THE ATYPICAL ACTIONS OF TRIMIPRAMINE ON THE TELENCEPHALIC PROJECTION OF THE RAT LOCUS COERULEUS

K. Hauser, R.S.G. Jones and H.R. Olpe
Biology Research Laboratories, Pharmaceuticals Division, CIBA-GEIGY Ltd., 4002 Basel.

Trimipramine is a clinically well established antidepressant lacking noradrenaline- and serotonin-uptake inhibiting properties. On the other hand it shares with several other antidepressants pronounced antimuscarinic and antihistaminic properties and it blocks dopamine-uptake in corpus striatum. Despite its lack of effect on noradrenaline-uptake, we recently found that the drug does affect the cortical noradrenergic projection of the locus coeruleus after both acute and chronic treatment.

Experiments were performed on male rats (RAI (SPF) , 250-320 g) anaesthetized with chloral hydrate. Conventional electrophysiological and stereotaxic techniques were used to record from noradrenergic neurons of locus coeruleus and to administer noradrenaline micro-iontophoretically to cingulate cortical neurons (NA : 0.5 M, pH 3.5). Acute injections of trimipramine (30 mg/kg i.p.) strongly increased the spontaneous discharge rate of the 10 presumed noradrenergic neurons recorded in locus coeruleus. The mean increase in firing rate determined 30 min following the drug injection was 76.5 $\pm$ 15 %. In the case of one neuron which was kept for two and a half hour, the activity was still increased two hours after the injection of the drug. A single intraperitoneal injection of trimipramine significantly reduced the depressant action of microiontophoretically applied noradrenaline on cingulate cortical neurons (n = 65 both prior and after the drug injection) recorded one to three hours following the drug administration. There was a 30% reduction in the response to 30 nA of noradrenaline and the reponse to 90 nA of noradrenaline was reduced by approximately 20 percent.Acute injections of desipramine (10 mg/kg i.p.) or of the vehicle (0.9% NaCl) potentiated the action of noradrenaline or had no effect respectively. Single daily injections of trimipramine (30 mg/kg) given for 4 weeks significantly increased the cingulate neurons sensitivity to micro-iontophoretically administered noradrenaline. With the lowest "dose" of noradrenaline (10 nA, 60 sec) there was a 52 % increase in the maximal firing depression of cingulate cortical neurons ($p < 0.01$).

Typical tricyclic antidepressants exert effects on the cortical projection of the locus coeruleus opposite to the ones found with trimipramine. However, it is not clear whether the global effect of trimipramine and classic antidepressants on noradrenergic transmission is different. Their effect remains to be elucidated. In the case of trimipramine, the adrenolytic action in the cortex, coupled with the activating effect on locus coeruleus may cancel each other out to a large extent.

The fact that the drug induces a super- rather than a subsensitivity to noradrenaline after long-term treatment indicates that the latter effect is not an absolute prerequisite for antidepressant efficacy.

EFFECTS OF DS 103-282 ON SOUND-INDUCED SEIZURES AND ON NMDLA INDUCED EPILEPSY IN MICE

M.J. Croucher, G.B. De Sarro & B.S. Meldrum, Department of Neurology, Institute of Psychiatry, De Crespigny Park, London, SE5 8AF.

Aspartic and glutamic acids have an excitatory action at spinal and supraspinal sites (Watkins & Evans, 1981). Compounds preferentially blocking excitation due to N-methyl-D-aspartic acid (NMDA) prevent sound-induced seizures in mice (Croucher et al, 1982) and protect against NMDA induced seizures in mice (Czuczwar & Meldrum, 1982). 5-Chloro-4-(2-imidazolin-2-yl-amino-2,1,3-benzothiodazole (DS 103-282, tizanidine) is a new muscle relaxant (Sayers et al, 1980), which in preliminary clinical trials is effective in the treatment of spasticity (Smolenski et al, 1981). DS 103-282 depresses polysynaptic excitation on spinal cord motoneurones, either by postsynaptic reduction in effectiveness of excitatory transmitter (Curtis et al, 1983) and/or by presynaptic inhibition of release of excitatory neurotransmitter (Davies et al, 1983). DS 103-282 also possesses anticonvulsant properties in mice (Sayers et al, 1980). The aim of the present experiments was to assess whether DS 103-282 was able to attenuate or suppress sound-induced seizures in DBA/2 mice and NMDLA induced seizures in albino Swiss mice. After exposure to auditory stimulation the incidence and timing of successive seizure phases (wild running, clonus, tonus and respiratory arrest) were recorded as previously described (Croucher et al, 1982). Animals injected with NMDLA were observed for 30 min and the occurrence and timing of clonic seizures evaluated (Czuczwar & Meldrum, 1982). DS 103-282 was administered intraperitoneally (0.1 ml/10g) 30 min before auditory stimulation or NMDLA administration. ED_{50} was calculated by log-probit analysis.

Administration of DS 103-282, 0.33, 0.66, 1, 1.5, 2.15 and 3.3 mg/kg, i.p., produced dose-dependent protection against wild running, clonic, tonic and respiratory arrest phases in DBA/2 mice (Table 1).

Table 1 Anticonvulsant activity of DS 103-282 in DBA/2 mice.

		% Response				
		WR	Clonic	Tonic	RA	n
Control		100	100	100	43	23
DS 103-282	0.33 mg/kg	100	100	80	30	10
"	0.66 " "	80	50*	40*	0**	10
"	1 " "	75	44**	25**	19	16
"	1.5 " "	29*	14**	0**	0**	7
"	2.15 " "	27**	9**	0**	0**	11
"	3.3 " "	6**	0**	0**	0**	16

* $p < 0.05$ ** $p < 0.01$

The protective effect of DS 103-282, 1.5 mg/kg, i.p., was maximal after 30 min and was absent after 2 h. DS 103-282, 3.3 and 10 mg/kg, did not significantly increase the NMDLA ED_{50} for clonic convulsions. In conclusion, the present results confirm an anticonvulsant effect of DS 103-282 but do not establish that postsynaptic blockade of excitatory transmission is responsible for this.

Croucher, M.J. et al (1982) Sci., 216, 899-901.
Curtis, D.R. et al (1983) Br. J. Pharmac., 79, 9-11.
Czuczwar, S.J. & Meldrum, B.S. (1982) Eur. J. Pharmacol., 83, 335-338.
Davies, J. et al (1983) Br. J. Pharmac. 78, 2P.
Sayers, A.C. et al (1980) Drug Res. 30, 793-803.
Smolenski, C. et al (1981) Curr. Med. Res. Opin. 7, 374-383.
Watkins, J.C. & Evans, R.H. (1981) Ann. Rev. Pharmacol. Toxicol. 21, 165-204.

RELATIVE ANTICONVULSANT ACTIVITIES OF TWO NEW DIPEPTIDE 'NMDA RECEPTOR' ANTAGONISTS

M.J. Croucher[1], A.W. Jones[2], B.S. Meldrum[1] & J.C. Watkins[3], [1]University Department of Neurology, Institute of Psychiatry, Denmark Hill, London SE5 8AF and Departments of [2]Pharmacology & [3]Physiology, The Medical School, University of Bristol, Bristol, BS8 1TD.

Receptors sensitive to excitatory amino acids have recently been classified into 3 types. Those selectively activated by N-methyl-D-aspartic acid (NMDA), those stimulated by kainic acid, and those preferentially activated by quisqualic acid (Watkins & Evans, 1981). A group of potent and specific 'NMDA receptor' antagonists (ω-phosphonic α-carboxylic amino acids) have been shown to possess anticonvulsant activity in a range of animal models of epilepsy (Croucher et al, 1982; Czuczwar & Meldrum, 1982; Meldrum et al, 1983). We report here the relative anticonvulsant actions of 2 new phosphonate dipeptides, γ-D-glutamyl-aminomethylphosphonate (GLU-AMP) and β-D-aspartyl-aminomethylphosphonate (ASP-AMP) in a rodent model of epilepsy following both central and systemic administration.

All experiments were performed with DBA/2 mice. Following exposure to auditory stimulation these animals exhibit a sequential seizure response consisting of an initial wild running phase, generalised myoclonus, tonic flexion and extension and frequently respiratory arrest (Croucher et al, 1982). The antagonists were administered either intracerebroventricularly (in 10 μl phosphate buffer, under light ether anaesthesia) or intraperitoneally (in 100 μl saline) 45 min prior to testing. Log dose-response curves were constructed and relative anticonvulsant activities estimated using the method of Spearman-Karber.

Following central administration the 2 phosphonate dipeptides showed similar or greater anticonvulsant activity than the 2 most potent NMDA antagonists known at present (Table 1). The relative anticonvulsant activities of the peptides are consistent with their relative antagonist potencies at central NMDA receptors (Jones, Smith & Watkins, unpublished) suggesting the former action is mediated via inhibition of NMDA receptor-mediated excitation. The peptides are also active following systemic administration (Table 1). This activity may be enhanced by the synthesis of prodrugs of these antagonists.

Table 1 Relative anticonvulsant activities of 'NMDA receptor' antagonsits (2APH, 2-amino-7-phosphonoheptanoic acid; 2APV, 2-amino-5-phosphono-valeric acid).

	Relative Potency Ratio (ED_{50} 2APH/ED_{50} x)					
	icv			ip		
Antagonist	WR	Clonic	Tonic	WR	Clonic	Tonic
2APH	1	1	1	1	1	1
2APV	0.09	0.07	0.05	0.11	0.10	0.10
GLU-AMP	0.39	0.83	0.40	0.28	0.15	0.24
ASP-AMP	1.32	2.50	1.43	0.58	0.23	0.24

Watkins, J.C. & Evans, R.H. (1981) Ann. Rev. Pharmacol. Toxicol. 21, 165-204.
Croucher, M.J. et al (1982) Sci. 216, 899-901.
Czuczwar, S.J. & Meldrum, B.S. (1982) Eur. J. Pharmacol. 83, 335-338.
Meldrum, B.S. et al (1983) Neurosci. Lett. 39, 101-104.

THE EFFECTS OF DL-α-MONOFLUOROMETHYLDOPA ON RAT PINEAL, HYPOTHALAMIC AND CORTICAL FUNCTION

Amanda J. Bradbury, R.E. Giddins and J.A. Smith, (introduced by Dr. B. Costall), Postgraduate School of Studies in Pharmaceutical Chemistry, University of Bradford, Bradford BD7 1DP.

In the assessment of the physiological role of the mammalian pineal gland it would be advantageous to be able to inhibit pineal melatonin (MT) synthesis without affecting amine synthesis in the central nervous system. We make use of the fact that the pineal gland is outside the 'blood brain barrier' and report the effect of DL-α-monofluoromethyldopa (MFMD), a potent irreversible inhibitor of aromatic L-amino acid decarboxylase, on pineal, hypothalamic and cortical indoleamine content both in the daytime when MT synthesis is low and at night when MT production is high.

Groups of male Sprague-Dawley rats were injected with 10mg kg^{-1} i.p. MFMD, a dose which has been shown to maximally inhibit pineal 5-hydroxytryptamine (5-HT) content (Smith et al unpublished data), 4 or 7h before killing by decapitation at 6h and 20h after lights on. (Animals were housed in a light:dark schedule of 14h:10h). Blood was collected from each animal at decapitation and assayed for serum MT, by the radioimmune assay of Ho and Smith (1982). The pineal gland, hypothalamus and cortex were dissected out and immediately homogenised in 0.2M perchloric acid. After centrifugation the levels of the indoleamines from each brain area were simultaneously assessed by high-performance liquid chromatography with electrochemical detection using a reverse phase system. (C18 hypersil ODS with 6% methanol in phosphate/citrate buffer pH 5.0 at 0.6v). The effect of 10 mg kg^{-1} i.p. MFMD, 4h and 7h pretreatment on the decarboxylase enzyme activity 6h after lights on was assessed using a modified radioenzymatic method of Snyder and Axelrod (1964).

Following 4 or 7h pretreatment of MFMD in the day, 6h after lights on, pineal 5-hydroxytryptophan (5-HTP) was elevated about 7 fold ($p < 0.01$) whilst 5-HT was reduced by 85% ($p < 0.01$), although 5-hydroxyindole acetic acid (5-HIAA) was significantly reduced only after the 7h pretreatment. Simultaneously hypothalamic 5-HT and 5-HIAA were not altered following the 4h pretreatment (but after 7h pretreatment the 5-HT was slightly but significantly reduced). 5-HTP was undetectable as were cortical 5-HTP, 5-HT and 5-HIAA. Serum MT which is produced exclusively in the pineal from 5-HT was not significantly reduced following the 4h pretreatment but did reach significance with the 7h pretreatment. The decarboxylase enzyme activity was reduced in all the brain areas examined following 4h or 7h pretreatment ($p < 0.05$).

4h pretreatment of MFMD before killing at 6h after lights off significantly increased 5-HTP and decreased 5-HT, 5-HIAA in the pineal. The changes were still significant but to a lesser degree with a 7h pretreatment of the drug. Hypothalamic or cortical indoleamines were unaffected by either pretreatment, the exception being cortical 5-HT which was significantly elevated with the 4h pretreatment. Serum MT, in the same animals, was unchanged after 4h pretreatment but showed a significant decrease following the 7h pretreatment.

Although MFMD appeared to penetrate the 'blood brain barrier' and inhibit decarboxylase activity in all three brain areas, only pineal 5-HT was significantly reduced both in the day and at night. Serum MT was also significantly reduced indicating that MFMD is a possible candidate for a chemical lesion of the pineal gland.

This work was supported by the Wellcome Trust and the M.R.C.

HO, A.K. and Smith J.A. (1982) Biochem. Pharmacol. 31,2251-55.
Snyder, H.S. and Axelrod, J. (1964) Biochem. Pharmacol. 13, 805-806.

IS HYPERLOCOMOTION INDUCED BY THE 5-HT_1 AGONIST RU24969 MEDIATED BY THE 5-HT_1 RECEPTOR

M.D. Tricklebank, Centre de Recherche Merrell International, 16 rue d'Ankara, 67084 Strasbourg Cedex, France.

On the basis of ligand binding studies, 5-methoxy-3-(1,2,3,6-tetrahydro-pyridin-4-yl)IH indole (RU 24969) has been proposed as a selective and highly potent agonist at the 5-HT_1 receptor (Hunt et al, 1981). Hyperlocomotion is the prominent feature of the behavioural response to the drug and has been suggested to be a functional correlate of activation at 5-HT_1 sites (Gardner and Guy, 1983). The present investigation was designed to explore further the mechanism of action of RU 24969 in producing hyperlocomotion and, in particular, to provide additional evidence for an involvment of the 5-HT_1 receptor in the response.

Male Sprague-Dawley rats (6-9 per group) were allowed 5 min in an observation box (1 animal per cage) before s.c. injection of RU 24969. Observations began 3 min after injection and ambulation was scored as previously described (Tricklebank, 1984). Antagonists were injected s.c. 30 min before RU 24969.

Hyperlocomotion was induced dose-dependently by RU 24969 at doses between 1 and 5 mg/kg. The effects of a submaximal dose (2.25 mg/kg), which increased ambulation approximately 3 fold, were reduced dose-dependently by reserpine (0.25-1 mg/kg, s.c., 18 h before hand), by the α_1 adrenoceptor antagonist, prazosin (0.5-1 mg/kg) and by the dopamine D_2 receptor antagonist, sulpiride (5-30 mg/kg), consistent with catecholaminergic neurons being a necessary component of the response.

Somewhat paradoxically, the 5-HT_2 receptor antagonists, ketanserin (1-2.5 mg/kg) and mesulergine (0.25-1 mg/kg), also inhibited hyperlocomotion. However, in view of the proposed selectivity of RU 24969 for the 5-HT_1 site, and the potent affinities of ketanserin and mesulergine for α_1 and/or dopamine recognition sites (Leysen et al, 1981; Closse, 1983), it seems unlikely that 5-HT_2 receptors are directly involved.

In order to determine the involvement of 5-HT_1 receptors, the effects of the (-) isomers of propranolol and pindolol, which show high affinity for the 5-HT_1 recognition site (Nahorski and Willcocks, 1983), were examined. Hyperlocomotion after RU 24969 was inhibited dose-dependently by (-) propranolol (4-16 mg/kg) and (-) pindolol (2-4 mg/kg) consistent with the findings of Gardner and Guy (1983) using racemic propranolol. However, similar inhibition was also seen following identical doses of the (+) isomers of both drugs, indicating a lack of stereoselectivity for the antagonism.

In general, these results suggest that hyperlocomotion following RU 24969 results from activation of a catecholaminergic pathway(s). However, the question of whether the 5-HT_1 receptor is involved must remain open since although propanolol and pindolol inhibit the response they do so non-stereoselectively, a finding inconsistent with this hypothesis.

Closse, A. (1983) Life Sci. 32, 2485-2495
Gardner, C.R. and Guy, A.P. (1983) Brit. J. Pharmacol. 78, 96P
Hunt, P. et al (1981) Proc. 8th Int. Congr. Pharmaol. Tokyo
Leysen, J.E. et al (1981) Life Sci. 28, 1015-1022
Nahorski, S.R. and Willcocks, A.L. (1983) Brit. J. Pharmacol. 78, 107P
Tricklebank, M.D. (1984) This meeting

AUTORADIOGRAPHIC LOCALIZATION OF SEROTONIN RECEPTORS IN MOUSE BRAIN

S.Patel & P.Slater, Department of Physiology, Medical School, University of Manchester, Manchester M13 9PT

Brain contains two serotonin (5-HT) receptor subtypes. [^{3}H]5-HT labels selectively the 5-HT_1 receptor (Peroutka et al,1981). Ketanserin is a selective antagonist for the 5-HT_2 receptor, and [^{3}H]ketanserin is the most suitable ligand for binding to this receptor subtype (Leysen et al,1982). The distribution of the 5-HT_1 receptor has been determined in rat brain using quantitative autoradiography (Biegon et al, 1982),whereas the precise distribution of the 5-HT_2 receptor is unknown. We have used autoradiography to determine and compare the detailed distribution of the two serotonin receptors in mouse brain.

Slide-mounted, cryostat-cut sections of brain from female mice were incubated for 30 min at 20°C in 0.17 M Tris-HCl buffer (pH 7.6) containing 4 mM $CaCl_2$. For 5-HT_1 receptors, each section was covered with 0.1 ml of Tris buffer containing 2 nM [^{3}H]5-HT (20 Ci/mmol), 4 mM $CaCl_2$, 10 µM pargyline, 0.01% ascorbate and 1 µM clomipramine in the presence or absence of 1 µM cold 5-HT. For 5-HT_2 receptors, each section was covered with 0.1 ml of Tris buffer containing 2 nM [^{3}H]ketanserin (17.9 Ci/mmol) and 4 mM $CaCl_2$ in the presence or absence of 1 µM ketanserin tartrate. After incubation for 1h at 20°C, the sections were washed twice (3 min) in cold (5°C) buffer, dipped briefly in cold water and dried. Sections were exposed to tritium-sensitive film for 18-23 weeks at 4°C.

5-HT_1 receptors were highly concentrated in the substantia nigra (zona reticulata) and the globus pallidus. Other areas with a high density of 5-HT_1 sites were the lateral geniculate nucleus, subthalamic nucleus and lateral preoptic area. The entopeduncular nucleus had a moderate density of 5-HT_1 receptors, as did the hippocampus and dentate gyrus in which the receptors were distributed evenly. There were few 5-HT_1 sites in the striatum, cerebral cortex and the substantia nigra zona compacta.

5-HT_2 receptors were spread evenly throughout many brain areas. Exceptions were the striatum and nucleus accumbens which had a very high density of sites. By contrast, the globus pallidus and entopeduncular nucleus had few 5-HT_2 receptors. The receptors were also concentrated in the locus coeruleus and the dorsal raphe nucleus. The medial raphe, lateral hypothalamus, interpeduncular nucleus, hypothalamus and substantia nigra had moderate levels of 5-HT_2 sites; the zona compacta having more than the zona reticulata.

The findings show that mouse brain has a heterogenous distribution of serotonin receptor subtypes. The density of the two receptors differ widely in some areas, implying different functional roles for serotonin.

[^{3}H]Ketanserin was provided by Janssen Pharmaceutical Ltd. S.P. is supported by S.E.R.C.

Biegon,A. et al (1982) Brain Res. 242, 197
Leysen, J.E. et al (1982) Molec.Pharmac. 21, 301
Peroutka, S.J. et al (1981) Science 212, 827

CORTICAL LESIONS MODIFY MYOCLONUS INDUCED BY THE FOCAL INJECTION OF PICROTOXIN INTO STRIATUM OR CORTEX

P. Jenner, N. Leigh, C.D. Marsden, S. Muscatt & C. Reavill, University Department of Neurology, Institute of Psychiatry & The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London, SE5, U.K.

Controversy exists as to whether myoclonus produced by focal injection of picrotoxin into the striatum is initiated from the striatum or from the overlying motor cortex (Tarsy et al, 1978; Palfreyman et al, 1980; Crossman et al, 1983). We now report the effect of cortical and striatal lesions on myoclonus initiated by the focal injection of picrotoxin into these areas.

Female Wistar rats (151-175 g) either received a unilateral kainic acid (1 ug in 0.5 ul 0.9% saline) lesion of the striatum or unilateral undercutting of the cortex overlying the striatum 7 days prior to behavioural examination. At the same time all animals were fitted with bilateral guide cannulae (A 8.5; L 2.5) to allow injection of picrotoxin (1 ug in 1 ul 0.9% saline)into the striatum (V 4.7) or overlying cortex (V 1.0).

Unilateral kainic acid lesions of the striatum reduced the incidence, duration and frequency of myoclonus induced by injection of picrotoxin into the striatum compared to the intact forebrain (Table 1). Onset time and intensity of myoclonus were unaffected. In most animals the lesion spread to cause some cortical damage. Kainic acid lesions of the striatum did not alter the incidence, onset time, duration or intensity of myoclonus produced by focal injection of picrotoxin into the overlying cortex. The frequency of myoclonus, however, was reduced. Following unilateral undercutting of the cortex only 2 of 8 animals exhibited myoclonus following intrastriatal administration of picrotoxin. In these 2 animals the onset of myoclonus was abnormally delayed and the duration and intensity of myoclonus markedly reduced. Focal injection of picrotoxin into the undercut area of cortex did not produce myoclonus in any animals.

Table 1 Effect of cortical and striatal lesions on picrotoxin-induced myoclonus

Lesion and injection site		Incidence	Onset time (min)	Duration (min)	Frequency (jerks/min)	Intensity (0-4)
Striatal lesions						
Striatum	I	8/12	7.0±1.0	58.9±5.0*	35.0±3.0*	2.1±0.3
	C	12/12	5.8±0.5	78.3±5.0	54.3±3.9	2.1±0.2
Cortex	I	10/10	5.7±1.9	64.7±7.5	30.9±4.3*	1.7±0.3
	C	10/10	2.0±0.3	87.2±8.1	43.2±2.5	1.8±0.2
Cortical lesions						
Striatum	I	2/8	30.5	12.5	30	0.5
	C	8/8	4.3±0.6	67.9±3.7	46.0±4.3	2.3±0.3
Cortex	I	0/8	-	0*	0*	0*
	C	8/8	2.1±0.1	71.5±7.0	45.3±3.4	2.6±0.3

* $p < 0.05$ compared to intact contralateral forebrain. I = ipsilateral to lesion; C = contralateral to lesion.

The data supports the hypothesis that picrotoxin-induced myoclonus is critically dependent on cortical events.

