-(cyclopentylcarbonyl)piperidin-1-yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride, were studies in urethane-anaesthetized normotensive rats (NR) and in conscious spontaneously hypertensive rats (SHR). In NR the left femoral artery was cannulated for the direct measurement of systolic, diastolic and mean blood pressure as well as heart rate. Both OR H3088 and OR N0676 produced a dose-dependent fall in blood pressure at the doses of 0.01-10 μmol/kg i.v. After the smallest dose the maximal decrease in mean arterial pressure was approximately 25-30% by both drugs. After each dose the blood pressure remained lowered for the whole observation period of 30 min. The fall in diastolic pressure was proportionately more pronounced than the fall in systolic pressure. The heart rate was not significantly affected by the doses of 0.01 or 0.1 μmol/kg. After the doses of 1 or 10 μmol/kg the heart rate was slightly decreased.

In SHR the systolic blood pressure and heart rate were recorded with a tail cuff method (W+W instrument, Model 8002). Before each measurement the rats were kept for 30 min at 35°C to make the pulsations of the tail artery detectable. OR H3088 was administered at the dose of 10 μmol/kg p.o. on 5 consecutive days. Each administration of the drug produced a fall in blood pressure. The maximum fall, 20-40 mm Hg, was observed at 1-3 h, and the blood pressure remained significantly lowered at least for 5 h. No decrease in the antihypertensive activity of OR H3088 could be seen upon repeated administrations. The heart rate of SHR was not significantly affected by OR H3088.

OR N0676 had an antihypertensive effect in SHR even at the very low dose of 0.3 μmol/kg p.o. The dose of 1 μmol/kg p.o. was administered on 5 consecutive days. Each administration of the drug produced a significant fall of blood pressure. The maximum fall, 20-30 mm Hg, was seen at 1-5 h. The antihypertensive effect of OR N0676 was not attenuated upon repeated administrations. The compound did not significantly affect the heart rate of SHR.

The acute oral toxicity of these compounds is very small so that LD_{50} in rats is at least 10000 times bigger than the doses which exert antihypertensive effects. The results indicate that the compounds OR H3088 and OR N0676 are potent orally effective antihypertensive agents with small toxicity and without tachycardic properties in SHR. The antihypertensive effect of these agents may be, at least partly, due to a decrease in peripheral resistance, as suggested by the more pronounced fall in diastolic as compared to systolic blood pressure in NR.

CARDIOVASCULAR RESPONSES TO THE INTRAHYPOTHALAMIC ADMINISTRATION OF ADRENALINE IN CONSCIOUS RATS

K. R. Borkowski, Department of Physiology, University of Leicester, Leicester, LE1 7RH.

Adrenaline, injected directly into the anterior hypothalamus (AH) of anaesthetised normotensive rats, has been shown to induce decreases in blood pressure and heart rate (Struyker Boudier & Bekers, 1975), lending some support to the postulated existence of hypothalamic cardiodepressor "adrenaline-receptors" (Bolme et al, 1974). However, selective agonist and antagonist agents for the postulated "adrenaline-receptor" are, as yet, unavailable and while an involvement of hypothalamic α-adrenoceptor mechanisms could not be completely ruled out, hypothalamic β-adrenoceptors were implicated in mediating the adrenaline-induced hypotension and bradycardia (Borkowski & Finch, 1978). Since anaesthesia is capable of modifying cardiovascular responses in the rat (Barrett, 1971), the present study describes and attempts to characterise the cardiovascular effects of adrenaline injected into the AH of conscious, unrestrained normotensive rats.

In conscious normotensive rats, prepared for direct recording of blood pressure by the method of Popovic & Popovic (1960), the bilateral injection of adrenaline (0.001 - 10μg), in a volume of 1μl of 0.001M HCl, directly into the AH, via stereotaxically implanted guide-cannulae (coordinates:- A + 6.8, V - 2.0, L ± 1.0 selected from the stereotaxic atlas of Pellegrino & Cushman), caused a dose-related reduction in heart rate. These reductions in heart rate (up to -60 ± 10 beats/min., n = 11) and the hypotensive effect (up to -15 ± 4mm Hg, n = 11) of the higher doses of adrenaline were comparable to the adrenline-induced cardiovascular depression reported in anaesthetised rats (Struyker Boudier & Bekers, 1975) and further support the postulated existence of hypothalamic "adrenaline-receptors" mediating cardiovascular depressor effects (Bolme et al, 1974).