Crossman, A.R. et al (1983) Galway Meeting BPS
Palfreyman, M.G. et al (1980) Brain Res. Bull. 5, 613
Tarsy, D. et al (1978) Brain 101, 143

SELECTIVE EFFECTS OF GABA ON PUTATIVE SUBTYPES OF BENZODIAZEPINE RECEPTORS IN RAT CEREBELLAR AND HIPPOCAMPAL MEMBRANES

R. Mitchell and L. Wilson, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ (Introduced by B.L. Ginsborg)

The existence of interactive mechanisms between GABA receptors and benzodiazepine binding sites is demonstrated by the increase in affinity of benzodiazepine binding induced by GABA (e.g. Martin and Candy, 1978). Subdivision of benzodiazepine binding sites into BZ_1 and BZ_2 receptors has been proposed by Braestrup and Nielsen (1981), largely on the basis of selective affinities for β-carboline derivatives. It is unclear however, whether both types of site are linked to GABA receptor mechanisms.

Cerebellum and hippocampus were removed from male Wistar rats (150-250g) and stored frozen at -20°C. Tissue was homogenised in 100 volumes of ice-cold Tris HCl buffer (25mM, pH 7.1, containing 1mM EDTA) using an Ystral high frequency homogeniser. Samples were centrifuged at 48,000g for 10 minutes and washing of the membrane pellet repeated a further three times. Equilibrium binding assays were carried out using [^{3}H] flunitrazepam ([^{3}H]FNM), 0.25nM and membranes equivalent to 5mg tissue (≃ 400μg protein) in a total assay volume of 2ml. After incubation at 0°C for 90 min, samples were filtered through GF/B filters and washed with three X 5ml of ice-cold buffer. Specific binding was defined with 0.2μM clonazepam.

The displacement of bound [^{3}H]FNM by ethyl β-carboline-3-carboxylate (ECC) was compared in the two regions. Hofstee plots indicated a single component in cerebellar membranes but good fit to a two component model in hippocampus. However, as reported previously, under different conditions, (Mitchell and Wilson, 1983), the affinities of both of the components in hippocampus (0.29 ± 0.06 and 7.45 ± 0.63nM) were significantly different from that in cerebellum (0.91 ± 0.11nM), $p < 0.001$ in each case, $n = 3$), suggesting that a simple BZ_1/BZ_2 classification may be inappropriate. GABA (10nM - 100μM) had no significant effect on the binding of [^{3}H]ECC in either region at ligand concentrations from 0.5 to 20nM, but caused concentration-dependent increases in [^{3}H] FNM binding. The potency of this effect of GABA was significantly greater in cerebellum (Km 0.23 ± 0.06μM) than in hippocampus (Km 1.64 ± 0.19 μM) ($p < 0.001$, $n = 4$), suggesting that BZ receptor subtypes may interact differentially with GABA receptors. To investigate the effect of GABA on each component, experiments were carried out with concentrations of ECC which gave partially selective displacement from the subtypes of receptor. In hippocampal membranes, Hofstee plots indicated that [^{3}H]FNM displaceable by 1.0nM ECC was ≃ 70% from the high affinity type receptor, whilst that further displaceable by 20nM ECC was ≃ 85% low affinity type. 30μM GABA produced a 58.7 ± 3.1% increase in total specific [^{3}H] FNM binding, whilst that fraction displaced by 1.0nM ECC showed only a minor increase of 26.3 ± 4.4% ($p < 0.05$ against total). The fraction displaced by 20 but not 1.0nM ECC, however, showed a 63.9 ± 3.8% increase ($p < 0.05$ against 1nM-sensitive), ($n = 4$ in each case). In cerebellar membranes, the corresponding fractions of [^{3}H]FNM binding all showed very similar increases of 75.0 ± 5.3% ; 70.8 ± 5.8% and 76.1 ± 4.4% respectively ($n = 4$). This suggests that whilst the cerebellar BZ receptor and the low affinity hippocampal receptor interact prominently with GABA receptor mechanisms, the high affinity hippocampal receptor shows little or no such interaction.

Braestrup, C. and Nielsen, M. (1981), J.Neurochem. 37, 333.
Martin, I.L. and Candy, J.M. (1978), Neuropharmacol. 17, 993.
Mitchell, R. and Wilson (1983), submitted to Eur. J. Pharmacol.

L.W. was supported in part by S.H.H.D.

GLUCOCORTICOID-INDUCED HYPERTENSION IN RATS : EX VIVO EFFECTS OF RU 38486 ON ALTERED IONIC FLUXES FROM VASCULAR SMOOTH MUSCLE

L. Eloi[2], J.P. Grünfeld[2], A.M. Moura[1], & M. Worcel[1]. 1. Centre de Recherches Roussel-Uclaf, BP.9, F93230 Romainville. 2. Inserm U.90, Hôspital Necker, F75015 Paris, France.

We studied the action of the antiglucocorticoid RU 38486 [17β-hydroxy-11β-(4-dimethylaminophenyl) 17α-(1-propynyl)estra-4,9-dien-3one] on arterial hypertension induced by the specific glucocorticoid receptor agonist RU 26988 [11β, 17β-dihydroxy-17α-(1-propynyl)androesta-1,4,6 trien-3-one]. The administration of RU 26988, at 20mg/kg/day p.o. during 3 days, results in a rapid rise in BP [controls : 109±5 mmHg (n=10) ; RU 26988 : 142±6 mmHg (n=10)]. When administered in association with RU 26988,the antagonist RU 38486,at 100mg/kg/day po, prevents glucocorticoid induced hypertension [RU 26988+RU 38486: 119±5 mmHg (n=10)]. We have studied, ex vivo, ^{22}Na and ^{86}Rb effluxes from rat tail arteries of normotensive, hypertensive and treated rats. Using a superfusion technique (Garay, Moura et al., 1979), we have observed that the hypertensive effect of RU 26988 is associated with an increase in ^{22}Na efflux rate [controls : 0.135±0.003 min^{-1} (n=10) ; RU 26988 : 0.155±0.004 min^{-1} (n=10) $p < 0.001$] which is due exclusively to an increase in the ouabain sensitive fraction [controls : 0.035±0.003 min^{-1} (n=10) ; RU 26988 : 0.059±0.003 min^{-1} (n=10) $p < 0.001$]. The glucocorticoid antagonist RU 38486 suppresses the glucocorticoid induced increase in ^{22}Na efflux rate [RU 26988+RU 38486 : 0.127±0.003 min^{-1} (n=10) $p < 0.001$] as well as ouabain sensitive fraction [RU 26988+RU 38486 : 0.038±0.002 min^{-1} (n=10) $p < 0.05$]. Similarly, RU 26988 treatment increases ^{86}Rb efflux rate [controls :0.0103±0.0001 min^{-1} (n=10); RU 26988 :0.0138±0.004 min^{-1} (n=10) $p < 0.001$] which appears reduced by antiglucocorticoid treatment [RU 26988+RU 38486 : 0.0111±0.0002 min^{-1} (n=10) $p < 0.001$].

In conclusion, the present results show that glucocorticoid induced hypertension as well as the associated changes in ionic permeabilities of arterial smooth muscle are blocked by the antiglucocorticoid RU 38486.

Garay RP, Moura AM, Osborne-Pellegrin MJ, Papadimitriou A & Worcel M. (1979) J. Physiol. 287, 213-229.

OCTOPAMINE ACTIONS AND ITS INTERACTIONS WITH NORADRENALINE ON THE RAT VAS DEFERENS

M.A.K. Lafi & L.D. Leake, Department of Biological Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth PO1 2DY.

Octopamine (OA) is a normal constituent of the nervous system (Axelrod & Saavedra, 1977) but its physiological function is not clear. In some invertebrates it is likely to be a neurotransmitter (Robertson & Jurio, 1977) while in vertebrates it has been traditionally called a "false transmitter". OA is known to co-exist with noradrenaline (NA) in central and peripheral neurones and may be co-released with it. Certainly specific OA receptors exist in the mammalian brain (Dao & Walker, 1980) and OA has been shown to potentiate NA action centrally (Jones, 1982). Jones suggests a neuromodulatory effect in the CNS. We have investigated the actions of OA and its interactions with NA and sympathetic stimulation in the rat peripheral nervous system.

Vasa deferentia from Sprague-Dawley rats (200-300g) were set up in Mg^{2+}-free Tyrodes. Transmural stimulation was effected using parallel platinum-wire electrodes and a Grass SD9 stimulator modified to deliver trains of pulses (2ms, 2-8Hz, 4-8 pulses) at 15s intervals.

Exogenous OA and NA produced concentration-dependent contractions of the vas deferens, NA being some ten times more potent than OA. Contractions induced by OA showed two components: phasic, rhythmic activity superimposed on and partly masked by a tonic increase in tension. The tonic contractions were inhibited by uptake blockers (DM1, guanethidine), reserpine ($5mg.kg^{-1}$ i.p. 20h before experiment), β-blockers (DC1, propranalol, H35/25) and low doses ($10^{-8}M$) of α-blockers (phentolamine and prazosin). They were potentiated by lowering bath temperature to 30° or 25°C, or by combined treatment with reserpine and pargyline (an MAO inhibitor) followed by exposure to NA. Phasic OA-induced contractions were inhibited by low temperature (25°C) but unmasked by uptake blockers, reserpine pretreatment, β-blockers and low doses of α-blockers, although they were inhibited by higher doses. These experiments suggest the tonic action of OA is presynaptic while the phasic is postsynaptic.

OA at low concentration ($5x10^{-6}M$) potentiated the response to NA. At the same concentration it inhibited contractions induced by transmural stimulation. This inhibition was mimicked by clonidine (α_2-agonist). In the presence of 5-20μM metoclopramide (an α_2-blocker, see Spedding, 1980) OA potentiated the transmurally induced contractions. NA responses were not inhibited at up to 250μM. If OA causes release of NA and blocks NA uptake by the presynaptic terminal, then more NA will accumulate at the nerve-muscle junction. This level of NA may not be sufficient to have a postsynaptic action but it will act on the presynaptic α_2-receptors to inhibit further NA release (Marshall et al, 1978). Thus OA causes a reduction in the height of transmurally-induced contractions but when the α_2-receptors are blocked a potentiating effect is unmasked.

Axelrod, J. & Saavedra, J.M. (1977) Nature 265, 501.
Dao, W.P.C. & Walker, R.J. (1980) Br.J. Pharmac. 68, 132P.
Jones, R.S.G. (1982) European J. Pharmacol. 77, 159.
Marshall, I. et al (1978) Br. J. Pharmac. 64, 145.
Robertson, H.A. & Jurio, A.V. (1977) Int. Rev. Neurobiol. 19, 173.
Spedding, M. (1980) Br. J. Pharmac. 71, 113.

PROTECTIVE EFFECTS OF DILTIAZEM AGAINST ADRENALINE AND CALCIUM-EVOKED DYSRHYTHMIAS

I. Cavero, A. Grosset and Françoise Lefèvre-Borg, Laboratoire d'Etudes et Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France

Verapamil is often used clinically to terminate re-entrant supraventricular tachycardia and to slow the ventricular rate in atrial flutter and fibrillation. The same beneficial effects are produced by other calcium antagonists such as diltiazem and tiapamil (Singh et al., 1983). The purpose of this communication is to present data which show that diltiazem reduces significantly electrocardiographic abnormalities evoked in dogs by a dysrhythmogenic dose of adrenaline and in rats by the infusion of a cardiotoxic dose of $CaCl_2$.

Beagle dogs (10-15 kg) were anaesthetized with pentobarbitone (35.0 mg/kg + 6.0 mg/kg/hr i.v.) placed under artificial respiration and prepared for the recording of heart rate and electrocardiogram. Dysrhythmia was induced by an i.v. bolus injection of adrenaline (50.0 µg/kg, i.v.) 10 min after starting an i.v. infusion of either saline (0.05 ml/kg/min) or diltiazem (30.0 µg/kg/min) lasting 15 min. In a second series of experiments i.v. saline (0.5 ml/kg) or diltiazem (300.0 µg/kg) were given as bolus injections 30 sec after adrenaline. Results are reported as a number of abnormal QRS complexes produced by adrenaline over a 5 min period.
Male Wistar rats (350-400 g) were anaesthetized with pentobarbitone (60.0 mg/kg i.p.), nephrectomized and prepared for heart rate and electrocardiogram recordings. $CaCl_2$ (20.0 mg/min/rat i.v.) was infused until the cessation of cardiac activity. Nephrectomy was performed to reduce the interanimal variability in the cardiac responses to $CaCl_2$ (Caparencu et al. 1978). The administration of $CaCl_2$ was initiated 15 min after starting the infusion of either i.v. saline (0.05 ml/kg/min) or diltiazem (25.0 µg/kg/min) which were given throughout the duration of the experiment. The percentage of animals exhibiting ventricular tachycardia, fibrillation or cardiac arrest was calculated for the treated and control groups.

Diltiazem significantly ($p<0.05$, (t-test) reduced the percentage of abnormal cardiac QRS complexes measured during the 5 min which followed the injection of adrenaline. This value was 15 $\pm$ 4% (n=6) and 61 $\pm$ 7% (n=6) in diltiazem (30.0 µg/kg/min i.v.) and saline treated dogs, respectively. Similar positive results were obtained when diltiazem (300.0 µg/kg i.v.) was injected 30 sec after the administration of adrenaline.
In nephrectomized rats the infusion of $CaCl_2$ produced various pathological electrocardiographic changes. Death occurred within 5 to 7 min and, in 67% of the saline-perfused animals (n=6), was due to ventricular fibrillation. In contrast, all rats given diltiazem died of cardiac arrest albeit within the same time period as the control. However, diltiazem reduced significantly ($p<0.05$, Kruskall Wallis test) (from 73 $\pm$ 28, n=6, to 13 $\pm$ 7, n=5) the number of abnormal QRS complexes occurring within a 5 min infusion of $CaCl_2$.

These results indicate that diltiazem can protect the heart against serious electrocardiographic abnormalities evoked in dogs by a large i.v. bolus injection of adrenaline and in rats by an infusion of a cardiotoxic dose of $CaCl_2$.

Singh, B.N. et al (1983) Drugs 25, 125-153
Caparencu B. et al (1978) Agressologie 19, 367-378

PHARMACOLOGICAL INTERVENTIONS TO ANTAGONIZE ADVERSE ELECTROCARDIOGRAPHIC EFFECTS OF HIGH DOSES OF DILTIAZEM IN CONSCIOUS DOGS

I. Cavero and Josette Sabatier, Laboratoire d'Etudes et de Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France.

The slow inward calcium current plays an important role in the depolarization of the sino-atrial and atrio-ventricular (AV) nodes and in the excitation-contraction coupling of the vascular and cardiac muscle. Verapamil and diltiazem at certain plasma concentrations prolong AV conduction time and cause AV blockade of various degrees in dogs (Narimatsu & Taira, 1976; Hariman et al. 1979). These undesirable effects may also be observed in man given therapeutic doses of verapamil (Da Silva et al 1979; Morris & Goldschlager, 1983). The aim of this communication is to discuss the effects of some pharmacological interventions useful to antagonize AV abnormalities produced in conscious dogs by the i.v. administration of a large dose of diltiazem.

Three beagle dogs of 9.5-15.5 kg body weight were anesthetized with pentobarbitone to implant under sterile conditions a catheter into the femoral artery. After an appropriate period of recovery from the surgical procedure they were prepared for i.v. administrations (catheter placed into the brachial vein) and for the recording of blood pressure and electrocardiogram (lead II) using skin electrodes. Diltiazem was infused i.v. at 1.0 mg/dog/min for 60 min (corresponding to a 4.5 mg/kg) and 30 min later the animals were given either no treatment (control) or i.v. adrenaline (0.2-0.4 µg/kg/min during 15-45 min), methylatropine (0.5 mg/kg given over 10 min), glucagon (30.0 µg/kg over 15 min) or calcium chloride (45.0 mg/kg over 20 min).

In the three dogs used in this study, the chosen dose of diltiazem always produced an approximately 50% prolongation of PR interval within the initial 10 min of infusion and subsequently numerous second- and third-degree AV blocks which persisted for over 4 hours but were no longer present 24 hours later. These effects could be easily reproduced upon re-administration of diltiazem at weekly intervals. In these animals methylatropine suppressed all AV blocks within the infusion period. This effect lasted for over 2 hours and was accompanied by an increase in heart rate. Administration of glucagon eliminated most of the AV blocks produced by diltiazem. This favorable effect lasted at least 30 min. The infusion of calcium chloride was not promptly effective in antagonizing diltiazem-induced AV blocks, however, it appeared to accelarated the recovery of a normal rhythm. An infusion of adrenaline abolished all diltiazem-induced AV blocks and this effect lasted for over 30 min following the end of its administration. Finally, it should be noted that the described treatments did not restore to normality the prolongation of PR interval produced by diltiazem.

These results indicate that, in the conscious dog, the most serious electrocardiographic disturbances produced by a large dose of diltiazem can be easily antagonized by blockade of cardiac parasympathetic drive, stimulation of cardiac adrenoceptors with adrenaline and glucagon but not calcium chloride. These findings are of potential clinical interest for the management of adverse cardiac effects produced by toxic doses of diltiazem as well as verapamil or other calcium antagonists.

Da Silva, O.A. et al. (1979) Clin.Toxicol. 14, 361.
Narimatzu, A. & Taira, N. (1976) Naunyn-Schmiedeberg's Arch.Pharmacol. 294, 169.
Hariman, R.J. et al. (1979) Circulation 59, 797.
Morris, D.L. & Goldschlager, N. (1983) J.A.M.A, 249, 3212.

DISSOCIATION BETWEEN CHANGES IN DENSITY OF ^{3}H-QNB BINDING AND SUPERSENSITIVITY TO METHACHOLINE IN RAT SALIVARY GLANDS

J. Ekström[+], S.Z. Langer and C. Pimoule, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris, France and Institute of Physiology, Sölvegatan 19, University of Lund, Sweden[(+)].

There is marked supersensitivity to the muscarinic agonist methacholine for the secretory responses in the parotid and submaxillary glands of the rat following parasympathetic denervation (DEN) or decentralization (DEC). After sympathetic DEN the degree of supersensitivity is smaller and following sympathetic DEC even less pronounced (Ekström, 1980 ; Ekström and Malmberg, 1981).

We studied the effects of these surgical procedures and of salivary duct-ligation on the number of muscarinic receptors (Bmax) and their binding affinity (Kd) in male adult rats by measuring the binding of ^{3}H-quinuclidinylbenzilate, ^{3}H-QNB, (Pimoule et al., 1980). Specific binding was defined as that inhibited in the presence of 10 µM atropine and represented 70% at 0.6 nM ^{3}H-QNB. Comparisons were made between operated and contralateral control gland of the same animal.

In the parasympathetically DEN parotid glands (5 rats) Bmax of ^{3}H-QNB binding was reduced ($p<0.001$) by 47% 3 weeks postoperatively (DEN : 92.0±13.5 fmoles/mg prot. ; cont. : 174.0±19.0 fmoles/mg prot.), while the Kd was unchanged (DEN : 0.59±0.09 nM ; cont. : 0.80±0.12 nM). In another group of 10 rats already 1 week after DEN Bmax of ^{3}H-QNB binding was reduced ($p<0.02$), by 40%. In the parasympathetically DEC submaxillary glands, n=5, (3 weeks) neither Bmax (DEC : 284.0±22.9 fmoles/mg prot. ; cont. : 290.2±12.2 fmoles/mg prot.) nor Kd (DEC : 0.67±0.06 nM ; cont. : 1.1±0.4 nM) were changed. Three weeks after sympathetic DEN or DEC no significant changes in the ^{3}H-QNB binding parameters were observed in the parotid glands. In the submaxillary glands the Bmax of ^{3}H-QNB binding was increased ($p<0.05$), by 27%, after sympathetic DEN but not after DEC (4 rats in each group). The parasympathetically DEN parotid gland lost 32 (1 week) and 51% (3 weeks) in wet weight, while the parasympathetically DEC submaxillary gland lost 24%. Duct-ligation of the parotid gland reduced the weight by 52% and Bmax of ^{3}H-QNB binding by 84% in 6 rats (2 glands pooled), while the Kd remained unchanged. This marked receptor loss after duct-ligation indicates a predominant postsynaptic localization of the ^{3}H-QNB binding sites. When in 8 rats (2 glands pooled) bilateral parotid duct-ligation for 4 weeks was combined with unilateral parasympathetic DEN the last week, the Bmax was lowered in the DEN gland.

The present findings on the decrease in Bmax of ^{3}H-QNB in parasympathetically DEN parotid glands are in agreement with the original observations of Talamo et al. (1979). The decrease in receptor number following parasympathetic DEN may suggest the existence of muscarinic autoreceptors in the parotid gland.

Supersensitivity to muscarinic agonists following parasympathetic DEN is observed concomitantly with a decrease in Bmax of ^{3}H-QNB binding. After parasympathetic DEC supersensitivity to methacholine is observed without changes in Bmax of ^{3}H-QNB binding. This lack of correlation between muscarinic receptor density and supersensitivity to agonists is further supported by the results with sympathetic DEN in submaxillary glands : increase in Bmax of ^{3}H-QNB binding with a modest degree of supersensitivity to methacholine.

These data suggest that the supersensitivity of the salivary glands to the muscarinic agonist is not causally related to the receptor density and reflects a change beyond the muscarinic recognition site.

Ekström, J. (1980) Acta Physiol. Scand. 108, 253-262
Ekström, J. and Malmberg, L. (1981) Acta Physiol. Scand. 113, 103-110
Pimoule, C. et al (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 15-18
Talamo, B. et al (1979) Life Sciences 24, 1573-1580

TOLBUTAMIDE IS A GOOD MODEL DRUG FOR THE STUDY OF ENZYME INDUCTION AND ENZYME INHIBITION IN THE RAT

D.J. Back, F. Sutcliffe & J.F. Tjia, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

Oxidation of tolbutamide to hydroxytolbutamide is the rate limiting step in the elimination of tolbutamide (Rowland & Matin, 1977). In the rat, 80% of an orally administered dose of tolbutamide is excreted in urine predominantly as hydroxytolbutamide. However, in man, hydroxytolbutamide is further metabolised to carboxytolbutamide and this metabolite is present in urine to a greater extent. The metabolic profile of tolbutamide means that the pharmacokinetics of this drug are readily influenced by alterations in hepatic microsomal enzyme activity. For example, sulphaphenazole is reported to markedly increase the half life of tolbutamide in man (Pond et al., 1977) and rats (Sugita et al., 1981). We have examined the effect of various drugs on the pharmacokinetics of tolbutamide in the rat with the aim of demonstrating that this compound is a good model drug for assessing drug interactions in vivo both qualitatively and quantitatively.

Male Wistar rats were used in two studies. In study 1, rats were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}) and the carotid artery and a peripheral vein cannulated. Groups of rats were either controls, pretreated with phenobarbitone (40 mg kg^{-1} twice daily for 4 days), or given cimetidine, chloroquine or primaquine (50 mg kg^{-1}; i.p.) 30 min before the administration of tolbutamide (50 mg kg^{-1}) into the peripheral vein. Blood samples were collected at intervals to 5 h. Plasma tolbutamide and hydroxytolbutamide were measured by h.p.l.c substantially according to the method of Nation et al., (1978). In study 2, rats individually housed in metabolism cages, were either controls or given phenobarbitone , cimetidine or primaquine as described above. Tolbutamide (50 mg kg^{-1}) was injected i.p. and urine collected at intervals for 24 h. Hydroxytolbutamide wasdetermined by h.p.l.c.

Phenobarbitone pretreatment caused significant decreases in tolbutamide half life ($t_{\frac{1}{2}}$; from 128 ± 25 to 86 ± 9 min; mean ± S.D.) and area under the curve (AUC; from 513 ± 34 to 307 ± 47 μg ml^{-1} h) and significant increases in plasma clearance (Clp; from 1.21 ± 0.18 to 2.09 ± 0.22 ml min^{-1} kg^{-1}) and volume of distribution (Vd; from 220 ± 12 to 259 ± 21 ml kg^{-1}). Primaquine increased $t_{\frac{1}{2}}$ to 247 ± 60 min (92% increase) and AUC to 678 ± 56 μg ml^{-1} h (32% increase) and decreased clearance to 0.70 ± 0.15 ml min^{-1} kg^{-1} (42% decrease). The changes produced by cimetidine ($t_{\frac{1}{2}}$, 46% increase; AUC, 22% increase; Clp, 31% decrease) were less than those produced by primaquine. Chloroquine was without significant effect. Hydroxytolbutamide concentrations in plasma reflected the overall pharmacokinetic changes, being 3-fold greater following phenobarbitone and significantly reduced after primaquine. The urinary recovery data were also consistent with the effects seen on plasma concentrations. Excretion of hydroxytolbutamide was significantly increased by phenobarbitone up to 8 h. Both primaquine and cimetidine significantly reduced excretion of the metabolite.

We conclude that in pharmacokinetic interaction studies in laboratory animals, particularly when the interacting drug does not cause appreciable displacement from protein binding, the use of tolbutamide with its mono pathway metabolite profile gives distinct advantages over other substrates with more complex metabolic profiles (eg. antipyrine).

Nation, R.L. et al (1978) J. Chromatogr. 146, 121-131.
Pond, S.M. et al (1977) Clin. Pharmac. Ther. 22, 573-579.
Rowland, M. & Matin, S.B. (1973) J. Pharmacokin. Biopharm. 1, 553-567.
Sugita, O. et al (1981) Biochem. Pharmac. 30, 3347-3354.

DOPAMINE STIMULATES POSTSYNAPTIC α_2-ADRENOCEPTORS IN THE FEMORAL BUT NOT THE RENAL VASCULAR BED OF THE ANAESTHETISED DOG

N. Duval, P.E. Hicks and S.Z. Langer, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 58, rue de la Glacière, 75013 Paris, France.

Previous work has shown that the vasoconstrictor effects of dopamine (DA) injected intraarterially in the mesenteric bed of the anaesthetised dog are predominantly mediated by postsynaptic α_2-adrenoceptors (Shepperson et al., 1982). It was therefore of interest to determine whether the vasoconstrictor effects of DA in other vascular beds also involved postsynaptic α_2-adrenoceptors.

Mongrel dogs (12-20 kg) were anaesthetised with pentobarbitone (35 mg/kg i.v. ; and maintained with an injection of 6 mg/kg/h i.v.). The animals were ventilated with room air and a femoral artery and vein cannulated for measurement of blood pressure and administration of drugs. Laparotomy was performed and the left renal artery located and an electromagnetic flow probe (Carolina Medical) was placed around the artery. A 26G needle was inserted in the artery, distal to the flow probe to facilitate intraarterial (i.a.) injections. A similar protocol was adopted for the contralateral femoral artery.

The animals were ganglion blocked with chlorisondamine (1 mg/kg i.v.), treated with atropine (1 mg/kg i.v.) and propranolol (1 mg/kg i.v. plus 0.5 mg/kg/h i.v.). In some experiments the DA-receptor antagonist d-butaclamol (0.1 - 0.3 mg/kg i.v.) was also administered. Mean basal values for blood pressure (127 $\pm$ 5 mmHg), renal blood flow (131 $\pm$ 9 ml/min), and femoral blood flow (71 $\pm$ 8) were recorded after 30 min stabilisation, n=13.

Dose related decreases in blood flow were induced in femoral and renal vascular beds by i.a. injections of DA (0.3 - 300 µg/kg). DA was a more potent vasoconstrictor in the femoral than renal bed. The dose causing 50% decrease in flow (EC_{50} + 95% confidence limits) were 4.7 (3.1 - 5.4) µg i.a.. n=12 for femoral and 74.7 (65.4 - 85.8) µg i.a., n=13 for renal flow ; $P<0.01$. In the femoral vascular bed the vasoconstrictor effects of DA were not antagonised by prazosin (30 - 300 µg/kg i.v., 15 min) however after α_1-receptor blockade, these effects were progressively antagonized by the α_2-receptor antagonist RX 781094 (Chapleo et al., 1981) over the dose range 30 - 300 µg/kg i.v. 15 min. The blockade by RX781094 of the vasoconstriction induced by DA in the femoral bed was not modified by pretreatment with d-butaclamol (300 µg/kg i.v.). In contrast, in the renal bed DA-induced vasoconstriction was preferentially antagonised by prazosin (30 - 300 µg/kg i.v.) and was resistant to blockade by RX781094 (300 µg/kg i.v.).

The results indicate that local injections of DA to the femoral vascular bed cause vasoconstriction through stimulation of postsynaptic α_2-adrenoceptors as previously shown in the mesenteric vasculature of the dog. As the dose of DA is increased, the vasoconstrictor effects of DA are, not exclusively α_2-adrenoceptor mediated and stimulation of α_1-adrenoceptors takes place. In support of this view, in the renal bed, where there is so far little evidence for the existence of postsynaptic α_2-adrenoceptors even after DA-receptor blockade, the vasoconstrictor effects of DA are mediated through prazosin sensitive α_1-receptors.

It is concluded that DA behaves as a preferential α_2-adrenoceptor agonist in vascular smooth muscle but can also stimulate α_1-adrenoceptors at higher doses. Conclusions about the relative potency of DA and NA at α-adrenoceptors should be re-evaluated separately for α_1 and α_2-adrenoceptor subtypes.

Chapleo C.B. et al (1981) Br.J.Pharmacol. 74, 842P
Shepperson N.B. et al (1982) Eur.J.Pharmacol. 81, 627-635

BINDING OF L-TRYPTOPHAN TO ALBUMIN: EFFECT OF PROTEIN CONCENTRATION AND IMPLICATIONS FOR BRAIN UPTAKE

C.A. Fenerty & W.E. Lindup, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

The fraction of total plasma tryptophan which is not bound to albumin appears to be available for uptake into the brain. Pardridge (1979) suggested that uptake of L-tryptophan into the brain was underestimated if predicted from unbound L-tryptophan measured in vitro. However, the unbound fraction of L-tryptophan was calculated on the assumption that the apparent association constant (Ka) and the number of binding sites (n) of albumin for the amino acid were unaffected by protein concentration. Bowmer & Lindup (1978; 1980) have shown that there is an inverse dependence of nKa with L-tryptophan for both human (HSA) and bovine albumin (BSA) but the extent of the dependence upon protein concentration was not measured. The aim of this study was to quantitate the change in nKa with albumin concentration and to determine how it affected the prediction of L-tryptophan uptake into the brain.

The binding of a range (0.5-50μM) of concentrations of L-[5-^{3}H]-tryptophan (0.025μCi/ml) to a series of single concentrations of defatted albumin (75-600μM) was measured by equilibrium dialysis for 4 hr at 37°C in 0.05M saline phosphate buffer pH 7.4. Scatchard plots were used to obtain values of n and Ka for each of the albumin concentrations studied.

Table 1 Effect of protein concentration on the values of nKa for the interaction of human albumin with L-tryptophan

HSA (μM)	nKa (l/mol x 10^{-4})	n
600	0.79	0.26
450	0.87	0.41
150	1.11	0.60
75	1.29	0.70

The inverse dependence of nKa upon albumin concentration for the binding of L-tryptophan to both human (Table 1) and bovine albumin (data not shown) has been confirmed. Table 1 shows some of the results for human albumin and it can be seen that an increase in protein concentration affects n somewhat more than Ka. There is a decrease in n of about 60% as the albumin concentration is raised from 75μM to 600μM (0.5-4%).

Pardridge (1979) calculated the brain uptake index for L-tryptophan on the basis of a single Ka value (7.7 x 10^{3} l/mol for 300μM (2%) BSA) but both n and Ka change with protein concentration and so the fraction of unbound L-tryptophan is a function of nKa. This inverse dependence of nKa upon albumin concentration means, therefore, that the unbound fraction of L-tryptophan at a particular concentration of albumin cannot be accurately predicted by the use of a Ka value obtained from an experiment at a single protein concentration which is different from the one for which Ka is required.

Bowmer, C.J. & Lindup, W.E. (1978) Biochem. Pharmacol., 27, 937-942

Bowmer, C.J. & Lindup, W.E. (1980) Biochim. Biophys. Acta, 624, 260-270

Pardridge, W.M. (1979) Life Sci., 25, 1519-1528

INTRACELLULAR STUDIES OF THE ACTION OF K^+ CHANNEL BLOCKERS ON FROG MOTONEURONAL RESPONSES TO EXCITATORY AMINO ACIDS

M.S. Arenson & A. Nistri, Department of Pharmacology, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Excitatory amino acids such as glutamate and chemically-related analogues depolarize spinal motoneurones with a rather small increase in their input conductance (Nistri & Constanti, 1979). One explanation is that excitatory amino acids may depress outward K^+ fluxes while activating inward depolarizing currents (Shapovalov et al., 1978, Engberg et al., 1979): the net result would be a small change in total neuronal conductance. Although ion-sensitive microelectrodes have not suggested a significant depression of K^+ permeability following glutamate application (Bührle & Sonnhof, 1983), another approach to studying the role of K^+ in these responses would be to test the effect of K^+ channel blockers. For this purpose, we investigated excitatory amino acid responses in the presence of tetraethylammonium (TEA), 4-aminopyridine (4-AP) or caesium.

Experiments were carried out on motoneurones of the frog spinal cord slice preparation continuously superfused with oxygenated Ringer solution at 7°C (Nistri & Arenson, 1983). Intracellular recordings were obtained with 3 M KCl or 3 M CsCl filled microelectrodes. Motoneurones had a resting membrane potential of -74 ± 6 mV and a resting input conductance of 64 ± 12 nS (mean $\pm$ s.e.m.). Bath-applied TEA (3-5 mM) or 4-AP (0.5 - 1 mM) produced large increases in motoneuronal electrical activity with continuous fast depolarizing potentials (often with superimposed spikes) and slow oscillations of the cell membrane potential. Some of these phenomena probably had a presynaptic origin as they were blocked by 3 μM tetrodotoxin or 2 mM Mn^{2+}. Motoneurones were usually depolarized by TEA, 4-AP or intracellularly-applied Cs^+ although the depolarization amplitude was variable and apparently influenced by the degree of presynaptic discharge. Cell conductance was depressed by 20 to 80% of its resting value. The decay phase of the antidromic spike was greatly prolonged by the three K^+ antagonists and block of the spike afterhyperpolarization ensued (Cs^+ appeared to be the weakest agent of the three). Mn^{2+} blocked synaptic transmission and also shortened the spike decay phase when prolonged by 4-AP but not by TEA. TEA did not block the depolarization produced by glutamate (0.7 - 2 mM), quisqualate (30-45 μM) or N-methyl-D-aspartate (100 μM). Similar results were noted when 4-AP was used. Conversely, intracellular iontophoresis of Cs^+ reversibly reduced by 30 to 90% the depolarizing effect of glutamate, quisqualate or DL-homocysteate (0.1 mM) and depressed any associated conductance change.

In conclusion, motoneuronal K^+ conductances could be apparently blocked by TEA, 4-AP or Cs^+. Only Cs^+ was able to depress excitatory amino acid responses, perhaps by an action on a distinct K^+ conductance and/or by an effect on sodium movements responsible for excitatory amino acid depolarization (Arenson et al., 1983).

This work was supported by a grant from the Joint Research Board of St. Bartholomews Hospital.

Arenson, M.S. et al. (1983). J. Physiol. In press.
Bührle, C.P. & Sonnhof, V. (1983). Pflügers Arch. 396, 154-162.
Engberg, I. et al. (1979). J. Physiol. Lond. 288, 227-261.
Nistri, A. & Arenson, M.S. (1983). Neuroscience, 8, 115-122.
Nistri, A. & Constanti, A. (1979). Progr. Neurobiol. 13, 117-235.
Shapovalov, A.I. et al. (1978). J. Physiol. Lond. 279, 437-455.

DESENSITIZATION OF NICOTINIC-EVOKED CATECHOLAMINE RELEASE FROM THE RABBIT ADRENAL GLAND

A.R. Collett, M.J. Rand & D.F. Story, Department of Pharmacology, University of Melbourne, Victoria, 3052, Australia.

An isolated perfused preparation of the rabbit adrenal was used to study the nicotinic receptor desensitization produced by a low concentration of 1,1-dimethyl-4-phenylpiperazinium (DMPP) on catecholamine secretion evoked by splanchnic nerve stimulation or perfusion with potassium (K^+) or higher concentrations of DMPP. The catecholamine stores of the adrenal gland-splanchnic nerve preparation were radiolabelled with ^{3}H-adrenaline and subsequently evoked release of catecholamines was deduced from the amounts of radioactivity in fractions of the perfusate (Collett & Story, 1982). Splanchnic nerve stimulation at a frequency of 5 Hz for 8 min periods resulted in an efflux of radioactivity which peaked within 2 min and was maintained throughout the period of stimulation. The efflux evoked by 8 min periods of perfusion with K^+ (30 mM) or DMPP (100 µM) also peaked within 2 min; however, in contrast to the stimulation-evoked release, the efflux with these agents was not maintained but returned substantially to resting levels in their continued presence. To study the desensitizing effect of pre-exposure to DMPP, release was evoked by two brief periods of nerve stimulation (1 min) or by 2 min periods of perfusion with DMPP (100 µM) or K^+ (30 mM) : the interval between the periods was 30 min and the efflux in the second period was expressed as a percentage of that in the first. Pre-infusion of the glands with DMPP (10 µM) for 10 min periods, terminating 5 min before the second evoked release period, produced negligible catecholamine release but markedly reduced the release evoked by nerve stimulation and by 100 µM DMPP : in contrast, the release evoked by K^+ was unaltered (shown in Table 1).

Table 1 Effect of DMPP pre-infusion on secretory responses

Efflux evoked by	Evoked efflux (second period as % of first period)
DMPP (100 µM, 2 min)	
control	57 ± 2
DMPP (10 µM) pre-infusion	8 ± 3*
Stimulation (5 Hz, 1 min)	
control	91 ± 5
DMPP (10 µM) pre-infusion	29 ± 6*
K^+ (30 mM), 2 min)	
control	83 ± 9
DMPP (10 µM) pre-infusion)	90 ± 9

Asterisk denotes a significant difference from control at $P < 0.05$

The results suggest that in the continued presence of DMPP, nicotinic receptors on the chromaffin cells are desensitized. Thus, DMPP-evoked release is not maintained and prior contact with a low concentration of DMPP inhibits catecholamine secretion evoked by both transmitter acetylcholine and exogenous nicotinic agonists but not by K^+. It has been proposed that the persistence of release evoked by prolonged nerve stimulation may be dependent upon co-release from the splanchnic nerves of substance P. Substance P has been shown to protect against agonist desensitization of nicotinic receptors (Livett et al., 1983).

Collett & Story (1982). J. Auton. Pharmac. 2, 25-34.
Livett et al (1983). J. Auton. Nerv. Syst. 7, 59-86.

THE RELATIONSHIP BETWEEN AGONIST OCCUPATION OF CEREBRAL α_1-ADRENOCEPTORS AND PHOSPHOINOSITIDE HYDROLYSIS

E. Brown, D.A. Kendall & S.R. Nahorski, Department of Pharmacology and Therapeutics, University of Leicester, Medical Sciences Building, University Road, Leicester.

Recent studies from this laboratory have demonstrated that alpha$_1$ adrenoceptors are linked to phosphoinositide (PI) hydrolysis in rat cerebral cortical slices (Brown et al. 1983). Agonist dose-response curves suggested that there was probably little receptor reserve, so in the present communication we have employed the irreversible alpha$_1$ antagonist, phenoxybenzamine, to more fully examine the relationship between receptor occupation and the 'PI' response.

Rat cerebral cortex slices (350 x 350 μM) were preincubated for 1 h at 37^o in Krebs/Henseleit buffer. Phenoxybenzamine was then added to achieve final concentrations of 10^{-8} or 3 x 10^{-8} M, and preincubation proceeded with occasional gassing with 95% O_2, 5% CO_2 for a further 60 min. The slices were washed three times by resuspension in fresh, phenoxybenzamine-free, buffer and were then transferred to tubes containing 0.18-0.25 μM ^{3}H-myo-inositol + 5 mM lithium chloride for 30 min, after which appropriate agonists were added for a further 45 min incubation. ^{3}H-inositol phosphates (IP), the products of ^{3}H-PI hydrolysis, were extracted and separated from ^{3}H-inositol by ion exchange chromatography.

In parallel experiments, slices were preincubated under the same conditions as previously described, then incubated for 60 min at 37^o with ^{3}H-prazosin for assessment of alpha$_1$ receptor binding. Incubations were terminated by rapid filtration of intact slice suspensions through Whatman GF/C filters, and specific ^{3}H-prazosin binding to the slices was defined as the difference between total binding and that remaining in the presence of 10^{-3} M noradrenaline.

^{3}H-Prazosin binding to control slices revealed a K_D of 0.04 nM and a B_{max} of 110 fmol/mg protein compared with a K_D of 0.05 nM and a B_{max} of 121 fmol/mg protein in a crude membrane preparation. The K_i values of a number of agonists and antagonists in displacing ^{3}H-prazosin binding were not significantly different in intact slices compared with membranes incubated under identical conditions.

Incubation of slices with 10^{-8} M and 3 x 10^{-8} M phenoxybenzamine reduced maximum ^{3}H-prazosin binding to 44% and 24% of control levels respectively. The maximum noradrenaline-stimulated accumulation of ^{3}H-IP in these slices was reduced to 51% and 24% respectively, of the maximum response in the absence of phenoxybenzamine. Phenoxybenzamine treatment did not affect the EC_{50} value for noradrenaline-stimulated ^{3}H-IP accumulation, nor did it influence the response to the muscarinic agonist carbachol. Similarly, the maximum accumulations of ^{3}H-IP elicited by the partial alpha$_1$ agonists phenylephrine and methoxamine, closely paralleled the fall in alpha$_1$ receptors caused by phenoxybenzamine. In separate experiments, 10^{-8} M phenoxybenzamine reduced ^{3}H-prazosin binding to 52% and 41% of the control level and the maximum responses to methoxamine and phenylephrine to 57% and 32% respectively. Again, the EC_{50} values of these partial agonists were unaltered by phenoxybenzamine.

The results presented show that an effective reduction of alpha$_1$ receptors due to irreversible blockade by phenoxybenzamine, leads to an almost identical percentage reduction in the maximum rate of accumulation of ^{3}H-IP by alpha$_1$ agonists. Although the details of the linkage between the alpha$_1$ receptor and the PI hydrolytic mechanism are at present unknown, the data suggests a very close association between receptor occupation and this response.

Brown, E. et al (1983) Br.J.Pharmac. 78, 108P

INHIBITORY INFLUENCE OF α_2-ADRENOCEPTORS ON STRIATAL CHOLINERGIC TRANSMISSION

D. Fage & B. Scatton, Biochemical Pharmacology Unit, Synthélabo-L.E.R.S., 31 avenue Paul Vaillant Couturier, 92220 Bagneux, FRANCE.

It is now well established that presynaptic α_2-adrenoceptors are involved in the modulation of cholinergic transmission in both the peripheral (Langer et al, 1981) and the central (Vizi et al, 1979) nervous systems. Since α_2-adrenoceptors are present in the striatum (U'Prichard et al, 1979), they may participate in the regulation of the activity of the striatal cholinergic interneurons. To explore this possibility we have studied the effect of the selective α_2-adrenoceptor agonist clonidine on the levels of acetylcholine (ACh) (an index of cholinergic neuronal activity) in the rat striatum.

Experiments were performed on male Sprague Dawley rats (150g). ACh levels were measured by a radioenzymatic technique (Guyenet et al, 1975). Surgical and chemical lesions were performed as described previously (Scatton and Bartholini, 1982).

Systemic administration of clonidine (0.01-0.3 mg/kg ip) caused a dose-dependent increase in striatal ACh concentrations (ED50 0.02 mg/kg). Moreover, clonidine (0.3 mg/kg ip) markedly retarded the rate of utilization of striatal ACh (as measured after intrastriatal infusion of hemicholinium-3, a choline uptake inhibitor). These data indicate that clonidine diminishes striatal cholinergic neuron activity. This effect appears to be mediated via stimulation of α_2-adrenoceptors as the clonidine (0.03-1 mg/kg ip)-induced increase in striatal ACh levels was completely antagonized by RX 781094 (20 mg/kg ip), a selective α_2-adrenoceptor antagonist.

The mechanism whereby clonidine diminishes striatal cholinergic transmission does not appear to involve the nigro-striatal dopaminergic neurons or other monoaminergic neuronal pathways controlling striatal cholinergic interneuron activity as the elevation of striatal ACh levels induced by clonidine (0.3 mg/kg ip) was not antagonized by haloperidol (2 mg/kg ip), reserpine (4 mg/kg ip) or by 6-hydroxydopamine induced lesion of the nigro-striatal dopaminergic pathway. Also, the effect of clonidine on striatal cholinergic interneurons is not mediated indirectly via the cortico-striatal glutamatergic pathway (which controls striatal cholinergic neuron activity, see Scatton and Lehmann, 1982) as ablation of the fronto-parietal cortex did not modify the ability of clonidine (0.3 mg/kg ip) to elevate striatal ACh levels. It is more likely that the effect of clonidine on striatal cholinergic neurons is intrinsic to the striatum. However, the fact that clonidine (up to 10 μM) failed to affect the potassium-evoked release of ^{3}H-ACh (newly synthesized from ^{3}H-choline) in rat striatal slices excludes the possibility that the effect of this drug is mediated via α_2-receptors located on the terminals and/or the dendrites of striatal cholinergic cells.

In conclusion, the present results suggest that α_2-adrenoceptors exert an inhibitoty influence on striatal cholinergic transmission. This influence is probably intratriatal in nature and mediated via local neuronal circuits controlling striatal cholinergic neuron activity.

Guyenet, P.G. et al (1979) Brain Research 84, 227-237.
Langer, S.Z. (1981) Pharmacol. Review 32, 337-361.
Scatton, B. & Bartholini, G. (1982) J. Pharmacol. Exp. Ther. 220, 689-695.
Scatton, B. & Lehmann, J. (1982) Nature 297, 422-424.
U'Prichard, D. et al (1979) Mol. Pharmacol. 16, 47-60.
Vizi, E.S. & Harsing, L.G. (1979) In Presynaptic Receptors, S.Z. Langer, K. Starke and M.L. Dubocovich (eds). Pergamon Press, pp. 151-157.

THE ACTION OF FPL 52694 AND DISODIUM CROMOGLYCATE ON GASTRIC ACID SECRETION BY RAT ISOLATED STOMACH

P. Canfield, G. Coruzzi', B. Curwain & T. Rundell, Department of Physiology, St. Mary's Hospital Medical School, London W2 1PG and 'Institute of Pharmacology, University of Parma, Italy.

FPL 52694 is a monochromone, mast cell stabilising agent which has been reported to have an inhibitory effect on gastric acid secretion in rat, dog and man (Nicol et al, 1981; Canfield & Curwain, 1982; Davies et al, 1981). In conscious dog it was more effective against pentagastrin stimulated secretion when given directly into the stomach than when given i.v. (Canfield & Curwain, 1983). However, its mode of action has yet to be established. The experiments described here were part of an investigation of this problem.