The bradycardia and blood pressure effects induced by adrenaline (0.01 - 1μg bilat. AH) were unaffected by pretreatments with piperoxan (10μg bilat. AH, 30 min), which itself induced a sustained decrease in heart rate (-40 ± 8 beats/min., n = 14) and a small reduction in resting blood pressure (-10 ± 3mm Hg, n = 14). However, following pretreatment with timolol (3.125μg bilat. AH, 30 min), which did not affect the resting blood pressure (-3 ± 2mm Hg, n = 17), but reduced the heart rate (-60 ± 6 beats/min., n = 17), the bradycardia induced by adrenaline (0.01 - 1μg bilat. AH) was completely reversed and only increases in blood pressure were observed.

These results indicate that the cardiovascular depressor effects of adrenaline injected into the AH of conscious normotensive rats do not appear to be mediated by hypothalamic α-adrenoceptors. A hypothalamic β-adrenoceptor involvement is however implicated, though the ability of adrenaline injected into the AH to induce a tachycardia, following pretreatment with timolol, suggests that this pretreatment not only blocks a hypothalamic heart rate lowering mechanism, normally activated by adrenaline, but also "unmasks" a cardiopressor mechanism. Whether the hypothalamic receptors blocked by timolol are analogous to the postulated "adrenaline-receptors" remains to be seen.

This work was aided by a grant from the British Heart Foundation.

Barrett, A.M. (1971). Eur. J. Pharmac. 15, 267-273.
Bolme, P. et al (1974). Eur. J. Pharmac. 28, 89-94.
Borkowski, K. R. & Finch, L. (1978). Clin. Exp. Hypertens. 1, 279-291.
Popovic, V. & Popovic, P. (1960) J. Appl. Physiol. 15, 727-728.
Struyker Boudier, H. A. & Bekers, A. D. (1975). Eur. J. Pharmac. 31, 153-155.

CARDIOVASCULAR EFFECTS OF INDORAMIN IN DOGS

D.W.G. Harron, R.G. Shanks, Department of Therapeutics & Pharmacology, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland.

Indoramin reduces blood pressure in animals and in man by competitive α_1 adrenoceptor blockade. The fall in BP is not accompanied by a reflex tachycardia (Baum et al, 1973; Carballo et al, 1974). It has been suggested that this lack of tachycardia is due to the local anaesthetic activity of indoramin on the cardiac conduction system (Alps et al, 1972; Algate et al, 1981). The present studies in anaesthetized dogs investigated: firstly, the effects of increasing doses of indoramin and mexiletine on mean arterial pressure (MAP) and heart rate (HR) and on the HR responses to right stellate ganglion stimulation; secondly, the effects of indoramin on MAP and HR following pre-treatment with atropine 0.04 mg/kg and propranolol 0.6 mg/kg; and thirdly, the effects of indoramin on the changes in MAP and HR produced by bilateral carotid artery occlusion, bilateral central simulation of the cut vagi and the intravenous administration of isoprenaline 0.5 µg/kg, noradrenaline 1.0 µg/kg and phenylethylamine 0.5 mg/kg. The results of the first study indicated that indoramin (cumulative dose 1.75 mg/kg) reduced ($p < 0.05$) MAP from 136.7 $\pm$ 21.3 to 63.3 $\pm$ 21.7 mm Hg and HR from 158.0 $\pm$ 6.1 to 129.3 $\pm$ 2.3 beats/min; in another group of dogs, mexiletine (cumulative dose 7.75 mg/kg) gave a small reduction in MAP from 127.5 $\pm$ 17.5 to 98.3 $\pm$ 13.3 mm Hg and HR from 153.3 $\pm$ 1.7 to 144.3 $\pm$ 3.5 beats/min. The tachycardia produced by right stellate stimulation (10 v, at 1.0, 2.5, 5.0 and 10.0 Hz) was not blocked by either indoramin (cumulative dose 1.75 mg/kg) or mexiletine (cumulative dose 7.75 mg/kg). In the second study indoramin (cumulative dose 1.75 mg/kg) administered following pre-treatment with atropine and propranolol reduced ($p < 0.05$) MAP from 158.7 $\pm$ 10.0 to 98.8 $\pm$ 9.8 mm Hg and HR from 138.7 $\pm$ 8.0 to 103.2 $\pm$ 5.1 beats/min. In the third study, carotid artery occlusion, central stimulation of the cut vagi, noradrenaline and phenylethylamine produced increases in MAP and HR, and isoprenaline a decrease in MAP and an increase in HR. Indoramin (cumulative dose 3.5 mg/kg) reduced ($p < 0.05$) the increase in MAP and HR produced by carotid artery occlusion, noradrenaline and phenylethylamine. Indoramin had no effect on the increases in MAP and HR produced by bilateral central stimulation of the vagi. The decrease in MAP produced by isoprenaline was increased from 20.3 $\pm$ 4.7 to 32.7 $\pm$ 9.6 mm Hg with indoramin and the increase in HR was reduced from 55.5 $\pm$ 3.3 to 33.0 $\pm$ 9.3 beats/min. In conclusion mexiletine, a drug with local anaesthetic activity, had little effect on MAP and HR in anaesthetized dogs, but indoramin reduced ($p < 0.05$) MAP and HR; neither drug reduced the tachycardia produced by stellate stimulation indicating that drugs with local anaesthetic activity do not necessarily prevent a tachycardia or cause bradycardia and that this property does not account for the absence of a reflex tachycardia with indoramin; the bradycardia after indoramin is not due to changes in vagal or sympathetic activity as it occurred after atropine and propranolol. The reduction by indoramin in increases in MAP produced by bilateral carotid artery occlusion, noradrenaline and phenylethylamine are due to peripheral α_1 blockade. The reduction by indoramin of the increases in HR produced by these procedures may indicate a possible direct effect on the myocardium.