Stomachs from rats weighing 35-45g were set up as described by Spencer (1982). All drugs were added to the buffered serosal bathing solution which was gassed with 95% O_2/5% CO_2 (pH 7.4: 34°C). The unbuffered mucosal saline was gassed with 100% O_2 and changed at 15 min intervals to determine acid output by titration. Results are expressed as the secretory ratio R, in each stomach where R = response to drug/preceding rate of secretion.

Test stomachs were incubated with FPL 52694 throughout the experiment (0.5mM) and responses to pentagastrin (0.2uM) and histamine (160uM) compared with those from control stomachs without FPL 52694. There were no significant differences ($P>0.05$) between test and control responses. Higher concentrations of FPL 52694 suggested that, contrary to expectations, it caused a stimulation of acid output and the effects of various concentrations of FPL 52694 were studied in a second series of experiments. Over the range 0.5-5mM, FPL 52694 caused a concentration related increase in acid secretion. This stimulatory action of FPL 52694 was not antagonised by metiamide (100uM), atropine (10uM) or propranolol (25uM); concentrations which significantly inhibited responses to histamine, bethanechol and isoprenaline respectively, in these preparations. Stimulation by FPL 52694 was also unaffected by tetrodotoxin (10uM).

The effect of disodium cromoglycate (DSCG), a dichromone with mast cell stabilising activity, was also investigated. DSCG (0.5 & 5mM) was without significant effect upon basal acid output or on responses to pentagastrin (0.5uM) or histamine (160uM)

In contrast to its action in vivo, FPL 52694 does not inhibit acid secretion in the rat stomach in vitro but acts as a stimulant to acid output. Similar findings have been reported for β-adrenoceptor agonists (Canfield, Hughes, Price & Spencer, 1981). This stimulatory action of FPL 52694 does not depend upon the intrinsic neural plexi nor on an action at adrenergic, cholinergic or histamine receptors. In contrast, the dichromone DSCG was without effect on basal acid secretion. Neither drug affected stimulated secretion. Preliminary experiments suggest that FPL 52694 may have an inhibitory effect in vitro when added to the mucosal bathing solution.

Canfield, S.P. & Curwain, B.P. (1982) Gut 23, A899
Canfield, S.P. & Curwain, B.P. (1983) Br. J. Pharmac. 78, Suppl., 177P
Canfield, S.P., Hughes, A.D., Price, C.A. & Spencer, J.E.(1981)J.Physiol.316,23-31.
Davies, H.A., Rhodes, J. & Thomas, M. (1981) Br. J. Clin. Pharmac. 1, 53-56.
Nicol, A.K., Thomas, M. & Wilson, J. (1981) J. Pharm. Pharmacol. 33, 554-556.
Spencer, J.E. (1982) J. Pharmacol. Methods 8, 197-212.

POSSIBLE MECHANISMS OF ACTION OF CALCITONIN IN HAEMORRHAGIC HYPOTENSION

R.F.L. Bates, G.A. Buckley, R.M. Eglen[1] and C.A. McArdle, Department of Life Sciences, Trent Polytechnic, Nottingham [1]present address, Syntex Research Centre, Heriot-Watt University, Edinburgh.

It has been reported that i.v. salmon calcitonin (SCT) produces a pressor response when administered to rats rendered hypotensive by haemorrhage (Bates et al, 1983). This effect was not due to peripheral vasoconstriction and was not observed in normotensive animals. We have investigated the mechanism(s) of this response by determining the effect of SCT after bilateral vagotomy, chemical sympathectomy (Kostrzewa et al,1974), and vasopressin antagonism (Kruszyski et al, 1980, Htzinikolaou et al, 1981).

Sprague-Dawley rats (♂,♀, 150-350g) were anaesthetised with urethane, cannulated for drug administration and blood pressure recording and bled to reduce the mean arterial pressure (MAP) as previously described (Bates et al,1983). After a 20 minute stabilisation period SCT (10 i.u. Kg^{-1} in 0.1ml, 0.15M NaCl with 1mg ml^{-1} (BSA) or vehicle were administered i.v. The change in MAP was monitored for a further 60 minutes.

Groups of 6-10 animals were pretreated with 6-Hydroxydopamine (6-OHDA,100mg Kg^{-1} i.p. in 1mg ml^{-1} ascorbic acid, 4,3 and 2 days prior to experimental use), bilateral vagotomy (5-10 minutes after bleeding) or the vasopressin antagonist [1-β-mercapto-β,β-cyclopentamethylene propionic acid), 2-(O-methyl) tyrosine] arginine vasopressin ($d(CH_2)_5$Tyr(Me)AVP, 0.1mg kg^{-1}, i.v. in 0.15M NaCl with 1mg ml^{-1} BSA, 5 minutes prior to bleeding). In each case control groups received appropriate pretreatment vehicle. There were no differences between these 3 groups which have therefore been combined as the pretreatment control.

Table 1. (Change in MAP After Drug Treatment ($\bar{x} \pm$ s.e., mmHg)

Pretreatment	Drug	5 minutes	15 minutes	60 minutes
Combined controls(n,18-21)	vehicle	+1.4±0.8	-0.3±1.1	-0.8±0.9
	SCT	+7.6±0.7 *	+6.2±0.8 *	+5.3±1.2 *
Bilateral vagotomy(n,6-8)	vehicle	+2.2±1.4	-0.2±1.8	-3.4±2.5
	SCT	+9.6±2.4 *	+8.7±1.6 *	+4.5±2.6 *
6-OHDA (n,10-12)	vehicle	+1.1±0.9	-0.3±1.0	+0.9±1.3
	SCT	+4.5±1.2 *	+1.8±1.5	-0.1±1.4
$d(CH_2)_5$Tyr-(Me)AVP(n,6-8)	vehicle	+2.0±2.0	+1.4±1.2	-0.3±2.4
	SCT	+7.7±0.8 *	+6.3±1.7 *	+7.4±2.1 *

*P<0.05, 2 tailed Students 't' test.

The pressor response to SCT was unaffected by bilateral vagotomy or $d(CH_2)_5$Tyr(Me) AVP (at a dose which, in pithed rats, has been shown to abolish for 3 hrs. the pressor response of 1-100 mU Kg^{-1} vasopressin). However after pretreatment with 6-OHDA the pressor response only reached statistical significance at 5 minutes. These results suggest that pressor response to SCT in rats rendered hypotensive by haemorrhagic shock may involve an increase in sympathetic tone, rather than modification of vagal afferents or the secretion of vasopressin.

Bates, R.F.L. et al (1983) Br.J.Pharmac. 79, 255P
Htzinikolaou,P et al (1981) Am.J.Physiol. 240, H827-831
Kostrzewa, R.M. and Jacobowitz, D.M. (1974) Pharmacol.Rev. 26, 199
Kruszynski, M. et al (1980) J.Med.Chem. 23, 364-368

FACTORS AFFECTING LITHIUM ABSORPTION IN THE RAT SMALL INTESTINE

N.J. Birch, I.P.L. Coleman, *M.E. Hilburn and A.R. Karim, Dept. of Biological Sciences, The Polytechnic, Wulfruna St. Wolverhampton WV1 1LY. *Dept. of Chemistry, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET.

Lithium is used orally in the treatment of manic-depressive psychoses. However, the absorption of lithium across the gastrointestinal tract has not been fully investigated. More complete information would be of value in resolving the controversy that surrounds 'slow release' lithium preparations and developing more rational lithium therapy.

Both the method and analytical proceedures adopted in this study are indicated by Birch etal, (1983).

The rate of mucosal to serosal (M-->S) directional transfer of lithium and tissue lithium uptake was linearly related to increasing concentration of the lithium cation from 5 to 100 mM. Little evidence of saturation was observed and there was no regional difference in transport (Birch etal, 1983), although in the humans a regional difference has been reported (Ehrlich and Diamond, 1983).

There was a good correlation between water movement and transport of lithium into the serosal compartment of both jejunum and ileum over the period of incubation ($r>0.94$). Serosal lithium transfer in the jejunum was unaffected by glucose depletion, anoxia, $\pm 10^{o}C$ variation in temperature, 2,4,6-triaminopyrimidine (TAP) at 25 mM and pH 6.1, Phloretin, 2,4 Dinitrophenol and Phloridzin, all inhibitors at 0.2 mM concentration. These observations indicate that the absorption of lithium in the jejunum is passive, but probably linked to water movement.

In the duodenum, lithium transfer M-->S was not dependent on oxygen or glucose metabolism, whereas a $10^{o}C$ increase in temperature showed a significant ($P<0.05$) decrease in lithium transfer. Of the inhihitors tested above, only Phloridzin at 0.2 mM in the duodenum increased serosal lithium transfer ($P=0.016$).

In the ileum, serosal lithium transfer was decreased ($P<0.05$) in the presence of 25 mM TAP^{+} and $10^{o}C$ changes in temperature, while the anoxic conditions and Phloridzin increased lithium absorption ($P<0.05$). Glucose depletion had no significant effect, suggesting that in the ileum, the transfer of lithium M --> S is to some extent dependent on temperature and may proceed via the paracellular pathway. The increase in lithium absorption into the serosal compartment as a consequence of anoxia may be due to the loss of integrity of the tissue, thus rendering the tissue more permeable.

Tissue uptake of lithium was largely unaffected by inhibitors or experimental conditions employed. This confirms that the passage of lithium across the small intestine is likely to be by passive diffusion linked to the concommitant movement of water.

REFERENCE:
Birch N J etal, (1983). Brit. J. Pharmacol. in press.
Ehrlich B.A. and Diamond J.M. (1983). Lancet 1:306

MODULATION OF (^{3}H)-DIHYDROERGOCRYPTINE AND (^{3}H)-YOHIMBINE BINDING SITES ON HUMAN PLATELETS DURING THE MENSTRUAL CYCLE

D.B. Barnett, S.R. Nahorski & A. Richardson, Department of Pharmacology and Therapeutics, University of Leicester, Leicester, LE2 7LX.

Oestrogens have been shown to alter the density of both rabbit and human platelet adrenoceptors labelled with ^{3}H-dihydroergocryptine (^{3}H-DHE) (Roberts et al, 1979; Peters et al, 1979; Barnett et al, 1983). These results, together with the observation that ^{3}H-DHE appears to also label a population of sites additional to those identified by ^{3}H-yohimbine (^{3}H-YOH) in human and rabbit platelets (Motulsky & Insel, 1982; Barnett et al, 1983) have prompted us to examine the possible effect that physiological changes in oestrogens may have in altering platelet alpha adrenoceptor density. We have, therefore, examined both ^{3}H-DHE and ^{3}H-YOH binding to platelet throughout the normal menstrual cycle.

Six healthy female volunteers who were receiving no medication were recruited to the study. Daily oral temperature and day 21 progesterone levels were measured during one complete cycle prior to the study cycle to establish normal ovulation was occurring. During the study cycle venous blood samples were taken on days 7, 14, 21 and 28. Washed platelet membranes were prepared and used in binding assays with ^{3}H-DHE and ^{3}H-YOH. Incubations were terminated by filtration. Specific binding was defined as that binding displaced by 10μM phentolamine and the density of binding sites (B_{max}) determined by non-linear saturation analysis. Plasma oestrogen and progesterone were measured by a RIA method.

^{3}H-DHE and ^{3}H-YOH bound specifically with high affinity to platelet membranes at all stages of the menstrual cycle (K_D : ^{3}H-DHE 7.93 (5.40 - 11.64) nM; ^{3}H-YOH 3.30 (2.94 - 3.71) nM). There was no significant difference between the K_D values for each ligand at any time during the cycle. The B_{max} of specific sites labelled by ^{3}H-YOH and ^{3}H-DHE varied during the cycle, ^{3}H-YOH being lower at the beginning, and ^{3}H-DHE higher at the end of the cycle than at other times (Table 1). Furthermore, on days 7 and 28, ^{3}H-DHE also labelled significantly higher numbers of sites than were identified by ^{3}H-YOH at the same times.

Table 1 Alpha Adrenoceptor Concentrations (B_{max}), and Oestrogen Levels Throughout the Menstrual Cycle

Ligand/Hormone	Day 7	14	21	28
^{3}H-DHE fmoles/mgP	233 ± 50†	218 ± 47	211 ± 21	362 ± 79*†
^{3}H-YOH fmoles/mgP	145 ± 13**†	185 ± 27	184 ± 20	186 ± 31†
Oestrogen pmols/l	255 ± 49	344 ± 42	612 ± 150	377 ± 105

* Significantly different from 3H-DHE on day 7, 14 and 21 ($P < 0.1$)
** Significantly different from ^{3}H-YOH on day 14 and 21 ($P < 0.1$)
† ^{3}H-YOH significantly different from ^{3}H-DHE ($P < 0.05$) : Mean ± SEM ($n = 4 - 6$)

These results suggest that modulation of 3H-DHE and ^{3}H-YOH binding sites on platelets can occur during the menstrual cycle, and may be related to the changing oestrogen titre. The functional significance of these findings and whether the extra ^{3}H-DHE sites identified here are classical alpha$_2$ adrenoceptors is being investigated.

Supported by the British Heart Foundation

Barnett, D.B. et al (1983) Brit J Pharmacol, 79, 229P
Motulsky, H.J. & Insel, P. (1982) Biochem Pharmacol, 16, 2591
Peters, J.R. et al (1979) Lancet, ii, 933
Roberts, J.M. et al (1979) Endocrinology, 104, 722

CHARACTERISATION OF THE BINDING OF ^{125}I-(-)PINDOLOL TO HUMAN PLATELET β-ADRENOCEPTORS

D.B. Barnett, N. Cook & S.R. Nahorski, Department of Pharmacology and Therapeutics, University of Leicester, Leicester, LE2 7LX.

It has been shown that isoprenaline can increase intraplatelet cyclic AMP levels and inhibit aggregation of human platelets induced by other agonists (Jakobs et al, 1978, Kerry & Scrutton, 1983) presumably by interacting with a beta adrenoceptor on the platelet surface membrane. Only recently, however, has the direct labelling of this receptor been reported in human platelets (Steer & Atlas, 1982). These workers claimed that the lipophilic ^{125}I-labelled ligands cyanopindol and hydroxybenzylpindolol identified different densities of sites which, furthermore, did not exhibit the pharmacological properties of either the $beta_1$ or $beta_2$ adrenoceptor. Using the hydrophilic ligand ^{125}I-(-)pindolol (PIN) we have characterised this receptor as being typical of the $beta_2$ subtype.

^{125}I-PIN was iodinated according to the method of Barovsky & Brooker (1980). All experiments were carried out using freshly prepared platelets. Blood (100mls) was drawn from healthy normal volunteers using a 19 gauge needle into 0.1 vols of 3.8% sodium citrate as anticoagulant. After centrifugation of blood at 250g for 15 min, the platelet rich plasma was recentrifuged at 150g for a further 20 min. Using this method all platelet preparations were completely free of white blood cell contamination as assessed by visual counting. Platelet lysates were prepared as previously described (Cheung et al, 1982). Membranes were incubated with ^{125}I-PIN and appropriate concentrations of drugs in a final volume of 250µl assay buffer (50mM Tris-HCl pH 7.8, 1mM ascorbate) for 40 min at room temperature. Assays were terminated by rapid vacuum filtration and filters were washed with 3 x 10ml buffer (25mM Tris-HCl, 154mM NaCl pH 7.8) and radioactivity counted by standard technique. Specific binding defined as total minus non specific binding (in the presence of 200µM isoprenaline) was generally 70-80% of total binding at the concentration of ^{125}I-PIN (30-40pM) used in the competition experiments.

Binding was saturable, reversible and of high affinity. Maximal binding capacity (B_{max}) derived from Scatchard analysis of saturation data was 8.6 ± 1.7 fmol/mg protein (mean ± SEM, n = 3) and equilibrium dissociation constant (K_D) 23.3 ± 2.7 pM. This compares with the K_D (6.5pM) derived from kinetic analysis of association and dissociation rates ($t_{\frac{1}{2}}$ on 12 min; $t_{\frac{1}{2}}$ off 45 min). ^{125}I-PIN binding was stereospecifically displaced by propranolol with approximately 100 fold difference in affinity between the isomers. Displacement of binding by adrenergic agonists indicated the pharmacological characteristics of the $beta_2$ adrenoceptor with a hierarchy of potency; isoprenaline > adrenaline > noradrenaline (IC_{50} 9.3 ± 1.2 x 10^{-8}M, 5.6 ± 0.4 x 10^{-7}M and 7.2 ± 1.4 x 10^{-6}M respectively). In addition highly selective beta adrenoceptor antagonists exhibited affinities for the binding site similar to those found at the $beta_2$ subtype in other tissues; ICI 118,551 ($beta_2$ selective) 3.8 ± 1.3 x 10^{-9}M and practolol ($beta_1$ selective) 1.9 ± 0.3 x 10^{-5}M.

These data demonstrate the presence of a high affinity, low capacity binding site on human platelet membranes with the pharmacological characteristics of the $beta_2$ adrenoceptor. The functional significance of these receptors and their interaction with the much higher density of platelet $alpha_2$ adrenoceptors is being investigated.

Supported by the British Heart Foundation

Barovsky, K. & Brooker, G. (1980) J Cyc Nuc Res, 6(4), 297
Cheung, Y.-D., Barnett, D.B. & Nahorski, S.R. (1982) Eur J Pharmacol, 84, 79
Jakobs, K.H., Saur, W. & Schultz, G. (1978) Naunyn-Schmeidbergs Arch Pharm, 302, 285
Steer, M.L. & Atlas, D. (1982) Biochem Biophys Acta, 686, 240

COMPARISON OF THE EFFECTS OF LASALOCID (X-537A) AND ENANATIO X-537A ON ADRENERGIC NERVE FUNCTION

B.J. Fitzsimmons[1], R.E. Ireland[1] and M. Spedding[2]; [1] Chemical Laboratories, California Institute of Technology, Pasadena, California, USA; [2] Centre de Recherche Merrell International, Strasbourg, France.

Lasalocid (X-537A) is an ionophore which transports divalent cations and complexes l-noradrenaline stereospecifically (Westley et al, 1977) and with high affinity (Pressman, 1973). X-537A may release noradrenaline by directly transporting the amine or indirectly by depolarizing adrenergic nerves (Thoa et al, 1974). The enantiomer of lasalocid (enantio X-537A, Ireland et al, 1983) has been prepared by total synthesis. As the enantiomer will have a different capacity to complex noradrenaline and to interact with biological membranes we have compared the two compounds in isolated tissues, with particular respect to adrenergic function.

Rat vas deferens preparations were set up in Tyrode solution (Spedding, 1980) and contractions obtained to field stimulation at 10 Hz for 1 sec every 2 min. X-537A (10 μM) and enantio X-537A (10 μM) caused a transient contraction of the vas deferens. This effect was small (14.4±3.1% and 8.0±0.9%, respectively of the maximum response to field stimulation) and was abolished by phentolamine (1 μM). The contraction was augmented by desmethylimipramine (1 μM) and occurred in the presence of tetrodotoxin (3.1 μM) and guanethidine (30 μM). X-537A and enantio X-537A (10 μM) also reduced the effects of field stimulation (time to 50% inhibition: 16.1±3.8 min and 19.6±4.2 min) and inhibited the contractile effects of a submaximal concentration of phenylephrine (10 μM) to the same extent. Thus, both compounds released noradrenaline and also had postsynaptic inhibitory effects.

X-537A (3-30 μM) and enantio X-537A (10-30 μM) increased the rate and developed tension of spontaneously beating rat atria preparations set up in Tyrode solution at 31°C. The increases in rate and tension were transitory and abolished by (±)-propranolol (1.7 μM). The compounds (30 μM) caused a slowly developing bradycardia which was resistant to atropine (3 μM) and terminated in asystole.

X-537A relaxes taenia preparations from the guinea-pig caecum by inhibiting oxidative metabolism in the smooth muscle cells (Ishida & Shibata, 1982). X-537A and enantio X-537A were equieffective as relaxants of Ca^{++}-induced contractions in K^{+}-depolarized preparations set up as previously described (Spedding, 1982). The EC 50s were 20.3±3.1 and 20.5±4.6 μM, respectively (n=4).

In conclusion, X-537A and enantio X-537A can both release noradrenaline from sympathetic nerves and have direct inhibitory effects on muscle cells. The configurationof X-537A is not important for either effect.

Ireland, R.E. et al (1984), J. Org. Chem., in press.
Ishida, Y. & Shibata, S. (1982), J. Physiol., 333, 293-304.
Pressman, B.C. (1973), Fed. Proc., 32, 1698-1703.
Spedding, M. (1980), Br. J. Pharmac., 71, 113-120.
Spedding, M. (1982), Naunyn-Schmiedeberg's Arch. Pharmac., 318, 234-240.
Thoa, N.B. et al (1974), Life Sci., 14, 1705-1719.
Westley, J.W. et al (1977), J. Am. Chem. Soc., 99, 6057-6061.

THE CARDIOTOXIC ACTION OF EXTRACT IV FROM THE SEA-ANEMONE TEALIA-FELINA

R.C. Elliott & Roseline S. Konya, Department of Applied Biology, Brunel University, Uxbridge, Middlesex, U.K.

Aldeen et.al. (1981a) and Elliott & Konya (1983) reported the partial purification of a toxin present in saline extracts of the sea-anemone Tealia felina. The toxin has in vitro histamionolytic and haemolytic actions which form the basis of a bioassay to determine the units of activity (AU) in extracts, Aldeen et.al. (1981a). In this communication we describe the toxic action of Extract IV (Elliott & Konya, 1983), on the blood pressure (BP) and the respiration of female Wistar rats (270-320g) anaesthetised with urethane. Doses below 20 AU/kg had no effect on the BP. All doses over 20 AU/kg were lethal, the time to death varying between 5 min. (90 AU/kg) and 2H (35 AU/kg). Following i.v. injection there was a rise in BP with a latency of 12s and a duration of 17 s.A. characteristic effect was marked bradycardia, with beats of irregular amplitude preceding death. The movement of the chest wall was recorded and a very transient increase in respiration rate was noted on injection, this was followed by a marked inspiratory effort lasting for c 12 s, this probably reflects the bronchoconstriction observed by Aldeen et.al. (1981b) following injections of Extract II. Respiration then showed a steady decline in amplitude and rate until death occurred. The amplitude of the contractions of the gastrocneumius muscle evoked by stimulation of the sciatic nerve only fell 10 min. after death indicating that paralysis of the respiratory muscles was an unlikely cause of death. A blood sample taken shortly before death did not show haemolysis. In some rats a marked fall in BP preceded cessation of respiration, in others respiration ceased before the complete decline in BP.

These experiments did not permit a conclusion to be drawn as to whether death was due to respiratory or cardiac failure. The in vitro rat heart preparation perfused with Krebs Hensleit solution at 37^{o}C was therefore used to study the effects of Extract IV. When a dose of 22.5 AU which would have been lethal to the rat was injected there was an immediate and progressive decline in the rate and force of the heart which ceased to beat after 1.2 min. If instead of injecting the extract the same dose was diluted in 1L of Krebs solution and continuously perfused through the heart, the same effect was observed. Six rat hearts were perfused with the extract, and cessation was observed in three after 19 mins., 10 mins. and 45 mins. respectively while there was no cessation in three of the preparations.

We conclude that cardiac failure was the most likely cause of death following injection of Extract IV in vivo. The cardiotoxic action of Extract IV resembles that of the toxin from the jellyfish Cyanea capillata studied by Walker (1977).

We are grateful to the Wellcome Trust for financial assistance in this work.

Aldeen, S.I. et.al. (1981a). Br. J. Pharmac. 72, 211-220.
Aldeen, S.I. et.al. (1981b). IRCS Med.Sci. 9, 703-704.
Elliott, R.C. & Konya, R.S. (1983). Br. J. Pharmac. 79 Proc.Suppl., 367P
Walker, M.J.A. (1977). Toxicon, 15, 15-27.

PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS CAN BE DIFFERENTIALLY ANTAGONISED

V.A. Alabaster and C.J. Peters, Department of Medicinal Biology, Pfizer Central Research, Sandwich, Kent, CT13 9NJ.

The activities of three antagonists, phentolamine, yohimbine and Wy 26703 (Lattimer et al. 1982), were compared at pre- and post-junctional alpha$_2$ sites in vitro to determine if these subtypes can be differentiated. Antagonist activity at the pre-junctional alpha$_2$ receptors was determined using the rat left atrium and vas deferens preparations. Post-junctional alpha$_2$ receptors are found on the saphenous vein of the dog (De Mey and Vanhoutte 1980) and the rabbit (Cambridge unpublished). The rabbit saphenous vein was used to assess antagonist potency at the post-junctional alpha$_2$ receptor. The selective, full agonist, UK-14,304 (Cambridge 1981) was used in all experiments.

The left atrium of the rat was bathed in Tyrode solution containing atropine 1uM and equilibrated with 95% O_2; 5% CO_2 at 31^{o}C under a resting tension of 0.5g. The tissue was paced at 1Hz, pulse width 0.1 mS at a threshold voltage. The atrium was field stimulated at the same frequency as the pacing electrodes, pulse width 2 mS, 5-6 volts, by electrodes placed on either side of the preparation. The two stimuli were synchronous. The field stimulus was applied for 1 min at intervals of 10 mins, and produced an inotropic response of about 0.5g tension, which in separate experiments was shown to be abolished by propranolol. The prostatic half of rat vasa deferentia was mounted in magnesium free Krebs-bicarbonate solution maintained at 37^{o} under a basal tension of 0.5g. The tissue was field stimulated via parallel platinum electrodes by single pulses at 0.1 Hz, 2 ms duration using a supramaximal voltage. One dose-response curve to UK-14,304 was obtained in atria and vasa deferentia 30 min after treating with a single dose or solvent control.

Saphenous vein rings of the rabbit were bathed in Krebs-bicarbonate solution containing propranolol 0.4uM, desipramine 0.1uM and normetanephrine 10uM, equilibrated with 95% O_2, 5% CO_2 at 37^{o}C at a resting tension of 2g. Dose-response curves to UK-14,304 were constructed before and 30 min after the addition of a single concentration of antagonist. Three concentrations of each antagonist were studied, and dose-ratios used to calculate pA_2 values according to the Schild relationship (Arunlakshana and Schild 1959). The results are shown in Table 1.

Table 1. pA_2 values and (slope) at post- and pre-junctional alpha$_2$ receptors.

Compound	Rabbit Saphenous Vein	Rat Left Atrium	Rat Vas Deferens
Phentolamine	7.42 (0.87)	7.50 (0.92)	------
Yohimbine	7.67 (0.92)	7.77 (1.05)	7.64 (1.13)
Wy 26703	6.25 (1.16)	8.52 (0.99)	8.21 (0.87)

The three antagonists were competitive in all preparations giving parallel shifts of the dose response curves and also Schild slopes not significantly different from unity. Phentolamine and yohimbine showed similar potency at both pre- and post-junctional alpha$_2$ sites, whereas Wy 26703 showed a 186 to 91 fold selectivity for the pre-junctional alpha$_2$ receptor. This large difference in antagonist affinity provides good evidence for a difference in receptor type between pre- and post-junctional alpha$_2$ adrenoceptors.

Arunlakshana O. and H.O. Schild (1959). Br. J. Pharmacol. Chemother. 14, 48.
Cambridge D. (1981). Eur. J. Pharmacol. 72, 413.
De Mey J. and P.M. Vanhoutte (1980). Arch. Int. Pharmacodyn. 244, 328.
Lattimer N. et al. (1982). Br. J. Pharmacol. 75, 154P.

THE EFFECT OF INCREASED PLASMA TOTAL TRYPTOPHAN ON PLASMA NON ESTERIFIED FATTY ACIDS IN MALE MICE

P.R. Gard† & S.L. Handley, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET

Displacement of tryptophan (TRP) from the albumin molecule by non-esterified fatty acids (NEFA) was reported *in vitro* by McMenamy and Oncley (1958). Thus in rats increased plasma NEFA produced a significant increase in plasma free (unbound) TRP, increased brain TRP and slightly decreased plasma total TRP (Knott & Curzon, 1972). Conversely Madras et al., (1973) reported that decreased plasma NEFA produced decreased plasma free TRP and increased plasma total TRP in rats, whilst in humans decreased plasma NEFA resulted in decreased total and free TRP. The possibility of a converse effect of TRP on plasma NEFA does not appear to have been investigated.

Male TO mice (30-35 g) were injected with L-TRP s.c., plasma samples were obtained thirty minutes post treatment and NEFA concentrations were determined by the fluorimetric method of Curzon and Kantamaneni (1977). The remaining plasma was then pooled and assayed for total TRP using the fluorimetric method of Denkla & Dewey (1967), as modified by Bloxam & Warren (1974).

Table I Effects of L-TRP s.c. on plasma NEFA. Differences from vehicle control *p = <.05; **p = <.005; ***p = <.0005

Dose L-TRP (mg/kg) s.c.	Pooled plasma total TRP (µg/ml)	Mean plasma NEFA (mEq/dm^3 ± sem)	N
Vehicle control	36.00	0.767 ± 0.053	6
10	30.11	0.590 ± 0.121	4
100	93.10	0.548 ± 0.131	4
150	98.85	1.177 ± 0.041***	6
200	119.54	1.165 ± 0.050***	6
300	212.64	1.040 ± 0.082*	6
400	258.62	1.238 ± 0.120**	5

The results presented in Table 1 showed that doses of L-TRP above 100 mg/kg all significantly increased plasma NEFA compared with the saline vehicle control. There was no significant linear relationship between plasma total TRP and NEFA (Pearson's r = .638, NS).

Fatty acids arise from three sources: dietary fat, hepatic de novo synthesis or synthesis in adipose tissue itself (Goodman, 1970). All of the animals received standard diet cubes *ad libitum*, hence it is unlikely that this effect is a result of dietary carbohydrate intake. Similarly NEFA may be released following the action of noradrenaline on β-adrenoceptors of adipose tissue, this is the mechanism by which NEFA are mobilised following stress (Bogdonoff et al., 1960). Again it is unlikely that the stress of the injection technique produced the rise in plasma NEFA, as no significant effects on NEFA were seen at the lower doses of L-TRP. The mechanism of this effect may be via hormonal control of NEFA mobilisation or due to an effect on sympathetic nervous system action.

Bloxam, D.L. & Warren, W.H. (1974) Anal. Biochem. 60, 621-625
Bogdonoff, M.D. et al (1960) J. Clin. Endocrinol. metab. 20, 1333-40
Denkla, W.D. & Dewey, H.K. (1967) J. Lab. Clin. Med. 69, 160-69
Goodman, H.M. (1970) The Physiologist 13, 75-88
Knott, P.J. & Curzon, G. (1972) Nature 239, 452-3
Madras, B.K. et al (1974) Adv. Biochem. Psychopharmacol. 11, 143-151
McMenamy, R.H. & Oncley, J.L. (1958) J. Biol. Chem. 233, 1436-47

† Present address: Department of Pharmacy, Brighton Polytechnic

ASSESSMENT OF α-ADRENOCEPTOR AGONIST ACTIVITY IN THE ISOLATED, PERFUSED GUINEA PIG PORTAL VEIN OF α_2-ADRENOCEPTOR ANTAGONISTS

R.M. Eglen, S. Fraser and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

RS-21361 (Michel & Whiting, 1981), RX-781094 (Doxey et al, 1983) and Wy 26703 (Lattimer et al, 1982) are selective α_2-adrenoceptor antagonists. RX-781094 has been reported to possess α_1-adrenoceptor agonist activity both *in vitro* (Dalrymple et al, 1983) and *in vivo* (Dalrymple et al, 1983; Paciorek & Shepperson, 1983).

The present study was designed to compare the α-adrenoceptor agonist activity of RS-21361, RX-781094 and Wy 26703 with standard α_1- and α_2-adrenoceptor agonists using the isolated, perfused guinea pig portal vein. The technique used was that of Patmore & Whiting (1983). Helically cut portal veins from female Dunkin-Hartley guinea pigs were perfused at a rate of 6 ml.min^{-1} under 0.5 g tension with warm (37^{0}C) physiological salt solution. Compounds were assessed for α_1-adrenoceptor agonist activity using a 4 min contact period and a 15 min dose cycle. Each tissue was exposed to only one agonist. In experiments where prazosin or yohimbine were used, at least 30 min was allowed for equilibration.

The preparation responded in a biphasic manner, producing both a fast and slow response, to either phenylephrine (10^{-8} - 10^{-4} mol.litre^{-1}) or noradrenaline (2.1×10^{-6} - 2.1×10^{-3} mol.litre^{-1}). The α_2-adrenoceptor agonist, xylazine (10^{-8} - 10^{-4} mol.litre^{-1}) was without effect. RX-781094 (10^{-7} - 10^{-4} mol.litre^{-1}) produced a slow response only whereas RS-21361 and Wy 26703 (10^{-8} - 10^{-4} mol.litre^{-1}) were without effect. The EC_{50} values, determined by regression analysis, for all compounds are shown in Table 1.

COMPOUND	EC_{50} (mol.litre^{-1}) FAST	SLOW	n
Phenylephrine	6.04×10^{-7}	3.77×10^{-7}	4
Noradrenaline	2.23×10^{-5}	2.68×10^{-5}	4
RX-781094	No response	5.15×10^{-6}	4
Wy 26703	No response	No response	4
RS-21361	No response	No response	4

Table 1 EC_{50} values for contractile responses in guinea pig portal vein *in vitro*

The biphasic responses to phenylephrine and noradrenaline were antagonised by the α_1-adrenoceptor antagonist, prazosin (10^{-8} - 5×10^{-7} mol.litre^{-1}), but not by the α_2-adrenoceptor antagonist, yohimbine (10^{-8} - 5×10^{-7} mol.litre^{-1}). Therefore, the perfused guinea pig portal vein preparation produces a biphasic contraction only in response to α_1-adrenoceptor stimulation and is devoid of postsynaptic α_2-adrenoceptors. The slow response to RX-781094 was antagonised by prazosin but not by yohimbine. Therefore, the contracture evoked by RX-781094 was due to α_1-adrenoceptor stimulation. In contrast to RX-781094, neither RS-21361 nor Wy 26703 exhibited any α_1-adrenoceptor agonist activity in this preparation.

Dalrymple, H.W. et al (1983) Br.J.Pharmac. 78, 128P.
Doxey, J.C. et al (1983) Br.J.Pharmac. 78, 489-505.
Lattimer, N. et al (1982) Br.J.Pharmac. 75, 154P.
Michel, A.D. & Whiting, R.L. (1981) Br.J.Pharmac. 74, 255P.
Paciorek, P.M. & Shepperson, N.B. (1983) Br.J.Pharmac. 79, 12-14.
Patmore, L. & Whiting, R.L. (1983) Fed.Proc. 42, 5577.

THE ACTION OF SULPHASALAZINE, INDOMETHACIN AND BW-755C ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL ACTIVATION BY PHA

K. Hillier, D. B. Jones[1] and S. Pacheco[2], Clinical Pharmacology Group, University of Southampton, Southampton SO9 3TU, [1]Pathology Department, Southampton General Hospital and [2]Department of Biology, University of Vicosa, 36570 MG, Brazil.

Sulphasalazine (S) which is used empirically in the treatment of inflammatory bowel disease (IBD) inhibits activation of peripheral blood mononuclear cells (PMNC) by PHA in guinea pig (Ali et al, 1982) and human (Holdstock et al, 1981). Prostaglandin E_2 (PGE_2) shares this action (Goodwin et al, 1977). It is suggested that enhanced eicosanoid levels in the mucosa of patients with IBD is derived mainly from mononuclear cells and that this may be important in the pathogenesis of the disease (Hillier et al, 1982; Zifroni et al, 1983).

In cultured tissues S has been shown to variously enhance or reduce PG levels and we are attempting to determine if S inhibits activation of PMNC by mitogens via effects on cyclooxygenase or lipoxygenase derived metabolites of arachidonic acid. PMNC were treated with PHA and activation quantitated by determining (^3H)-thymidine uptake after 48h culture. S inhibited activation dose dependently. 50ug/ml S added at 0h or 24h of culture inhibited by 49.5% $\pm$ 18.3 (mean $\pm$ SD; N = 15) and 45.5% $\pm$ 20.2 (N = 9); 100µg/ml similarly inhibited by 89.5 $\pm$ 16.3 (N = 12) and 83.8 $\pm$ 15.7 (N = 9) respectively.

Indomethacin (0.01 to 10µg/ml) present throughout culture had no significant effect on activation and 1 and 5µg/ml did not prevent S inhibiting activation when the latter was added at 0 or 24h.

Measurement of PGE_2 by radioimmunoassay showed that S (50µg/ml) enhanced PGE_2 levels by 56.27% $\pm$ 29.37 (N = 4) after 5h of culture in non-activated PMNC.

BW755C (2.5µg/ml) did not affect activation but 80µg/ml inhibited activation by 96.5% $\pm$ 4.8 (n = 8). The lower dose did not significantly affect inhibition of activation by 50µg/ml S.

Our results suggest that the inhibitory effect of S on PHA-induced activation is not mediated by cyclooxygenase derived products of arachidonic acid metabolism. The inhibition of activation by BW755C in dosage likely to inhibit most lipoxygenase enzymes may implicate the lipoxygenase products in activation processes.

Cell viability was not affected by drug dosage employed as measured by the trypan blue technique.

S.P. is supported by The British Council and MEC, Brazil.

Ali, A. G. M. M. et al (1982) Lancet, i, 506-507.
Goodwin, J. S. et al (1977) N. Engl. J. Med. 297, 963-968.
Hillier, K. et al (1982) Br. J. Pharmac. 76, 157-161.
Holdstock, G. et al (1981) Gut, 22, 1025-1030.
Zifroni, A. et al (1983) Gut, 24, 659-664.

HISTAMINE RELEASE FROM LEUCOCYTES OF PATIENTS SUFFERING FROM CATERPILLAR DERMATITIS

Pamela Graham, J.M. Harris & Dianne G. Nelson, Clinical Pharmacy Unit, Brighton General Hospital and Department of Pharmacology, School of Pharmacy, Brighton Polytechnic, Brighton BN2 4GJ

Dermatitis due to the browntail moth caterpillar (*Euproctis chrysorrhoea*) continues to be a seasonal problem particularly along the south-east coast. The rash comprises intensely pruritic maculo-papules or weals which may last for weeks (Blair, 1979). There is speculation whether or not the underlying mechanisms include an immunological component. Earlier studies suggest that dermatitis results from contact of human skin with specialised 'nettling' hairs found on tubercles along the caterpillar's abdomen. Caterpillar skin can synthesize histamine and also contains 5-hydroxytryptamine and other mediators of inflammation (Graham et al, 1981). We have now measured the amount of histamine and 5HT in tubercles and nettling hairs alone and have investigated the capacity of human leucocytes to release histamine when incubated with an extract of the hairs.

Aqueous extracts for histamine assay and n-butanol extracts for 5HT assay were made, 70 caterpillars being needed to provide enough hairs for one 5HT determination. The histamine content of the nettling hairs, 889 µg/g dry weight, was much greater than that of the tubercles, 310 µg/g, and of whole skin, 122 µg/g. The pattern was similar for 5HT though the differences were less marked, mean values being 5.55 µg/g; 4.02 µg/g and 3.47 µg/g respectively.

Using the method of May et al (1970), measurements were made of histamine release from leucocytes of 12 patients with dermatitis and from 4 of their neighbours who were symptom-free after direct contact with caterpillars. Leucocytes (0.5 - 1.0 x 10^7) were incubated at 37°C in Tris ACM buffer, pH 7.6, with or without 1.0 mg of dialysed, lyophilized nettling hair extract reconstituted in buffer. After centrifugation the histamine in the supernatant was assayed fluorimetrically. Released histamine was expressed as a percentage of the total cell content with a correction for spontaneous release. Values ranged from 4% to 36.5% with a mean of 17.0% in the patients and from 0 to 1.2%, mean 0.6% in the symptom-free. The difference was statistically significant ($p < 0.05$). One of the 4 non-reactors and 9 of the 12 patients had a history of atopy. Although *specific* histamine release in response to extract was not higher in atopic subjects, a significant difference ($p < 0.05$) in *spontaneous* release was observed, 21.84% ± 6.58 compared with 5.17% ± 1.62 for non-atopics.

Our results demonstrate that the histamine and 5HT contained in caterpillar skin are concentrated in the nettling hairs, but considerably less than 1 µg would be introduced into human skin even if all the hairs from a caterpillar penetrated at one site. This evidence strengthens our previously stated belief that these mediators are not responsible *per se* for the widespread reaction produced. It was shown by de Jong & Bleumink (1977) that these hairs contain phospholipase A_2 and they postulated that this releases histamine indirectly by generating lysolecithin. However in this case one would expect the leucocytes of all individuals to behave similarly, whereas our results clearly show a difference. An immunological mechanism remains a distinct possibility and we are currently measuring serum immunoglobulin levels in some 30 of the many local people affected by the caterpillars during 1983.

Blair, C.P. (1979) Clin.Exp.Derm. 4, 215-222
Graham, P., Harris, J.M. & Nelson, D.G. (1981) Br.J.Pharmac. 74, 983P
Jong, M.C.J.M. De & Bleumink, E. (1977) Arch.Derm.Res. 259, 263-281
May, C.D., Lyman, M., Alberto, R. & Cheng, J. (1970) J.Allergy 46, 12-20

ALTERATIONS IN GASTROINTESTINAL MOTILITY ASSOCIATED WITH ERYTHROMYCIN

M.A. Pilot, H.D. Ritchie, H.H. Thompson & G.P. Zara (Introduced by M. Chaput de Saintonge), Surgical and G.I. Science Research Units, The London Hospital Medical College, 26 Ashfield St., London E.1 2AJ

Erythromycin is known to cause gastrointestinal side-effects, namely abdominal cramps, diarrhoea, nausea and vomiting, mostly with oral therapy although these symptoms have also been reported with intravenous administration. The mechanism responsible for these effects is unclear. The present studies were undertaken to investigate whether erythromycin has an effect on the motility patterns of the stomach and small intestine in the fasted conscious dog. During fasting, mammalian gastrointestinal motility is characterised by the migrating motor complex (MMC). This consists of periodic bursts of contractions which originate proximally in the stomach and propagate distally to the terminal ileum.

Silver electrodes were implanted on the serosa of the small intestine of 5 dogs under halothane/nitrous oxide anaesthesia. Two of the dogs also had force transducers on the proximal stomach, antrum and duodenum. Two weeks were allowed for recovery. In each study, after a fast of 18 hours, electrical and mechanical activity was recorded on magnetic tape. Following a basal period of three hours, erythromycin lactobionate was infused i.v. at the rate of 1 or 7 mg/kg over 30 minutes. The infusion was started 7 to 30 minutes following an MMC in the duodenum, and was followed by a further 3 hours of recording.

During i.v. infusion of the lower dose of erythromycin (1 mg/kg), a burst of contractions appeared in the proximal stomach and was propagated distally to the ileum. Propagation of the previous MMC was abolished, and the only propagation seen was that of the premature erythromycin-induced MMC. Premature MMC's are also known to be triggered by motilin and morphine. Following this, normal fasting activity was altered, and continuous irregular activity was observed at all recording sites. With the dose of 7 mg/kg there was an immediate increase in activity at all electrode sites, which was more pronounced proximally, and all the animals vomited. Regular fasting activity did not return for at least 3 hours after the infusion. Continuous irregular contractile activity occurred in the stomach and proximal intestine. In the distal intestine, a period of inactivity followed by an occasional MMC starting in the lower jejunum and propagating distally was observed. Two hours after the infusion at both doses the shape of the electrical slow wave became irregular and disorganised.

These observations suggest that i.v. erythromycin stimulates the motility of the canine gastrointestinal tract. Such alterations may be partly responsible for the gastrointestinal side-effects reported by patients receiving treatment with this antibiotic.

DISOPYRAMIDE PRODUCES NON-COMPETITIVE, VOLTAGE-DEPENDENT BLOCK AT THE NEUROMUSCULAR JUNCTION

A.L. Harvey, S.V.P. Jones & I.G. Marshall, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW

Disopyramide in addition to its Class 1 and 3-type antiarrhythmic actions is known to produce an anticholinergic effect on the heart (Mirro et al, 1980). The compound has also been shown to possess nicotinic blocking actions at the skeletal muscle neuromuscular junction (Healy et al, 1981) and at autonomic ganglia (Byrne et al, 1981). A local anaesthetic action has also been reported (Baines et al, 1976). We have now studied the effects of disopyramide in greater detail at the neuromuscular junction in an attempt to elucidate the mechanism of its blocking action at this site.

In the indirectly stimulated (0.1 Hz) chick biventer cervicis preparation, disopyramide (10^{-4}M-10^{-3}M) produced a concentration-dependent reduction of twitch amplitude. Twitches elicited directly in the presence of erabutoxin-b (1 µg/ml) were only reduced by concentrations of disopyramide above those producing complete block of indirectly elicited twitches. Equieffective twitch blocking concentrations of both disopyramide (6×10^{-4}M) and tubocurarine (3×10^{-6}M) greatly reduced agonist responses to acetylcholine (90 ± 4% and 47 ± 6% respectively, mean ± s.e. n = 6) and carbachol (95 ± 4% and 100 ± 0% respectively, n = 6) whereas when twitches were reduced by magnesium (7×10^{-3}M) there was a lesser reduction of responses to acetylcholine (9 ± 3%) and carbachol (38 ± 5%). Twitch block produced by disopyramide was not reversed by neostigmine (3×10^{-8}M) whereas tubocurarine-induced block was reversible by the anticholinesterase.

Concentration-response lines to acetylcholine and carbachol were shifted to the right in a non-parallel fashion and the maximal response depressed by disopyramide (5×10^{-5}M-10^{-4}M). In contrast tubocurarine produced parallel shifts and yielded a pA_2 value against carbachol of 6.17 ± 0.15 (n = 5).

Intracellular recording studies were carried out in the cut costocutaneous muscle of the garter snake (Fiekers et al, 1983), voltage clamped at membrane potentials from -50 to -130 mV. Epcs decayed as a single exponential at all holding potentials in the absence and presence of disopyramide. Disopyramide (5×10^{-5}M - 5×10^{-4}M) produced a concentration and voltage dependent reduction of the amplitude of neurally evoked endplate-currents (epcs) and of the time constant of decay (τ) of epcs. For example disopyramide (10^{-4}M) reduced τ_{epc} from 1.28 ± 0.15 ms to 0.73 ± 0.10 ms (n = 5) at -50 mV (a 43% reduction) but at -90 mV reduced τ_{epc} from 2.13 ± 0.35 ms to 0.74 ± 0.08 ms (a 65% reduction). Thus disopyramide produced a greater shortening of τ at hyperpolarised membrane potentials. The voltage dependence of τ_{epc} conformed to the relationship $\tau(V_m) = \tau(0)\, e^{-V_m/H}$ where H is the change in membrane potential required to produce an e fold change in τ. In disopyramide (10^{-4}M) H increased from -80 ± 6mV to 1058 ± 394mV i.e. the voltage dependence of τ was reduced.