Algate et al, (1981) J. Pharm. Pharmacol. 33, 256-239.
Alps et al, (1972) Cardiovasc. Res. 6, 226-234.
Baum et al, (1973) Arch. Int. Pharmacodyn. Ther. 204, 390-406.
Carballo et al, (1974) Curr. Med. Res. Opin. 2, 437-443.

EFFECTS OF FELODIPINE ON THE HIND LIMB VASCULAR RESISTANCE OF DOGS

B.S. Jandhyala & B. Ljung (introduced by D.E. Clarke). Department of Pharmacology, University of Houston, Tx. 77004 and AB Haessle, S-431 83 Molndal, Sweden.

Felodipine (4-(2,3-dichlorophenyl-1, 4-dihydro-2, 6-dimethyl-3-ethoxycarbonyl-5 -methoxycarbonyl-pyridine) is a potent vasodilator and has been shown to lower arterial pressure in hypertensive animals as well as man. The present study was undertaken to evaluate the efficacy of this agent to reduce vascular resistance in the presence and in the absence of neurogenic control of the hind limb vasculature.

Dogs were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and anesthetic level was maintained constant by continuous infusion of the anesthetic at a dose of 4 mg/kg/hour. One of the hind limbs was acutely denervated while the other remained intact. Under these conditions, pressure-flow characteristics were simultaneously established by recording perfusion pressures at various controlled flow levels (20-150 ml/min) in the denervated as well as innervated limbs (Jandhyala, etal, 1976). Intravenous administration of 0.01 μmol/kg of felodipine failed to produce any significant changes in the resistance (measured as changes in perfusion pressure) of denervated vasculature, whereas the resistance in the innervated limb was significantly reduced as indicated by the shift of the pressure-flow curves to the right (30 to 55 mm Hg. change depending upon the flow level). However, i.v. administration of 0.1 μmol/kg of the drug reduced resistances in the denervated as well as innervated limbs. But, the shifts in the denervated limb were significantly lesser than those observed in the innervated limb. (20-50 mmHg. and 40 to 120 mmHg. respectively). The data seemed to suggest that an inhibition of neurogenic component contributed to the reduction in the vascular resistance following felodipine administration in the pentobarbital anesthetized dogs. In contrast, in dogs anesthetized with morphine, (3 mg/kg, i.m.) plus chloralose (100 mg/kg., i.v.), i.v. administration of felodipine (0.1 μmol/kg.) produced significant reductions in the vascular resistances of both the denervated as well as innervated limbs and the magnitude of these shifts were not statistically different. Further analysis of the data indicated that unlike that occurred in pentobarbitol dogs, the neurogenic component was not significantly affected by this agent in morphine-chloralose dogs. However, it is also possible that any impairment of neurogenic tone by felodipine was to a degree, compensated by reflexogenic increases in the nerve discharge in morphine-chloralose dogs and such compensation was perhaps depressed under pentobarbital anesthesia. In similar studies in morphine-chloralose anesthetized dogs, Hydralazine (2.5 mg/kg, i.v.) a potent vasodilator, was as effective as felodipine (0.1 μmol/kg) in reducing vascular resistance in the denervated limb and it is less effective in the innervated limb. Thus it appears that vasodilator effects of hydralazine in the innervated leg were probably compensated by a reflex increase in the neurogenic activity.