We conclude that disopyramide possesses a non-competitive blocking action at the neuromuscular junction, which is not reversible by anticholinesterase agents. The voltage dependent nature of the block suggests that it is mediated via blockade of the open form of the acetylcholine activated receptor-ion channel complex.

SVPJ holds an SERC studentship.

Baines, M.W. et al (1976) J.Int.Med.Res. 4(Suppl 1), 5-7
Byrne, A.J. et al (1981) Acta Anaesth.Scand. 25, 275-278
Fiekers, J.F. et al (1983) J.Pharmac.exp.Ther. (in press)
Healy, T.E.J. et al (1981) Br.J.Anaesth. 53, 495-498
Mirro, M.J. et al (1980) Circ.Res. 47, 855-865

ANTAGONISM BY MDL 72222 OF 5-HT-INDUCED DEPOLARISATIONS OF THE RAT ISOLATED VAGUS NERVE AND SUPERIOR CERVICAL GANGLION

D.H. Fortune & S.J. Ireland, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODS.

5-Hydroxytryptamine (5HT) receptors on mammalian peripheral neurones appear distinct from those present on smooth muscle, although subtypes of neuronal receptor may exist (Wallis, 1981). Recently, Fozard (1983) has reported that tropine 3,5-dichlorobenzoate (MDL 72222) was a potent and selective antagonist of the neuronally mediated stimulant effect of 5HT on the rabbit isolated heart. 5HT-induced depolarisation of the rat isolated vagus nerve (VN) and superior cervical ganglion (SCG), and 5HT-induced stimulation of the rabbit heart are resistant to methysergide, although metoclopramide behaves as a competitive antagonist of 5HT at all three sites (Fozard & Mobarok Ali, 1978; Ireland et al., 1983). It was therefore of interest to examine the effect of MDL 72222 on 5HT responses on the VN and SCG. The effects of the compound against depolarisations induced by GABA, DMPP and muscarine have also been examined on the SCG.

Male Lister hooded rats (270-330g) were anaesthetised with chloral hydrate (300mg/kg ip) and the VN or SCG was excised and desheathed. Agonist-induced depolarisations were recorded extracellularly from VN or SCG preparations mounted in 2-compartment perspex baths, (Ireland et al., 1982). On the SCG, 5HT uptake was prevented by the inclusion of paroxetine ($1x10^{-6}M$) in the superfusion medium (Ireland et al., 1983).

On the VN, MDL 72222, $3x10^{-8}M$-$1x10^{-6}M$, caused rightwards shifts of the 5HT concentration - depolarisation curve, although this was accompanied by a fall in both the slope and maximum response (E_{100}). In the presence of MDL 72222, $1x10^{-6}M$, the maximum response approximated to only 50% of the control E_{100}. Therefore 5HT equipotent molar ratios were measured from the control curve E_{25}. A plot of log (dose-ratio-1) against log concentration of antagonist (Arunlakshana & Schild, 1959) appeared to be non-linear, thereby preventing the estimation of a pA_2 value. As an indication of potency, the mean 5HT dose-ratio for MDL 72222, $3x10^{-7}M$, was 28.7 $\pm$ 7.0 (n=4).

On the SCG, MDL 72222, $3x10^{-7}M$, caused a rightwards shift of the 5HT concentration-depolarisation curve. The mean 5HT dose-ratio measured at the E_{25} was 12.7 $\pm$ 3.9 (n=4) which is similar to the result obtained with this concentration of the 5HT antagonist on the VN. In contrast, for the rabbit heart, Fozard (1983) quoted a pA_2 value of 9.27 for MDL 72222. Therefore, the 5HT dose-ratio expected for MDL 72222, $3x10^{-7}M$, would be approximately 560 - clearly greater than that obtained on the rat neuronal tissues. On the SCG, MDL 72222, $3x10^{-7}M$, had no significant effect against depolarisations induced by GABA, or muscarine, although at $3x10^{-5}M$, MDL 72222 produced a weak inhibition of the effects of DMPP and muscarine.

In conclusion, MDL 72222 was found to be a potent and selective antagonist of 5HT-induced depolarisations of the rat VN and SCG. However, on the VN, the behaviour of the 5HT antagonist was not consistent with a simple competitive action. Therefore the significance of the apparent difference in the 5HT antagonist potency of MDL 72222 on the rat and rabbit neuronal preparations remains to be determined.

Arunlakshana, O. & Schild, H.O. (1959) Br.J.Pharmac. Chemother. 14, 48-58.
Fozard, J.R. & Mobarok Ali, A.T.M. (1978) Eur.J.Pharmac. 49, 109-112.
Fozard, J.R. (1983). Br.J.Pharmac., Proc. Suppl. in press.
Ireland, S.J. et al. (1982) Br.J.Pharmac. 75, Proc. Suppl. 16P.
Ireland, S.J. et al. (1983) Br.J.Pharmac. 78, Proc. Suppl. 68P.
Wallis, D. (1981) Life Sci. 29, 2345-2355.

THE ELECTROPHYSIOLOGICAL EFFECTS OF PROPAFENONE ON GUINEA-PIG PAPILLARY MUSCLES

C. Delgado, J. Tamargo & T. Tejerina .Department of Pharmacology , Medical School ,Universidad Complutense ,Madrid-3 ,Spain .

Propafenone,(2´-[3-propylamino)-2-(hydroxy)-propoxy]-3-phenyl-propiophenone HCl, is a new antiarrhythmic agent which is highly effective against chronic recurrent supraventricular tachycardia (Waleffe et al, 1981) .We have studied the electrophysiological effects of propafenone in guinea-pig isolated atria obtained from non-treated animals and in atria obtained from guinea-pigs treated with propafenone (1.5 and 5 mg/kg twice daily i.p. for 24 days).

Guinea-pig atria were perfused with Tyrode solution at 34°C, bubbled with 95%O_2 and 5%CO_2 and stimulated at a basal rate of 1 Hz .Action potentials were recorded with conventional glass microelectrodes .

In non-treated atria , propafenone (10^{-7}M - $5x10^{-5}$M) caused a concentration-dependent decrease in amplitude and maximum rate of rise of the action potential and reduced the resting membrane potential . At 10^{-5}M and $5x10^{-5}$M, propafenone prolonged the action potential duration measured at 90% of repolarization (APD_{90}) and the effective refractory period without altering the APD_{50} .At 10^{-4}M, the resting membrane potential was decreased to -68.7 $\pm$ 2.2 mv (n=18. $p < 0.001$) and the fibres became inexcitable within 10 min .Propafenone (10^{-6}M-10^{-5}M) not only caused a negative chronotropic effect but also suppressed the abnormal automaticity induced by isoprenaline ,$BaCl_2$ or ouabain in atrial fibres .Furthermore ,propafenone (10^{-6}M - 10^{-4}M) depressed the amplitude and maximum rate of rise of the slow action potentials elicited by isoprenaline in high K (27 mM) Tyrode solution ; at concentrations higher than 10^{-6}M these effects were accompanied by a progressive reduction of the resting membrane potential and by a shortening of the APD_{50} and APD_{90}.

Action potential characteristics obtained from pretreated guinea-pig atria were not statistically different from the values obtained in non-treated atria .Propafenone, as in non-treated atria, reduced the amplitude and maximum rate of rise of the action potential ,depolarized the resting membrane potential and prolonged the APD_{90}, but all these changes were more marked in treated than in non-treated atria . Moreover ,in treated atria, propafenone (10^{-5}M - $5x10^{-5}$M) significantly prolonged the APD_{50} .

It therefore appears that in guinea-pig atria the cardiodepressant effects of propafenone are mainly related to its ability to inhibit Na and Ca conductances, this effect being potentiated in pretreated atrial preparations .

Waleffe et al (1981)Eur.Heart J. 2, 345-352.

EFFECT OF PY 108-068, A NEW CALCIUM ANTAGONIST, ON ISOLATED CARDIAC PREPARATIONS

J. Tamargo & T. Tejerina .Department of Pharmacology ,School of Medicine ,Universidad Complutense ,Madrid-3 ,Spain .

PY 108-068 (PY) is a new benzoxadiazolyle dihydropyridine which has been shown to exhibit potent and selective calcium antagonistic properties on vascular smooth mucsle fibres (Hof et al, 1982 ; Hof,1983). In the present report the effects of PY were studied on isolated rat atria and guinea-pig papillary muscles .

Spontaneously beating right atria and left atria driven at 3 Hz were suspended in Tyrode solution (32°C) .Guinea-pig papillary muscles were perfused at 7 ml/min with Tyrode solution (34°C) and driven at a basal rate of 1 Hz. Slow contractions and slow action potentials were elicited by adding isoprenaline to high K (27 mM) Tyrode solution and the muscles were stimulated at 0.2 Hz .Action potentials were recorded with glass microelectrodes .

Cumulative addition of PY (10^{-10}M - 10^{-5}M) to spontaneously beating right atria caused a concentration-dependent decrease in atrial rate, peak contractile force ,work index and maximum following frequency, and at concentrations higher than 10^{-7}M prolonged the sinus node recovery time .In isolated left atria,PY also caused a negative inotropic effect which was accompanied by a parallel decrease in peak df/dt. No effect was observed in the time to peak contractile force ,time for total contraction or the threshold current required to elicit a contractile response .PY produced a parallel shift in the relation between contractile force and Ca concentration (0.9 - 7 mM) to the right and decreased the amplitude of the slow contractions in depolarized atria (IC50 = 4.2×10^{-8}M) .In the same conditions, IC50 of verapamil was 4.3×10^{-7}M .The negative inotropic effect of PY was observed at all frequencies of stimulation (0.2 - 5 Hz) but it was more pronounced at higher frequencies of stimulation .

In guinea-pig papillary muscles PY (10^{-10}M - 10^{-5}M) caused a progressive shortening of the action potential duration measured at both 50% and 90% levels of repolarization and of the effective refractory period .No effect was observed at these range of concentrations on phase 0 characteristics of the action potential .However ,PY decreased the amplitude and maximum rate of rise and shortened the duration of the slow action potentials elicited by isoprenaline .

These results indicate that PY exerted on isolated rat atria and guinea-pig papillary muscles potent and selective calcium antagonistic properties and thus confirm previous results obtained in vascular smooth muscle .

Hof, R. et al (1982) J.Cardiovasc.Pharmac. 4,344-351.
Hof, R. (1983) Br.J.Pharmac. 78, 375-394 .

AFFINITY OF BUCINDOLOL TO α- AND β-ADRENOCEPTOR SUBTYPES AS EVALUATED BY RADIO-LIGAND BINDING STUDIES

O.-E.Brodde & N.O'Hara. Div. Renal & Hypertensive Diseases,Med. Klinik & Poliklinik, University of Essen, D-4300 Essen, Fed. Rep. Germany

Bucindolol, 2-[2-hydroxy-3-[(2-(3-indolyl)-1,1-dimethylethyl)amino] propoxy] benzonitrile hydrochloride, a new antihypertensive agent currently under investigation, has been shown to exhibit potent ß-adrenoceptor (R) antagonistic activity (Oates et al.,1981). Recently, however, Hamilton & Reid (1983) provided evidence that the affinity of bucindolol to α_1-R may be even higher than to ß-R. The aim of the present study was therefore, to further characterize the adrenergic profile of bucindolol. For this purpose the affinity of bucindolol to α- and ß-R subtypes was determined by radio-ligand binding studies.

ß-R - using (±)-^{125}iodocyanopindolol binding - were investigated in rabbit lung membranes (heterogeneous β_1- and β_2-R population, Brodde et al., 1983), rabbit right ventricle membranes (homogeneous β_1-R population, Brodde et al., 1982a) and human lymphocytes (homogeneous β_2-R population, Brodde et al., 1981). α_1-R were studied in rat liver membranes by ^{3}H-prazosin binding (labelling only α_1-R, Hoffman et al., 1981) and α_2-R were studied in human platelet membranes by ^{3}H-yohimbine binding (labelling only α_2-R, Brodde et al., 1982b). From the displacement curves IC_{50}-values were calculated and converted into K_I-values by the equation of Cheng & Prusoff (1973). The results are summarized in Table 1.

Table 1. K_I-Values (nM) for Inhibition of Binding to α- and ß-R Subtypes by Bucindolol, Propranolol, Prazosin and Yohimbine

R-Subtype	Bucindolol	Propranolol	Prazosin	Yohimbine
β_1- and β_2	0.55±0.07	3.2±0.22	---	---
β_1	2.2 ±0.33	9.7±1.1	---	---
β_2	1.2 ±0.1	4.5±0.31	---	---
α_1	673 ± 86	---	0.57±0.07	884 ± 96
α_2	1811 ±244	---	1500 ±123	3 ±0.16

Given are means ± S.E.M. of 3-7 experiments.

These results demonstrate that bucindolol is an non-selective ß-R antagonist with an affinity slightly higher than propranolol. Its affinity to α-R is only moderate.

Brodde,O.-E. et al. (1983). N.-S. Arch. Pharmacol. 322, 20-28.
Brodde,O.-E. et al. (1982a). J. Cardiovasc. Pharmacol. 4, 34-43.
Brodde,O.-E. et al. (1982b). Arch.int.Pharmacodyn.Ther. 257, 193-207.
Brodde,O.-E. et al. (1981). Life Sci. 29, 2189-2198.
Cheng,Y. & Prusoff,W.H. (1973). Biochem. Pharmacol. 22, 3099-3108.
Hamilton,C.A. & Reid,J.L. (1983). Br. J. Pharmacol., 80 Suppl. 536P.
Hoffman,B.B. et al. (1981). Life Sci. 28, 265-272.
Oates,H.F. et al. (1981). Arch.int.Pharmacodyn.Ther. 251, 95-102.

ONTOGENESIS OF HISTAMINE, HISTIDINE DECARBOXYLASE AND HISTAMINE N-METHYLTRANSFERASE IN CHICK BRAIN

J.M. Brine, D.J. Keeling[1], Judith A. Poat, I.R. Smith[1] and G.N. Woodruff[2], Department of Physiology and Pharmacology, University of Southampton, Southampton, SO9 3TU, [1]Smith Kline and French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR and [2]Neuroscience Research Centre, Merck Sharpe and Dohme Ltd., Hoddesdon, herts.

Histamine (HA) may play a neurotransmitter role in brain. Levels of established neurotransmitters and their biosynthetic enzymes are known to increase as synaptogenesis occurs (Coyle and Axelrod, 1972). The present study reports the developmental court of HA and its associated enzymes in chick brain.

Fertilised eggs and Rhode Sussex chicks of either sex were used. Whole brains were weighed and homogenised in either 10 vol. 0.1 M phosphate buffer pH 7.2 (for enzyme assay) or in 2 vol. 0.1 M perchloric acid (for HA determination). Histamine N-methyltransferase (HMT) activity was determined according to Snyder and Axelrod (1965). Histidine decarboxylase (HDC) activity was determined by the coupled assay method of Keeling et al. (1982). HA in homogenates was determined by the radioenzymatic method using a modification of procedure described by Gristwood et al. (1981).

Measurements were made from 10 days prior to hatching to 16 days post hatching. Brain weight rose steadily throughout the period studied, and most rapidly 8-4 days prior to hatching. Similarly protein concentration showed a marked increase in the 4 days prior to hatching, varying only slightly in the post hatch phase (72 ± 3 mg/g wet wt. at 4 days pre-hatch, 104 ± 3 at 3 days old and 124 ± 7 in adult birds, mean ± s.d., n = 6). HA concentration rose rapidly during both pre- and post-hatch phases (1.08 ± 0.14 ng/mg protein at 3 days old, this being 10 times greater than 10 days pre-hatch brain concentrations). Both HMT and HDC showed marked increases in activity in the pre-hatch phase. Thus HMT activity increased 2.6 fold 7-4 days prior to hatching (2.05 ± 0.30 p.mol/min/mg protein at 7 days pre-hatch). Activity was greatest in 3 day old chicks (7.6 ± 0.5 compared with 3.9 ± 0.5 p.mol/min/mg in 16 day old birds). HDC activity increased 2.8 fold in the 8 days prior to hatching (0.095 ± 0.016 f.mol/min/mg protein at 8 days pre-hatch). The greatest activity coincided with hatching (0.27 ± 0.04 compared with 0.11 ± 0.01 f.mol/min/mg at 16 daus old).

These sharp rises in enzyme activity in the pre-hatching period coincide with periods of synaptic maturation and proliferation in chick embryo spinal cord as assessed histologically (Oppenheim and Foelix, 1972) and the onset of spontaneous electrical activity in the cerebral hemispheres. Thus the results are not inconsistent with a neurotransmitter role for histamine in chick brain.

J.M.B. is an SERC/CASE Award student in conjunction with SK and F.

Coyle, J.T. & Axelrod, J. (1972) J. Neurochem. 19, 449-459
Gristwood, R.W., Lincoln, J.C.R., Owen, D.A.A. & Smith, I.R. (1981) Br. J. Pharmac. 74, 7-9
Keeling, D.J., Smith, I.R. and Tipton, K.F. (1982) Br. J. Pharmac. 77, Proc. Suppl. 561P
Oppenheim, R. & Foelix, R. (1972) Nature, 235, 126-128
Snyder, S.H. & Axelrod, J. (1965) Biochim. Biophys. Acta. 111, 416

ETHANOL INHIBITS THROMBIN-STIMULATED BREAKDOWN OF PLATELET MEMBRANE PHOSPHOLIPIDS

C.G. Fenn & J.M. Littleton, Department of Pharmacology, King's College, Strand, London WC2R 2LS.

Experiments on the inhibitory effect of ethanol on platelet aggregation induced by a variety of aggregating agents suggest that ethanol inhibits a platelet phospholipase probably of the A_2 type (Fenn & Littleton, 1983). Work on radiolabelled phosphatidylcholine incorporated into synaptosomes (Littleton & Nhamburo, 1983) also showed that ethanol inhibited a phospholipase A_2 in brain. Here we report the effects of ethanol in vitro on the breakdown of radiolabelled phospholipids in platelets stimulated by thrombin.

[1-^{14}C] arachidonic acid ([^{14}C]AA) was obtained from Amersham and 1-acyl-2-([^{3}H]-oleoyl) phosphatidylcholine ([^{3}H]oleoyl PC) was prepared by the method of Lands (1960). Human platelet-rich plasma was incubated for 60 minutes with [^{14}C]AA and with [^{3}H]oleoyl PC which had previously been dispersed by sonication in platelet poor plasma. Platelets were then gel-filtered and investigated for the effects of ethanol on thrombin-induced breakdown of phospholipids. The concentration of thrombin used (1 Uml^{-1}) caused rapid aggregation in stirred platelet preparations which was inhibited by the presence of ethanol (100 mM). All subsequent experiments were performed on unstirred platelets. After 5 minutes incubation with saline (controls), thrombin, or thrombin plus ethanol, the reactions were stopped with butylated toluene in chloroform:methanol and lipids were extracted overnight at 0°C. Phospholipids were separated on thin-layer silica gel chromatographic plates, visualised with iodine and scraped for scintillation counting.

The incorporation of [^{14}C]AA into platelets was mostly into PC (60.25 ± 5.6%) with similar amounts (11-12%) in each of the other phospholipids separated (P.S., PI, PE). Thrombin caused a significant fall in the [^{14}C]AA associated with phospholipids and this was prevented by ethanol. The incorporation of [^{3}H]-oleoyl PC into platelets was mainly (92.1 ± 2.6%) into PC but small amounts (3-4%) were also found in PI and PE presumably by transacylation reactions. Thrombin caused a significant fall in the [^{3}H]-oleic acid associated with phospholipids and this was almost completely prevented by the presence of ethanol. These results indicate that ethanol markedly inhibits the thrombin-stimulated breakdown of PC when labelled in the 2 position with [^{3}H]-oleic acid and probably also when labelled with [^{14}C]AA. However, stimulation of platelets with thrombin reduced the [^{14}C]AA in PI to 59.3 ± 14.1% of the level in control platelets and this reduction was not prevented by the presence of ethanol, (reduction to 59.0 ± 10.4%). The [^{3}H] oleic acid associated with PI (and presumably transacylated from PC) was reduced to 50.6 ± 5.0% of that in controls by stimulation with thrombin. This was only partially prevented (reduction to 75.8 ± 10.2%) by the presence of ethanol.

The results suggest that ethanol does not inhibit the utilisation of AA from PI when this is stimulated by thrombin, but that it does inhibit the cleavage of oleic acid from PI and the cleavage of oleic acid and AA from PC. These results are consistent with an inhibitory action on the platelet membrane phospholipase A_2 rather than other phospholipases.

Acknowledgement. This work was supported by the Medical Research Council.

Fenn, C.G. & Littleton, J.M. Alcohol. Clin. Exp. Res. 7 59-64 (1983).
Lands, W.E.M. J. Biol. Chem. 235 2233-2237 (1960).
Littleton, J.M. & Nhamburo, P.T. Br. J. Pharmacol. 78 118P (1983).

ALTERED CATECHOLAMINE RELEASE BY INDIRECT SYMPATHOMIMETICS FROM BRAIN SLICES OF ETHANOL-TREATED RATS

J.M. Littleton & M.A. Lynch, Dept. of Pharmacology, King's College, London WC2R 2LS.

The Ca^{2+} dependent depolarisation-induced release of neurotransmitters from brain slice preparations is enhanced when these are taken from animals which have been made tolerant to ethanol (Clarke et al., 1977; Lynch et al., 1983). Since similiar effects are produced when release of transmitter is induced by the Ca^{2+} ionophore A23187, we have suggested that the change associated with ethanol tolerance may be due to an increased ability of Ca^{2+} entry to stimulate transmitter release from the nerve terminal (Lynch & Littleton, 1983). However recently it has been shown that amphetamine stimulated release of [^{3}H]-dopamine from rat striatal synaptosomes is reduced when these are obtained from ethanol-tolerant animals (Mullin & Ferko, 1983). Since amphetamine-stimulated release is usually considered to be at least partly Ca^{2+} dependent, this result is difficult to reconcile with our hypothesis. We therefore investigated the Ca^{2+} dependence and releasing ability of amphetamine and another indirect sympathomimetic, tyramine, on release of [^{3}H]-dopamine (striatal slices) and [^{3}H] noradrenaline (cortical slices) from preparations from control rats and rats exposed to ethanol inhalation for 6 days. The methods have all been described previously (Lynch & Littleton, 1983).

Amphetamine, 1 μM, incorporated into the superfusate increased the fractional release of ^{3}H from slices preloaded with [^{3}H]-noradrenaline and [^{3}H]-dopamine. Only in the case of noradrenaline release from the cortex was there any evidence that this release was at all Ca^{2+} dependent (slightly reduced by EGTA, 1mM). The effect of ethanol treatment seemed to accentuate this Ca^{2+} dependence of [^{3}H] - noradrenaline release. The release in control preparations, studied in the absence of Ca^{2+}, was 0.385 ± 0.061%. min^{-1} whereas in ethanol-treated preparations it was 0.179 ± 0.027%. min^{-1} (means ± sem of 12 determinations, $p < 0.01$). The release in the presence of Ca^{2+} on the other hand rose from, in controls, 0.414 ± 0.085%. min^{-1} to, in ethanol-treated preparations, 0.623 ± 0.11%. min^{-1} (means ± sem of 6 determinations, NS). The release from the striatum of [^{3}H]-dopamine was similiarly reduced in preparations from ethanol-treated rats when studied in the absence of Ca^{2+} but in the presence of Ca^{2+} virtually no difference between the preparations was obtained.

Tyramine, 0.5 μM, produced similiar results to amphetamine in the sense that the release of either [^{3}H]-noradrenaline or [^{3}H]-dopamine was always significantly less from ethanol treated preparations studied in the absence of Ca^{2+}. Tyramine differed from amphetamine in that no evidence whatsoever for the Ca^{2+} dependence of transmitter release was obtained. Tyramine-induced ^{3}H release, when studied in the presence of Ca^{2+} was thus similiarly reduced in preparations from ethanol-tolerant animals.

It is therefore concluded that the development of ethanol tolerance is associated with altered characteristics of neurotransmitter release from brain slices by indirect sympathomimetics. Release which is Ca^{2+} independent is very definitely inhibited whereas release which is Ca^{2+} dependent is either unchanged or may be enhanced.

Acknowledgements. This work was supported by the Medical Council on Alcoholism.

Clarke, J.W. et al., Can. J. Physiol. Pharmacol. 55 758-768 (1977)
Lynch, M.A. et al., Brain Res., in press
Lynch, M.A. & Littleton, J.M., Nature, 303, 175-176 (1983)
Mullin, M.J. & Ferko, A.P., J. Pharmacol. exp. Ther., 225, 694-698 (1983)

MEASUREMENT OF IRREVERSIBLY OXIDISED SPECIES IN RAT BRAIN USING HIGH SPEED DOUBLE CYCLE VOLTAMMETRY AT CARBON FIBRE ELECTRODES

Z.L. KRUK & J.A. STAMFORD. Dept of Pharmacology, The London Hospital Medical College, Turner St, LONDON E1 2AD.

Ascorbic acid oxidises irreversibly at carbon fibre microelectrodes under physiological conditions, because of the hydration of dehydroascorbate. A consequence of this is that no reduction peak is observed on reversal of the voltage scan. Therefore a further oxidation peak is not detected until diffusion has replenished the ascorbate depleted by the initial oxidation. As a result, a double cycle high speed voltammogram with two anodic sweeps reveals a difference between the first and second oxidation currents which is related to the concentration of ascorbate in the solution. This forms the basis of a direct assay of ascorbic acid suitable for in vivo usage.

High speed cyclic voltammetry has been described in detail (Armstrong James et al 1981). In this study the input voltage comprised two cycles of a triangular waveform (75 Hz, ±1.0 V vs Ag/AgCl) sweeping initially in the cathodic direction. This waveform was applied once every thirty seconds and the working electrode was disconnected between sweeps.

The selectivity of the technique was tested initially in vitro. The clearest "difference current" was observed with ascorbate although NADH, NADPH, urate and glutathione also produced some difference current but only at high concentration and generally at a higher potential than ascorbate. The catechols did not interfere with the assay and the indoles only produced difference currents at concentrations that gave rise to a clear initial oxidation peak. Methylated catechols gave behaviour characteristic of methyl group elimination, the second sweep showing a new peak in the catechol oxidation region. These features made it unlikely that such compounds could be mistakenly identified as ascorbate on the basis of their signals.

In the CNS of anaesthetised rats a difference current was observed in all brain areas examined. This signal could be detected in the striatum, cortex and corpus callosum for five hours (longest period examined). Micro pressure ejection of sodium ascorbate from multibarrel carbon fibre microelectrodes increased the in vivo difference current in a dose dependent manner. The signal could also be increased by intraperitoneal injection of ascorbate (2g/kg). Conversely micro pressure ejection of ascorbate oxidase decreased the in vivo difference current, in the cortex by 81%. The remaining signal had different characteristics from that attributable to ascorbic acid. Some possible contributors to this remaining signal include NADH, NADPH, urate and glutathione, all of which are present in the rat CNS.

In conclusion, the assay appears capable of detecting extracellular ascorbic acid in vivo over several hours. Such a facility may also prove suitable for use in conscious unrestrained animals.

(The authors would like to thank Dr Julian Millar for the potentiostat design and construction and Mr Selwyn Mable for the voltammetric programmer).

Armstrong James M. et al (1981) J. Neurosci. Meth. 4 , 385-406.

A MODEL TO STUDY ENHANCED DRUG ELIMINATION DURING REPEATED DOSING WITH ACTIVATED CHARCOAL

D.M. Edwards and J. Feely, Department of Pharmacology and Therapeutics, Ninewells Hospital and Medical School, Dundee, DD1 9SY

Activated charcoal (AC) adsorbs a variety of drugs and may limit their absorption from the gastrointestinal tract. In man it has been shown also that repeated dosing with AC may enhance the subsequent elimination of drug already in the systemic circulation although the precise mode of action is not known (Neuvonen, 1982). The aim of this study was to devise a model to study the effect of repeated doses of AC on drug disposition.

Two groups of 5 fasted Wistar rats received 6 μCi of pheno-^{14}C-barbital (PB, specific activity 99 μCi/mg) intraperitoneally. One group was given AC (loading dose 57 mg/100 g at time 0 and 28 mg/100 g thereafter at 6,12,24,30, 48 and 54 hours) and the other (control) water, intragastrically (mixed with lactulose to prevent constipation) at these times. The animals were kept in silanised glass metabolic cages and before each dosing time complete urine and faecal collections and a 200 μl blood sample from the tail vein were taken. At 60 h the animals were killed and the major organs collected. PB (parent and metabolites) was measured by scintillation counting following extraction and thin layer chromatography.

Treatment with AC significantly ($p < 0.05$) reduced the PB elimination half-life from 31±2 to 16±1 h (mean±S.E.M.) and increased PB clearance from 0.11±0.03 to 0.22±0.03 ml/min. The total amount of PB excreted by the urinary route was reduced but there was no change in the PB parent/metabolite ratio. There was a marked increase in PB excretion in faeces in AC treated animals (Figure). At death there was less ($p < 0.05$) PB in the liver, kidney, lung, brain and plasma of AC treated animal and more PB in the gastrointestinal contents. There was no evidence of enterohepatic circulation of PB and there was greater than 85% recovery of ^{14}C. Preliminary results with ^{3}H-imipramine suggest that AC has only a small effect on elimination and does not reduce brain imipramine concentrations.

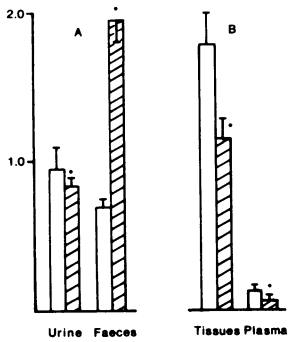

Figure: Effect of AC(▨) on (A) total urinary and faecal excretion over 60 h and (B) final tissue and plasma concentrations of pheno-^{14}C-barbital (uCi); mean ± S.E.M., n = 5; * $p < 0.05$ significantly different from Control (□)

These results suggest that orally administered AC acts as a gastrointestinal sink. Setting up a concentration gradient across the gut lumen it binds drugs and enhances elimination thereby reducing blood and tissue drug concentrations. By repeated dosing AC may be used to enhance drug elimination and reduce the extent and duration of toxicity following overdose. This model may facilitate the screening of other drugs that may prove to be susceptible to the effects of AC.

Neuvonen, P.J. (1982) Clin. Pharmacokinet. 7, 465

AGONIST AND ANTAGONIST PROPERTIES OF RX 801074 AT PERIPHERAL AND CENTRAL α_2-ADRENOCEPTORS IN THE RAT

T.L. Berridge, P.W. Dettmar, J.C. Doxey, B. Gadie, J.A.H. Lord, A.G. Roach, I.F. Tulloch and Narinder Virdee. Pharmacology Dept., Reckitt and Colman, Hull.

RX 801074 (2-[6-(1,4-benzodioxanylamino)] imidazoline maleate) has been shown to stimulate prejunctional α2-adrenoceptors in the rat isolated vas deferens but to block these receptors in the guinea-pig isolated ileum (Chapleo et al., 1983). We have studied the effects of RX 801074 at the following α2-adrenoceptor systems in the rat: prejunctional α2-adrenoceptors in the heart and vas deferens of pithed animals, peripheral α2-adrenoceptors mediating inhibition of gastrointestinal motility (g.i.m.; Doherty and Hancock, 1983), and central α2-adrenoceptors mediating hypotension, bradycardia and mydriasis.

All experiments have been performed in male Sprague Dawley rats. Rats were pithed and prepared for monitoring heart rate (HR) elevated (90-110 beats/min) by continuous stimulation of the spinal cord or for the measurement of electrically-evoked contractions of the vas deferens (Doxey et al., 1982). Inhibitory dose-response curves to RX 801074 were performed after saline or idazoxan (RX 781094: 0.1 mg/kg,i.v.). RX 801074 fully inhibited the stimulation-evoked tachycardia and this effect was competitively antagonized by idazoxan (cumulative ED50 values for RX 801074 after saline and idazoxan (0.1 mg/kg) were 2.3 ± 0.7 and 28.8 ± 9.3 μg/kg,respectively). In the vas deferens RX 801074 and clonidine reduced the twitch response (max. inhibitions 85 ± 2% and 100%, respectively). Control cumulative ED50 values for RX 801074 and clonidine were 0.4 ± 0.1 and 2.5 ± 0.4μg/kg, respectively. Both compounds were competitively antagonized by idazoxan (0.1 mg/kg) (RX 801074 and clonidine ED50 values being 11.1 ± 2.9 and 26.8 ± 3.4 μg/kg). RX 801074 (0.03-3 mg/kg,i.v.) reversed (20-30%) the inhibitory effects of clonidine (0.1 mg/kg,i.v.).

The charcoal meal test (Boura and Fitzgerald, 1966) was used to study the effects of RX 801074 on g.i.m. RX 801074 (up to 5 mg/kg,i.p.) did not affect g.i.m. whereas pretreatment with RX 801074 (5 mg/kg,i.p.) antagonized the inhibitory effects of clonidine on g.i.m. (clonidine ED50 values: control 11.8 μg/kg; RX 801074-treated 137 μg/kg).

In pentobarbitone anaesthetized rats, RX 801074 (1-10 mg/kg,i.v.) did not antagonize the hypotensive and bradycardic effects of clonidine (10 μg/kg,i.v.) nor did it affect the mydriatic dose-response curve to guanoxabenz. In contrast, i.c.v. administration of RX 801074 (100 μg) fully inhibited the clonidine-induced hypotension and bradycardia and effectively reversed the maximal mydriatic response produced by guanoxabenz (0.3 mg/kg,i.v.). RX 801074 (1-10 mg/kg,i.v. and 100 μg,i.c.v.) alone was without effect on pupil diameter and BP. RX 801074 (i.v.) lowered HR probably by stimulation of cardiac α2-adrenoceptors.

In conclusion, RX 801074 is a partial agonist at α2-adrenoceptors; its agonist/antagonist profile depends upon the peripheral or central α2-adrenoceptor system studied. It appears to be a full agonist at prejunctional α2-receptors in the rat heart but a partial agonist in the vas deferens. At peripheral α2-receptors inhibiting g.i.m. RX 801074 was a competitive antagonist. In the doses used RX 801074 did not enter the CNS; after i.c.v. administration it possessed only antagonist activity at central α2-adrenoceptors. That RX 801074 profiles predominantly as an α2-agonist in the rat heart and vas deferens but as a pure antagonist in the CNS and gut, illustrates the difficulty in predicting the central action of partial agonists from peripheral data.

Boura, A.L.A. and Fitzgerald, A.F. (1966). Br.J.Pharmac., 26, 307-321.
Chapleo, C.B. et al. (1983). Eur. J.Pharmac., 91, 123-128.
Doherty, N.S. and Hancock, A.A. (1983). J.Pharmac.exp.Ther., 225, 269-274.
Doxey, J.C. et al. (1982). Br. J. Pharmac., 77, Proc. Suppl., 532P.

AGGREGATION OF ALBUMIN AND THE INVERSE DEPENDENCE OF BINDING CONSTANTS (n Ka) UPON ALBUMIN CONCENTRATION

L.S. Clegg and W.E. Lindup, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

The apparent association constant (Ka) and number of binding sites (n) for some ligands, vary inversely with albumin concentration (Bowmer & Lindup, 1980). Shen & Gibaldi (1974) first postulated that such dependence of binding constants upon albumin concentration could be due to a concentration dependent aggregation or polymerization of albumin molecules. Aggregation of albumin could obscure binding sites for some ligands or alter the affinity of exposed sites by some form of allosteric modulation. We have investigated the possible contribution of dimerization/polymerization to this type of binding behaviour as shown by the ligand L-tryptophan (Bowmer & Lindup 1978, 1980).

Polyacrylamide gel electrophoresis was carried out on five different initial concentrations of ^{125}I labelled human albumin, each of which had been preincubated with L-tryptophan (10μM) (Table 1). The total protein in the dimer band and the proportion of the total bound L-tryptophan associated with this band were determined in each case. The binding of L-tryptophan to the HSA preparation was also determined by equilibrium dialysis.

Table 1 Proportion of the bound L-tryptophan which associates with the dimer

HSA (g/100ml)	Dimer Proportion(%)	L-tryptophan bound to dimer (%)
4.0	5.2 ± 1.6	6.1 ± 1
2.0	4.6 ± 0.9	6.3 ± 2
1.0	4.5 ± 0.9	6.1 ± 2
0.5	4.2 ± 0.4	5.9 ± 1
0.25	4.0 ± 0.4	6.3 ± 1

Values are expressed as mean (n = 4) ± S.D.

There was a slight increase in dimer proportion as the total protein concentration increased, but this change was not statistically significant. Furthermore, the dimer fraction accounted for only about 6% of the total binding of L-tryptophan at all albumin concentrations studied. Hence, it would seem that human albumin dimer binds L-tryptophan as well as the monomer. It is also unlikely that such a small dimer proportion could be responsible for the anomalous binding behaviour.

The binding of L-tryptophan to a bovine albumin 'monomer' preparation, when analysed by the method of Scatchard (1949) gave a positive plot which agreed well with that obtained for the binding to bovine albumin fraction V (Bowmer & Lindup, 1978). This suggests that a protein concentration dependent aggregation of albumin, whether or not it occurs, is not reponsible for the anomalous binding behaviour observed with this ligand. However, polyacrylamide gel electrophoresis of the 'monomer' preparation clearly showed the presence of a polymer band which was presumably the dimer. Although the amount of dimer could not be quantitated, visual inspection of the stained gels indicated that it represented a very small proportion of the total albumin content.

Bowmer, C.J. & Lindup, W.E. (1978). Biochem. Pharmacol. 27, 937-942.
Bowmer, C.J. & Lindup, W.E. (1980). Biochim. Biophys. Acta. 624, 260-270.
Scatchard, G. (1949). Ann. N.Y. Acad. Sci. 51, 660-672.
Shen, D. & Gibaldi, M. (1974). J. Pharm. Sci. 63, 1698-1703.

POTENT AND SELECTIVE α_2-ADRENOCEPTOR ANTAGONISTS IN A SERIES OF 2-SUBSTITUTED ANALOGUES OF IDAZOXAN

J.C.Doxey, A.G.Roach, M.R.Stillings, Diane A. Strachan, Narinder K. Virdee and A.P.Welbourn, Departments of Pharmacology and Medicinal Chemistry, Reckitt and Colman, Hull, HU8 7DS.

The selectivity and potency of idazoxan (RX 781094) as an antagonist at α_2-adrenoceptors and the influence of 2-alkyl substitution on these parameters have been described previously (Dettmar et al., 1983; Doxey et al., 1983a, 1983b; Gadie et al., 1983). It has been shown that idazoxan produces transient pressor responses in pithed rats due to partial agonist properties at α_1-adrenoceptors (Paciorek and Shepperson 1983). This communication describes the potency and selectivity of three analogues of idazoxan which possess either OMe (RX 821002), OEt (RX 811059) or OCH_2Ph (RX 821004) substituents in the 2-position.

The pre (α_2) and postjunctional (α_1)-adrenoceptor antagonist properties of the compounds were assessed in vitro by determining pA_2 values against UK 14304 and noradrenaline in the rat vas deferens (electrically-stimulated 0.1 Hz) and anococcygeus muscle, respectively (Doxey et al., 1983b). Antagonist pA_2 values were calculated from Arunlakshana and Schild (1959) plots. Male Sprague-Dawley rats (300-350g) were pithed, vagotomised and prepared for recording of arterial blood pressure and heart rate. Prejunctional α_2-adrenoceptor antagonist potency was determined against UK 14,304 on cardiac nerves and antagonism of cirazoline pressor responses was used to assess postjunctional α_1-adrenoceptor antagonist potency (Doxey et al., 1983b).The effects of the analogues on the resting blood pressure of pithed rats was used to assess possible partial α_1-adrenoceptor agonist properties. All drugs were injected via a cannulated femoral vein. The results obtained are shown in Table 1.

Table 1. Antagonist potencies at peripheral α_1/α_2-adrenoceptors.

	RAT ISOLATED TISSUES			PITHED RATS		
COMPOUND	VAS DEFERENS pA_2 (α_2) vs UK-14,304	ANOCOCCYGEUS pA_2 (α_1) vs NORADRENALINE	RATIO α_2/α_1	HR (α_2) DOSE for DR2 vs UK-14,304 (μM/kg iv)	DBP (α_1) DOSE for DR2 vs CIRAZOLINE (μM/kg iv)	RATIO α_2/α_1
IDAZOXAN	8·50	6·32	151	0·129	6·70	52
RX 821002	9·41	6·91	316	0·010	0·95	95
RX 811059	8·92	6·74	151	0·025	2·20	88
RX 821004	8·68	6·39	195	0·140	7·59	54

Both RX 821002 and RX 811059 were more potent than idazoxan at α_2-adrenoceptors both *in vitro* and *in vivo*; RX 821004 was of similar potency to the parent compound. Idazoxan (3-1000 μg/kg,i.v.) induced dose-related pressor responses in pithed rats the maximum elevation in diastolic blood pressure was 33 ±4 mmHg at a dose of 100 μg/kg. RX 821002, RX 811059 and RX 821004 had no significant effect on diastolic blood pressure over the same dose range.

The results obtained confirm previous observations that substitution at the 2-position of idazoxan can produce compounds with enhanced α_2-adrenoceptor antagonist potency and which, furthermore, have minimal effects on resting blood pressure of pithed rats.

Arunlakshana, O. and Schild, H.O. (1959). Br.J.Pharmac. Chemother., 14, 48-54.
Dettmar, P.W. et al.(1983). Neuropharmac., 22, 729-737.
Doxey, J.C. et al. (1983a). Br.J.Pharmac., 78, 489-505.
Doxey, J.C. et al. (1983b). Br.J.Pharmac., 79, 311P.
Gadie, B. et al. (1983). Br.J.Pharmac., 79, 312P.
Paciorek, P.M. and Shepperson, N.B. (1983). Br.J.Pharmac., 79, 12-14.

EFFECT OF FORSKOLIN ON THE INTRAOCULAR PRESSURE AND ON THE SYNTHESIS OF CYCLIC-AMP IN THE ALBINO RABBIT EYE

P. Mallorga, M.F. Sugrue and M.P. Viader. Centre de recherche, Laboratoires Merck Sharp & Dohme-Chibret, BP 127, 63 203 Riom Cédex, France.

The natural product forskolin has been shown to reversibly stimulate adenylate cyclase of several tissues independent of an action on cell surface receptors (Seamon et al, 1981). Consequently, forskolin offers a novel strategy for assessing the relationship between cAMP levels and physiological functioning. The objective of this study was threefold. Firstly, to characterize the in vitro effect of forskolin on adenylate cyclase present in membranes of rabbit ciliary processes, the site of aqueous humor secretion. Secondly, the measurement of cAMP in aqueous humor samples obtained from the anterior chamber of the rabbit eye at various times after the local administration of forskolin. Thirdly, to relate such changes to alterations in IOP.

Adult, male New Zealand rabbits were used in all experiments. IOP was measured by applanation pneumatography without local anaesthesia (Vareilles et al, 1977). Adenylate cyclase activity was assayed as described by Nathanson (1980) and cAMP levels were measured using a protein binding assay.

Forskolin stimulated adenylate cyclase present in homogenates of rabbit ciliary processes in a dose-dependent manner. The K_A for forskolin activation was 5 µM and the maximal stimulation was 20-fold (range: 10 to 36). (S)-timolol (1 µM), a concentration which blocked completely the stimulation of rabbit ciliary process adenylate cyclase by l-isoproterenol (3 µM), did not significantly affect the activation by forskolin (100 µM). Basal levels of cAMP in rabbit anterior aqueous ranged from nondetectable (<0.2) to 0.5 pmoles/50 µl of aqueous humor. The instillation (50 µl) of a 1% suspension of forskolin in 0.5% hydroxyethylcellulose (HEC) in water into the conjunctival sac of rabbits failed to significantly change the level of cAMP in the aqueous humor at 30, 60, 90 and 120 min post-administration. In contrast, cAMP levels in aqueous humor were significantly elevated when forskolin (10 µg solubilized in 50% ethanol) was injected directly (10 µl) into the anterior chamber of the eyes of rabbits pretreated with ketamine (25 mg/kg i.v.). Levels were significantly increased at 15, 30 and 45 min post-injection and at 1 h had returned to basal values. Levels reached a maximum of 8 pmoles/50 µl aqueous humor at 15 min. A 1% suspension of forskolin in 0.5% HEC was instilled into the conjunctival sac of normotensive, conscious rabbits and IOP was measured at 0.5, 1, 2, 3, 4 and 5 h post-instillation. Resting IOP before treatment was approximately 20 mm Hg. At no time point were values for forskolin-treated animals significantly different from vehicle-treated controls. In contrast, the IOP of rabbits significantly differed from vehicle-treated (10 µl of a 50% ethanol solution) at 2, 3, 4, 5 and 6 h after the injection of forskolin (10 µg) into the anterior chamber of the eye. The forskolin-induced reduction in IOP averaged 2 mm Hg. Our inability to confirm the observation that the IOP of rabbit, monkey and man is decreased following the instillation of a 1% suspension of forskolin into the conjunctival sac (Caprioli and Sears, 1983) probably stems from differences in the nature of the suspension.

In summary, forskolin stimulates rabbit ciliary process adenylate cyclase at a step beyond the beta-adrenoceptor. The injection of forskolin into the anterior chamber of the rabbit eye significantly decreased IOP and elevated the aqueous humor content of cAMP. Forskolin-induced changes in rabbit IOP occurred at a time when the induced elevation in cAMP content had returned to basal levels.

Caprioli, J. and Sears, M. (1983) Lancet I, 958-960.
Nathanson, J.A. (1980) Proc. Natl. Acad. Sci. USA, 77, 7520-7424.
Seamon, K.B. et al (1981) Proc. Natl. Acad. Sci. USA, 78, 3363-3367.
Vareilles, P. et al (1977) Exp. Eye Res. 24, 369-375.

EFFECTS OF NIFEDIPINE ON RELAXATION OF ISOLATED GUINEA-PIG TRACHEA INDUCED BY SALBUTAMOL

M.J. Peck and R. Towart, Miles Laboratories Limited, Stoke Court, Stoke Poges, Slough, SL2 4LY , England.

Recent reports have suggested that calcium antagonists may either inhibit, or potentiate, the relaxant effect of β-agonists in airways smooth muscle (Joubert et al, 1982; Kitamura and Ishihara, 1980). We have reexamined this question by comparing the relaxant effect of salbutamol in the absence and in the presence of various concentrations of nifedipine.