These studies demonstrated that while felodipine is very effective in reducing vascular myogenic activity, its ability to interfere with vasoconstriction induced by neurogenic mechanisms may also contribute to overall reduction in the vascular resistance. Since this latter mechanism is most pronounced only in dogs under pentobarbital anesthesia, possible interaction with the barbiturates cannot be ruled out.

Jandhyala, etal (1976). Europ.J.Pharmacol. 36, 21-32.

COMPARISON OF THE VASODILATOR EFFECTS OF AMRINONE, VERAPAMIL AND SODIUM NITROPRUSSIDE

M.G. Martorana, I.W. Rodger & M. Shahid, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW

We have reported previously that despite elevations in cyclic-GMP levels, the vasodilator effects of amrinone are not related to alterations in cyclic nucleotides and have suggested that its mechanism of action may be more related to inhibition of Ca^{2+} influx (Martorana et al, 1982). In this study, therefore, we have compared the effects of amrinone with those of sodium nitroprusside and verapamil on tension responses and cyclic nucleotide levels in vascular smooth muscle.

Pulmonary artery strips taken from male NZ white rabbits were suspended in Krebs-Henseleit solution at 37°C and bubbled with 5% CO_2 in O_2. After equilibration the preparations were constricted using phenylephrine (1×10^{-5}M). Concentration-dependent relaxations of the constricted arteries were induced either by verapamil (1×10^{-6}M to 1×10^{-4}M) or sodium nitroprusside (1×10^{-8}M to 1×10^{-5}M). Cyclic nucleotide levels were measured at different levels of drug-induced relaxation using standard assay kits (Radiochemical Centre, Amersham). The results are summarised in Table 1.

Table 1 Effects of amrinone, verapamil and sodium nitroprusside on tension responses and cyclic nucleotide levels in phenylephrine-constricted pulmonary arteries ± s.e. mean (n > 5)

Drug	Concentration (mol/litre)	% Relaxation	c-AMP (pmol/mg)	c-GMP (pmol/mg)
Control				
Phenylephrine	1×10^{-5}	-	0.23 ± 0.01	0.059 ± 0.005
Verapamil	1×10^{-6}	15.4 ± 2.5	0.24 ± 0.04	0.036 ± 0.004
	1×10^{-5}	33.5 ± 2.3	0.25 ± 0.02	0.035 ± 0.004
	4×10^{-5}	54.9 ± 3.7	0.23 ± 0.02	0.041 ± 0.008
	1×10^{-4}	92.8 ± 1.7	0.21 ± 0.01	0.040 ± 0.009
Sodium Nitroprusside	1×10^{-8}	13.9 ± 2.4	0.26 ± 0.01	0.061 ± 0.007
	1×10^{-7}	53.1 ± 6.1	0.28 ± 0.01	0.079 ± 0.008*
	1×10^{-6}	64.2 ± 5.1	0.26 ± 0.02	0.12 ± 0.01 *
	4×10^{-6}	89.5 ± 1.7	0.25 ± 0.01	0.15 ± 0.01 *
	1×10^{-5}	100	0.22 ± 0.02	0.19 ± 0.02 *
Amrinone	1.1×10^{-5}	9.8 ± 0.8	0.25 ± 0.02	0.047 ± 0.01
	2.6×10^{-5}	23.8 ± 1.5	0.22 ± 0.02	0.096 ± 0.02
	6.5×10^{-5}	51.1 ± 1.8	0.21 ± 0.02	0.082 ± 0.02
	2.6×10^{-4}	74.0 ± 1.7	0.21 ± 0.04	0.081 ± 0.02
	7.0×10^{-4}	97.2 ± 0.9	0.23 ± 0.02	0.084 ± 0.01

* significantly different from control ($P < 0.05$)

Sodium nitroprusside caused concentration-dependent increases in the levels of cyclic-GMP (without affecting cyclic-AMP levels) which correlated well ($r = 0.94$) with the vasodilator responses. In contrast, verapamil reduced the levels of cyclic-GMP. There was, however, no correlation between the vasodilator responses to verapamil and the corresponding levels of either cyclic nucleotide. In Table 1 the data for amrinone are taken from Martorana et al (1982) and are shown for comparison.

It may be concluded that the vasodilator effect of sodium nitroprusside is consistent with a mechanism of action involving cyclic-GMP. The lack of correlation between effects on tension and cyclic nucleotide levels with verapamil (and amrinone) is probably a reflection of an inhibitory action on Ca^{2+} influx.

M.G.M. is supported by an Organon grant and M.S. by a S.E.R.C. scholarship.

The gift of amrinone from Sterling-Winthrop is gratefully acknowledged.

Martorana, M.G. et al (1982) Br. J. Pharmac. in press

ETHANOL REDUCES AORTIC CALCIUM CONTENT: ITS RELEVANCE TO ATHEROSCLEROSIS

I.J. Lloyd, R. Weston, T.P.L. Thomas, Introduced by M.J. Lewis.
Department of Pharmacology & Therapeutics, Welsh National School of Medicine, Heath Park, Cardiff.

It now seems clear that ethanol either in low to moderate doses (Hennekens et al, 1979)or even in high doses (Ramsey, 1979) protects against myocardial infarction and atherosclerosis. There is currently no explanation for this effect.

Amongst the earliest events in atherogenesis is a generalised increase in glycosaminoglycans in arterial tissue (Lindner, 1977), and an increase in the extracellular calcium content of arterial tissue the so-called "primary calcification" (Meyer, 1977). Manoeuvres that reduce the arterial content of calcium are thought to reduce the incidence and severity of atherosclerosis (Kramsch et al, 1981).

Pig aortic tissue was incubated in physiological salt solution at 37°C for 3 hours in the absence and in the presence of varying amounts of ethanol. The concentrations of ethanol were varied between 0.86 mmol/l (4 mg%) and 43 mmol/l (200 mg%). Following incubation the tissue was dried, digested and the calcium, sodium and potassium contents measured by flame spectrophotometry.

A reduction in calcium content of 7.4% ($p < 0.05$) was noted in tissue incubated in the presence of 0.86 mmol/l of ethanol. This effect was not maintained in the presence of higher concentrations, namely 5.4, 21.7 and 43.5 mmol/l. At the two highest concentrations used there was an apparent increase in the calcium content. No significant change was noted in the sodium and potassium contents of aortic tissue incubated in the presence of alcohol at any of the concentrations tested.

Most of the evidence linking ethanol with a reduction in atherosclerosis has been derived from epidemiological studies. There are also some studies employing animal models of atherosclerosis which also suggest that alcohol may offer protection against atherosclerosis. The above data provide a further and more direct link between the two. They show for the first time to the author's knowledge a direct effect of small concentrations of ethanol on arteries. Turlapaty et al (1979) showed that large amounts of ethanol (170-430 mM) affected the calcium content of arteries. Finally they add support for the suggestion that ethanol is only effective in low to moderate doses.

References

Hennekens CH et al (1979). J Amer Med Assoc 242: 1973-4

Ramsey LE (1979). Amer Heart J 98: 402-3.

Lindner J (1977). Advance in Eper Med Biol 82: 782-5.

Meyer WW (1977). Advance in Exper Med Biol 82: 786-792.

Kramsch DM et al (1981). Science 213: 1511-3.

Turlapaty PDMV et al (1979). Experimentia 35: 639-40.

ACUTE EFFECTS OF CYCLOTHIAZIDE, TRIAMTERENE AND THEIR ASSOCIATION ON ARTERIAL SMOOTH MUSCLE PERMEABILITY: IONIC FLUXES EX VIVO

A.M. Moura & M. Worcel, Centre de Recherches Roussel Uclaf, 93230 Romainville, France.

The mechanism of the antihypertensive effect of diuretics is not well understood. It has been suggested that some of these compounds may decrease vascular resistance through a direct or indirect relaxant action on smooth muscle. The aim of the present work is to determine if cyclothiazide (CCT), triamterene (T) and their association may have a primary effect on arterial smooth muscle membrane permeability to ions.

We have studied ^{22}Na and ^{86}Rb (as a marker for K) effluxes from rat tail arteries obtained from normotensive male Wistar rats (200g) (Garay, Moura et al., 1979 ; Moura & Worcel, 1982). In order to reduce to a minimum the longterm alterations in salt metabolism, the action of CCT and T was studied acutely. Isotope experiments were performed ex vivo, on arteries excised from rats sacrificed 2h after the oral administration of diuretics. Under these conditions, ^{86}Rb efflux is markedly increased by CCT in a dose-dependent manner. On the other hand, by itself T at the doses of 2,10 and 25 mg/kg does not affect ^{86}Rb efflux. Only when given in association with CCT, T suppresses the thiazide induced increase in ^{86}Rb efflux (control: 0.0100 ± 0.0004 min^{-1}, (n=8) ; CCT 0.2 mg/kg : $0.0128 + 0.0001$ min^{-1} (n=8), ($p < 0.01$) ; CCT 0.2 mg/kg + T 10 mg/kg : 0.0103 ± 0.0002 min^{-2}, (n=8), (N.S.) ; CCT 0.5 mg/kg : $0.0137 + 0.0006$ min^{-1}, (n=8), ($p < 0.01$) ; CCT 0.5 mg/kg + T 25 mg/kg : 0.0105 ± 0.0004 min^{-1}, (n=8), (N.S.). All these effects of CCT, T and the association CCT + T are observed even in the absence of a diuretic action, when the compounds are administered to binephrectomized rats.

^{22}Na effluxes are not affected by the oral administration of CCT and T to normotensive Wistar rats. But both diuretics reduce total ^{22}Na effluxes, when given 4h after the sc injection of 10 µg/kg of aldosterone (ALDO) (ALDO : 0.162 ± 0.005 min^{-1}, (n=8); ALDO + CCT 0.5 mg/kg : 0.139 ± 0.001 min^{-1}, (n=8) ($p < 0.01$) ALDO + T 25 mg/kg : $0.136 + 0.003$ min^{-1}, (n=8), ($p < 0.01$) ; ALDO + CCT 0.5 mg/kg + T 25 mg/kg : 0.135 ± 0.003 min^{-1}, (n=8), ($p < 0.01$). The actions of CTT and T on ^{22}Na effluxes disappear after nephrectomy. On the other hand, aldosterone pretreatment does not modify the ex vivo effect of CCT and T on ^{86}Rb effluxes.

In conclusion, the present results show that CCT and T may exert a primary effect on K permeability of arterial smooth muscle. In particular, the "potassium sparing" compound triamterene appears to suppress the enhancement in membrane permeability to Rb, induced by cyclothiazide. The effect of both diuretics on Na permeability is secondary to their urinary action.

Garay, R.P., Moura, A.M., Osborne-Pellegrin, M.J., Papadimitriou, A. & Worcel, M. (1979), J. Physiol. (Lond) 287, 213-229.
Moura, A.M. & Worcel, M. (1982) Cl. Sc. & Mol. Med. (in press).

CAPSAICIN SENSITIVE SUBSTANCE-P NERVES: ANTIDROMIC VASODILATION AND INCREASED VASCULAR PERMEABILITY IN THE NASAL MUCOSA

L. Lundblad [1], J.M. Lundberg[2] & A. Änggård[1], Department of Otorhinolaryngology[1], Karolinska Hospital and Department of Pharmacology[2], Karolinska Institute, S-104 01 Stockholm, Sweden.

In immunohistochemical studies using antibodies to Substance-P (SP) it was found that a population of trigeminal ganglion cells in rat, cat and man were SP-immunoreactive (SP-IR). SP-IR nerve endings were also found in the spinal trigeminal nucleus, around sphenopalatine ganglion cells and around blood vessels as well as under or within the epithelium of the nasal mucosa in all three species. Ligation and denervation experiments in the cat indicate that the SP-IR nerves in the sphenopalatine ggl and the nasal mucosa originate from the trigeminal ggl. Neonatal capsaicin treatment in rats caused a loss of SP-IR neurones in the trigeminal system innervating the brain stem, the sphenopalatine ganglion and the nasal mucosa.

Antidromic stimulation of the maxillary branches of the trigeminal nerve in cat caused a frequency dependent vasodilation in the nasal mucosa. In contrast to the parasympathetic nerve the trigeminal branches did not respond to low threshold stimulation (2V, 0.2 msec). Combined trigeminal and parasympathetic nerve stimulation caused an additative vasodilatory response. The trigeminal vasodilation was hexamethonium and atropine resistant while the parasympathetic response was blocked by hexamethonium. Local intraarterial infusion of capsaicin in doses (0.1 - 10 nmol/min) caused a prolonged vasodilation with a duration of up to one hour. Local intraarterial infusion of SP (0.1 - 10 pmol/min) caused a hexamethonium and atropine resistant vasodilation. Antidromic maxillary nerve stimulation in rats caused an increased vascular permeability as shown by extravasation of Evans blue in the nasal mucosa.

In conclusion, the nasal mucosa contains peripheral branches of capsaicin sensitive SP-IR neurones of trigeminal origin around blood vessels and within the epithelium indicating a local axon reflex arrangement with a subsequent vasodilation and increased vascular permeability. This is further substantiated by the potent vasodilatory effect of SP and the vasodilation observed during antidromic trigeminal nerve stimulation. Activation of local sensory SP neurones may be of pathophysiological importance for patients with vasomotor and allergic rhinitis.

VARIABLE INHIBITORY EFFECTS OF FELODIPINE ON NORADRENALINE RESPONSES OF VASCULAR SMOOTH MUSCLES

B. Ljung, A. Kjellstedt, S.-L. Boström and M. Hallbäck-Nordlander, Dept of Pharmacology and Biochemistry, AB Hässle, S-431 83 Mölndal, Sweden

Felodipine (4-(2,3-dichlorophenyl-1,4-dihydro-2,6-dimethyl-3-ethoxycarbonyl-5-methoxycarbonyl-pyridine)) is a new antihypertensive vasodilating dihydropyridine which exerts its clinical effects in nM conc of free drug in plasma. In the isolated portal vein felodipine, in nM range, inhibits phasic contractile activity and contractures. In 1000-fold higher conc significant Ca^{2+} influx inhibition occurs (cf. Boström et al, 1981).

In these experiments the inhibitory effect of felodipine on noradrenaline (NA) induced responses have been studied in isolated portal veins of the rat and the rabbit and in helical strips of the thoracic aorta from these animals. Cumulative NA conc effect curves were obtained at 1 h intervals. The response patterns were compared to those obtained when Ca^{2+} of the Krebs-bicarbonate solution was lowered from 2.5 to 0.6 mM. The results are summarized in Table 1.

Table 1 Effects of felodipine (1 h exposure) and reduced $[Ca^{2+}]$ on NA sensitivity (pED_{50} (M)) and responsiveness (max, % of control). Mean ± s.e.mean; n = 6.

	Control	$[Ca^{2+}]$ = 0.6 µM		Felodipine			
				1 nM		1 µM	
	pED_{50}	pED_{50}	Max	pED_{50}	Max	pED_{50}	Max
V. portae rat	6.76±.06	6.53±.09	84±6.7	7.16±.08	38±5.0	5.87±.07	11±1.6
V. portae rabbit	6.66±.13	6.59±.17	67±5.2	6.16±.07	92±5.8	5.74±.12	58±5.5
Aorta rat	9.09±.17	8.29±.20	94±2.8	7.52±.13	76±3.6	7.13±.12	52±5.3
Aorta rabbit	7.57±.07	7.49±.09	111±4.6	7.27±.04	105±3.8	7.00±.10	99±5.4

In rat portal vein the low dose of felodipine (1 nM) suppressed all responses, but caused no rightward shift of the conc-effect curve, whereas 1 µM eliminated responses to NA in low and moderate conc. In rabbit portal vein 1 nM felodipine caused a rightward shift but no significant reduction of the maximum response. The high dose caused further shift and attenuation of max response. In the rat aorta, felodipine induced marked shifts and dose-dependent suppressions of the maxima. However, in the rabbit aorta, a small shift only was obtained. Reduction in $[Ca^{2+}]$ essentially caused rightward shifts, but no effect in the rabbit aorta. Two "calcium antagonists", verapamil and diltiazem were used as reference in equipotent conc (0.1 µM, 0.1 mM). Qualitatively the same pattern was observed except that both drugs markedly shifted NA curve also of the rabbit aorta when given in the highest conc.

It is concluded that felodipine in a "therapeutic conc" inhibits phasically active vascular muscle. It is virtually ineffective in muscle from some large arteries. It is suggested that felodipine, and so called calcium antagonists, exert their influence both at the membrane and at intracellular sites.

Boström, S.-L. et al (1981) Nature 292, 777-778