Single rings of isolated guinea-pig trachea were suspended in Tyrode's solution containing $3x10^{-7}$g/ml indomethacin to suppress the synthesis of prostaglandins, and contractions induced by the addition of carbachol (CCh, final bath concentration $3x10^{-8}$g/ml) (see Ahmed et al, 1983). The contractions were stable and reproducible and were attenuated by 5 minute preincubation of concentrations of nifedipine greater than 10^{-10}g/ml (Table 1). Cumulative concentration-response curves to the relaxant effects of salbutamol were in addition performed during the sustained phase of the CCh-induced contractions, both before and during incubation with nifedipine. The EC_{50} values and the maximum relaxation produced by salbutamol are shown in Table 1; pretreatment values on the left, and in the presence of DMSO or infedipine on the right. Concentrations of nifedipine producing significant relaxation of the guinea-pig trachea ($3x10^{-9}$,10^{-8}g/ml) slightly potentiated the relaxant effect of salbutamol; this was due to an increase in potency (lower EC_{50} values), not efficacy.

We conclude that calcium antagonists do not inhibit the relaxant effects of β-adrenoceptor agonists in guinea-pig trachea, but that a slight increase in β-agonist potency may be observed in the presence of effective concentrations of calcium entry blockers.

Contraction	Salbutamol EC_{50}	Salbutamol Max. Rel.	Treatment	Contraction	%	Salbutamol EC_{50}	Salbutamol Max. Rel.	n
0.99±0.13g	6.3	96%	DMSO	0.96±0.13g	97%	6.3	97%	9
1.02±0.20	5.4	92%	10^{-11}g/ml	0.99±0.19	98%	6.3	93%	6
1.04±0.17	5.4	94%	10^{-10}	0.89±0.14	88%	4.6	96%	6
1.19±0.12	6.8	88%	10^{-9}	0.95±0.08	81%	5.8	93%	10
1.08±0.18	6.6	88%	$3x10^{-9}$	0.53±0.13*	48%	3.8	95%	5
1.26±0.22	3.7	99%	10^{-8}	0.49±0.19*	38%	1.9	97%	6

Table 1 Effect of DMSO or various concentrations of nifedipine on the salbutamol-induced relaxation of contractions of guinea-pig trachea. Concentrations of salbutamol producing half-maximal response (EC_{50}) are expressed in ng/ml. The maximum relaxation (max. rel.) produced by salbutamol is given as an indication of efficacy. Results are expressed as means (±s.e.m. where shown) and * indicates $p < 0.05$ as compared to pretreatment contractions.

Ahmed, I., et al (1983). Proc. Br. Pharmacol. Soc. 1983.
Joubert, P., et al (1982). S.A. Med. J., 62, 247.
Kitamura, S., Ishihara, Y., (1980). Arzneim. Forsch. (Drug Res.) 30, 1088.

A METHOD OF COMPUTER AVERAGING BIOLOGICAL RESPONSES FROM CHART RECORDINGS

W. PIOTROWSKI (introduced by C.C. JORDAN), Dep't of Pharmacology, University College London, Gower Street, London, WC1E 6BT.

The time-correlated averaging of responses to repeated stimuli has proved to be an extremely powerful technique in electrophysiological studies, for example, recording of cortical evoked potentials. A major advantage of this technique is the dramatic reduction in the "noise" obtained when several responses to an identical stimulus are averaged. Few pharmacological laboratories have easy access online to a mainframe computer or to suitably interfaced and programmed mini- or micro-computers. It was therefore the aim of this work to devise a method of determining averaged responses of the contraction of isolated tissue preparations using only the chart recordings obtained during the experiments. We were interested in the longer term contractile responses of guinea-pig ileum to applied agonists, since these responses appeared to have more than one component. We wished to characterise these components and compare them for various agonists.

Chart recordings were made at suitable paper speeds and then digitised using a simply made reference grid, (1mm graph paper printed on tracing parchment) and a Tektronics 4954 digitising tablet attached to a Tektronics T4014 intelligent graphics terminal. This was connected to the host system of linked GEC 4190 minicomputers. Other hardware configurations could easily be utilised with suitable operating software. A series of Fortran programs were written to control the T4014/4954, manipulate data files, perform calculations on results, and to plot the digitised data. Programs could easily be adapted to other languages e.g. Basic or Pascal.

Obviously, the programs could be modified to suit virtually any purpose, but our protocol was as follows. In each experiment, a full agonist dose-response curve for contractile responses of isolated guinea-pig ileum to applied agonists was constructed. Individual doses were administered with a drug contact time of 90sec and a chart speed of 10mm/min. The recorder was operated for a further 30sec after washing out of the agonist. Successive doses were given in increasing concentrations in the progression 1,3,10,30 etc, though other progressions could easily be used with little modification to the programs. Recordings from several such experiments, performed on different days, were then digitised. Firstly, the maximum response was set so that the other responses could be expressed as a percentage of the maximum, and then each individual response was coded in 21 6 s increments (i.e. at 1mm intervals). The digitising puck was lifted clear of the tablet to signify the end of each response and generate a suitable code. The first point in each response was taken as the baseline for that response. When one dose-response sequence was digitised, the program used these data to update files containing data from previous days' experiments which had the same protocol. The files contained a suitable title followed by the responses, sorted and grouped into order of increasing concentration, including the means, and also standard errors if more than four such experiments had been performed. A separate Fortran program was written, using the GINO-F graphics library, to plot the data. A file "tidying-up" program was written to help the subsequent editing of data files.

Using the above technique, it was possible to resolve the response of isolated guinea-pig ileum to applied peptides into a number of components, and allowed further investigation of the "shape" of the response in a manner not possible without such a technique.

POSSIBLE MECHANISM BY WHICH POLYVALENT CATIONS INHIBIT ECCRINE SWEATING IN THE RAT

B.B. Mikolajczak, Department of Pharmacology, School of Pharmacy, Leicester Polytechnic, Leicester, LE1 9BH (introduced by B.J.Northover)

The inorganic salts of aluminium have a long history of use and form the basis, at least in part, of all antiperspirants now commercially available. Aluminium chloride and sulphate, which were used initially, were corrosive to both skin and clothing, and have since been replaced by less acidic salts such as the chlorhydrate. Salts of zirconium are used commercially to a much lesser extent.

Sweating in the plantar eccrine sweat glands was induced in anaesthetised rats by the subcutaneous administration of carbachol in a dose of between 2.5 ng and 2.5 mg per kg, or by local intradermal administration of carbachol or methacholine (0.75 μg in 0.01 ml of isotonic saline), or by localised heating. The effects of all these agencies were reduced or abolished by prior injection of atropine (100 - 750 μg/kg). Isoprenaline, administered subcutaneously in a dose of 0.4 mg/kg, was more effective than either adrenaline or noradrenaline at the same dose in promoting sweating in the foot pads. This effect was reduced by prior administration to the rat of propranolol (0.8 mg/kg).

Aluminium chlorhydrate applied topically to the foot pad in a concentration of 1-25 percent w/v at a pH between 1 and 4.6 inhibited carbachol-induced sweating. Lanthanum chloride was more effective mole for mole than either aluminium chlorhydrate or zirconium oxychloride. The antiperspirant effect of these 3 compounds was antagonized by simultaneous application of calcium chloride solution. This suggests that the polyvalent cations may inhibit sweating by antagonizing the action of endogenous calcium ions. Calcium chloride solutions applied alone had only a slight properspirant action, suggesting that the normal calcium ion concentration in mammalian extracellular fluids produces a near to maximal properspirant effect.

Other polyvalent cations studied were those of chromium, iron, barium, magnesium and titanium. All showed weak antiperspirant action in this rat preparation.

The site within the sweat gland and its duct at which these ionic interactions take place will be discussed in the context of current ideas about the mechanism of sweat production by the secretory coil and its subsequent compositional modification during passage along the duct.

EVALUATION OF CELLULAR INFILTRATION IN A GUINEA-PIG MODEL OF PULMONARY HYPERSENSITIVITY

A.K. Cane, D.J. Edwards, M.D. Hammond & W.A. Taylor, Research Department, Miles Laboratories Limited, Stoke Court, Stoke Poges, Slough, SL2 4LY, U.K.

Although pulmonary anaphylaxis and inflammation can be induced in guinea-pigs by various means (Sewall 1917, Richerson, 1972), the precise role of cellular infiltration in such models has not been fully elucidated. It seems likely that the oedema, haemorrhage and tissue damage which accompany the cellular infiltration (Shaw et al, 1980) are, at least in part, due to mediators released from these cells but the contributions from each cell type is not known. In addition, endemic pneumonitis in the guinea-pig can make histological interpretation difficult (Richerson, 1972).

By using ethanol fixation combined with stains to detect either peroxidase or phosphatase enzyme activity, in order to selectively stain eosinophils or neutrophils respectively, we have been able to measure significant changes in pulmonary cellular infiltration. Guinea-pigs were sensitised by an injection into each hind footpad of 0.25ml of ovalbumin (OA) in Freund's complete adjuvant. The total dose received by each animal was 5µg OA. Eight days later five groups of six animals were challenged with an aerosol containing either saline alone or OA at a concentration of 1mg, 100µg, 10µg or 1µg/ml in saline. They were killed 24 h later by an i.p. injection of sodium pentobarbitone (120mg/kg). The lungs were removed immediately and dilated by the intra-tracheal injection of 5ml of 70% ethanol and fixed in 70% ethanol. Paraffin sections 5µ thick were stained with haematoxylin and eosin for general morphology while eosinophils and neutrophils were identified enzymatically by either a peroxidase reaction (Undritz, 1952) or alkaline phosphatase activity (Gomori, 1952) respectively. Anaphylactic collapse occurred in 3/6 animals challenged with OA at 1mg/ml and 1 died. After challenge with aerosol from an OA solution at 100µg/ml, the animals were distressed but no collapse occurred. OA at lower doses produced no obvious response. The group with the maximum anaphylactic response (i.e. 1mg/ml) developed the highest concentration of inflammatory cells. Large numbers of neutrophils were found in the alveolar spaces whilst eosinophils had collected around the trachea and upper bronchial tract close to sites of first antigen contact. When the antigen concentration of the aerosol was reduced to 100µg/ml, the eosinophil level declined but a high neutrophil influx still occurred. At 10µg and 1µg no difference could be detected between challenged and control animals.

Thus cellular infiltration was only found in animals which had previously undergone marked immediate hypersensitivity reactions. Furthermore, distinct patterns of cellular infiltration were observed for eosinophils and neutrophils and the type of cell recruited was dependent on dose of antigen. It is hoped that by using this model it will be possible to relate the accumulation of cells to changes in lung function during the development of pulmonary inflammation.

Gomori, G., In Microscopic Histochemistry (1952) pp 175-189. Univ. of Chicago Press
Richerson, H.B. (1972) J. Lab. Clin. Med. 79, 745-757.
Sewall, H.J. (1917) J. Lab. Clin. Med., 2, 874-882.
Shaw, J.O. Henson, P.M. Henson, J. Webster. R.O. (1980) Lab. Invest. 42, 547-558.
Undritz, A. (1952) Sandoz Atlas, p. 28. Sandoz Ltd.

AGONIST-INDUCED INOSITOL PHOSPHOLIPID TURNOVER AND CALCIUM FLUX IN HUMAN PLATELET ACTIVATION

Lesley J. Brydon, A.H. Drummond, Karen A. Kirkpatrick, D.E. MacIntyre, W.K. Pollock & A.M. Shaw, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

Platelet activation is thought to depend upon elevation of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) (Gerrard *et al*, 1981). Agonist-induced degradation of phosphatidylinositol and/or polyphosphoinositides is a direct consequence of receptor occupancy which precedes and may evoke elevation of $[Ca^{2+}]i$ (Michell, 1982). More recent evidence indicates that protein kinase C (a Ca^{2+}-dependent, phospholipid-dependent enzyme that is activated by diacylglycerol (DAG) formed as a consequence of receptor-mediated inositol phospholipid hydrolysis) may act alone or synergistically with increased $[Ca^{2+}]i$ to mediate platelet responses to certain agonists (Nishizuka, 1983). To date there has been no systematic investigation of the effects of different agonists on these putative regulatory mechanisms in human platelets. In the present study we examined the effects of six platelet agonists, which act by combining with distinct receptors, on human platelet $[Ca^{2+}]i$ and inositol phospholipid hydrolysis.

All studies were performed using plasma-free suspensions of human platelets. $[Ca^{2+}]i$ was measured using the fluorescent Ca^{2+} indicator Quin 2 (Tsien *et al*, 1982), and inositol phospholipid hydrolysis was monitored as $[^{32}P]$-phosphatidic acid (PA) formation in platelets prelabelled with $[^{32}P]$-PO_4 (MacIntyre & Pollock, 1983). As PA is the product of diacylglycerol kinase action on DAG, $[^{32}P]$-PA formation can be taken as an index of inositol phospholipid degradation and DAG formation.

When suspended in medium of $[Ca^{2+}]o$ = 1 mM, platelet resting $[Ca^{2+}]i$ was 86 $\pm$ 6 nM (mean $\pm$ S.E. n = 16). 5HT ($\leq$30 μM); vasopressin (VP, $\leq$1 μM); platelet-activating factor (PAF, $\leq$2 μM); U44069, a TxA_2-mimetic ($\leq$3 μM) and thrombin ($\leq$1 μ/ml) but not adrenaline ($\leq$30 μM) induced rapid, concentration-dependent elevation of $[Ca^{2+}]i$ to a maximum of 500-1000 nM. Using platelets suspended in nominally Ca^{2+}-free medium, the magnitude of the increase in $[Ca^{2+}]i$ induced by all agonists was markedly reduced. Under these same conditions, all agonists with the exception of adrenaline, induced concentration-dependent formation of $[^{32}P]$-PA (maximum 2-20 fold over control levels depending on the agonist).

These results indicate that activation of human platelets by 5HT, VP, PAF, TxA_2 and thrombin involves elevation of $[Ca^{2+}]i$, derived predominantly via influx of extracellular Ca^{2+}, and inositol phospholipid hydrolysis. The inter-relationships between these events remains to be elucidated. Inositol phospholipid degradation may lead to Ca^{2+} flux. Alternatively, $[Ca^{2+}]i$ and diacylglycerol (via activation of protein kinase C) may act independently, synergistically or synarchically to induce platelet activation. Adrenaline, which fails to increase $[Ca^{2+}]i$ or stimulate inositol phospholipid turnover, must induce platelet activation by a different mechanism.

This study was supported by the M.R.C. and by the Medical Research Funds of the University of Glasgow.

Gerrard, J.M. et al (1981) in Platelets in Biology and Pathology 2 (ed. J.L. Gordon) 407-436, Elsevier, Amsterdam
MacIntyre, D.E. & Pollock, W.K. (1983) Biochem.J. 212, 433-437
Michell, R.H. (1982) Cell Calcium 3, 285-294
Nishizuka, Y. (1983) Trends Biochem. Sci. 8, 13-16
Tsien, R.Y. et al (1982) J. Cell Biol. 94, 325-334

INOTROPIC RESPONSES TO LOW AND HIGH CONCENTRATIONS OF OUABAIN: FREQUENCY DEPENDENCE AND FUNCTIONAL ANTAGONISM

J.Halliday & J.S.Williams, Department of Pharmacology,King's College,Strand, London WC2R 2LS.

Recent work by Godfraind et al (1982) has confirmed the involvement of more than one mechanism in the positive inotropic responses to certain cardiac glycosides. Low doses of ouabain appear to act by binding to high affinity sites inhibitable by dihydroouabain (Godfraind et al, 1982), whereas at higher doses, all cardiac glycosides, including dihydroouabain, bind to low affinity sites giving rise to inhibition of the Na^+K^+-ATPase enzyme. The mechanisms activated by binding to the high affinity site are not known, though stimulation of the sodium pump has been an often observed correlate (see Noble, 1980). In the present study, low and high dose responses to ouabain were compared in guinea pig atrial preparations with respect to their frequency dependence, as cardiac glycoside responses have long been considered to be markedly frequency-dependent (e.g. Koch-Weser, 1971). The responses of tissues, pretreated with different concentrations of ouabain, to the negative inotropic action of verapamil were also examined, as ouabain-treated tissues have been found to be relatively resistant to this agent (Halliday & Williams, 1982).

Guinea pig atrial preparations were set up in Krebs containing 100nM propranolol and electrically driven as previously described (Halliday & Williams, 1982). For the study of frequency dependence, tissues were driven at either 0.5 or 3Hz, in modified Krebs containing only 1mM $CaCl_2$ to optimise the positive inotropic effect of ouabain. After a 120min equilibration period, ouabain (75 or 250nM) was added to the tissues, and the responses followed for 120min. In the verapamil series, after equilibration in normal Krebs, ouabain (75 or 250nM) was added to half the tissues; its maximum effect was reached after a further 30min. At this point, verapamil (300nM) was added to both treated and control tissues, and responses were followed for 90min. Decreases in force were calculated as a percentage of the force of contraction immediately before addition of verapamil.

In the frequency studies, 75nM ouabain induced a maximal increase in force of 31.6 ± 11% (n=6) at 0.5Hz, and 34.1 ± 4.7% (n=7) at 3Hz, whereas 250nM ouabain induced a maximal increase of 154.1 ± 12.2% (n=6) at 0.5Hz and 91.1 ± 18.9% (n=5) at 3Hz ($p< 0.05$).

In control tissues, verapamil produced a fall in force of 57.3 ± 7.6% (n=6) over 90min. In tissues pretreated with 75nM ouabain, the fall in force (58.4 ± 5.2%, n=8) was not different from control. However, when tissues were pretreated with the higher dose of ouabain, the fall in force seen with verapamil (23.7 ± 4.9%, n=5) was significantly less than in control tissues ($p< 0.05$).

The fact that the response to the low concentration of ouabain does not show any frequency dependence suggests that neither the binding to the "receptor" responsible nor the mechanisms subsequently activated are dependent on factors such as depolarisation or intracellular sodium activity, which alter with the rate of beating. The verapamil findings suggest that the relative resistance of tissues treated with high concentrations of ouabain to the negative inotropic action of verapamil could be a consequence of inhibition of the Na^+K^+-ATPase enzyme, which is not seen at lower concentrations (Noble, 1980).

Godfraind,T. et al (1982) Nature, 299, 824
Halliday,J. & Williams,J.S. (1982) Br.J.Pharmac., 77, 524P
Koch-Weser,J. (1971) Circ. Res., 28, 34
Noble,D. (1980) Cardiovasc. Res., 14, 495

ACTIONS OF PUTATIVE NEUROTRANSMITTERS ON ADULT RAT DORSAL ROOT GANGLION NEURONES

R.D. Pinnock (introduced by P.C. Emson), MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QD.

Changing calcium (Ca^{2+}) influx into primary afferent neurone terminals has been suggested as a means of modifying sensory information as it enters the spinal cord. In cultured chick dorsal root ganglion (DRG) neurones, action potentials (APs) have a Ca^{2+} component which is reduced by putative neurotransmitters (Mudge et al 1979, Dunlap and Fischbach 1981). In contrast to cultured neurones, the majority of adult rodent DRG neurones do not possess a Ca^{2+} component (Yoshida and Matsuda 1979). While the DRG soma do not have synaptic inputs, there are Histamine and opiate receptors present on these cells (Ninkovic et al 1982).

In the present study the action of some putative transmitters was tested on a subpopulation of DRG neurones in DRGs excised from adult male rats and maintained acutely in vitro. Intracellular recordings were made from 27 neurones, identified by their slow conduction velocity ($<1ms^{-1}$), as being Aδ or C type primary afferent neurones. In 7 cases the dimensions of the neurone were accurately measured after lucifer yellow dye injections. 5 mM TEA added to the normal perfusing medium (NaCl, 124; KCl, 2; KH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 25; glucose 11; concentrations are mM) caused all the neurones to gain a significant Ca^{2+} component to APs generated by either root or square wave depolarizing stimuli. Drugs were applied by pressure from 7 barrelled micropipettes positioned within 200μ of the recording site. Drug concentrations in the pipettes were: GABA, 5HT, Histamine and Ach, all 10 mM; KCl, 20 mM; morphine sulphate, 1 mM. In agreement with previous work (Desarmenien et al 1982) GABA and KCl depolarized all the neurones, GABA producing a fall in membrane resistance and on 3 neurones in the presence of 10μM bicuculline methiodide, GABA reduced the Ca component of the APs. Histamine (15 cells), Ach (9 cells), 5HT (12 cells) and morphine (11 cells) had no consistent effect on the membrane potential or resistance of neurones before TEA, neither did they effect the Ca^{2+} component of action potentials. These results and previous work with opiates (Williams and Zieglensberger 1981) suggest that functional receptors for 5HT, Histamine, Ach and morphine probably do not occur on the DRG neurones sampled in this study. This implies the excised DRG is a poor model for studying presynaptic mechanisms in the spinal cord.

Desarmenien, M., Feltz, P., Loeffler, J.P., Occipinti, G. and Santangelo, F. (1982) Brit. J. Pharmacol. 76, 289P.
Dunlap, K. and Fischbach, G.D. (1981) J. Physiol. 317, 519-535.
Mudge, A.W., Leeman, S.E. and Fischbach, G.D. (1979) Proc. Natl. Acad. Sci. 76, 526-530.
Ninkovic, M., Hunt, S.P. and Gleave, R.W. (1982) Brain Res. 241, 197-206.
Williams, J. and Zieglensberger, W. (1981) Neurosci. Letts. 21, 211-216.
Yoshida, S. and Matsuda, Y. (1979) J. Neurophysiol. 42, 1134-1145.

CHANGES IN NEUROPEPTIDE CONTENT OF AMYGDALA IN SCHIZOPHRENIA

[1]B. Carruthers, D. Dawbarn, M. de Quidt, P.C. Emson, J. Hunter and G.P. Reynolds. MRC Neurochemical Pharmacology Unit and Brain Tissue Bank, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH and [1]Dept of Pathology, University of Bristol, Bristol BS8 1TD.

The underlying pathology of schizophrenia has commonly been associated with an abnormality in dopaminergic function. Such an abnormality has recently been demonstrated as an increased concentration of dopamine in post-mortem samples of amygdala of schizophrenics compared to a control group (Reynolds, 1983). The amygdala is known to contain high concentrations of neuropeptides, and the content of cholecystokinin (CCK) has been shown to be decreased in schizophrenia (Ferrier et al, 1983). The content of a range of neuropeptides has been measured by radioimmunoassay in post-mortem samples of amygdala taken from chronic schizophrenics and compared to control values.

For assay of CCK, samples were homogenized in 90% methanol, dried down at 55°C and reconstituted in veronal buffer. For assay of vasoactive intestinal polypeptide (VIP), substance P (SP), neurotensin (NT), methionine enkephalin (Met-E), somatostatin (SRIF) and neuropeptide Y (NPY), tissue was homogenized in 1M acetic acid, freeze-dried and reconstituted in phosphate buffer.

Significant reductions as defined by Student's T-test ($p<0.05$) were observed in the content of SP (-43.9%), NT (-39.4%), Met-E (-33.9%), and CCK (-23.8%) in the amygdala of schizophrenics compared to controls. No change was observed in the content of VIP, SRIF and NPY.

These results add further weight to the suggestion of abnormal limbic pathology in schizophrenia.

Ferrier, N., Roberts, G.W., Crow, T.J., Johnstone, E.C., Owens, D.G.C., Lee, Y.C., O'Shaunghnessy, D., Adrian, T.E., Polak, J.M. and Bloom, S.R. (1983). Life Sci., 33, 475-482.

Reynolds, G.P. (1983). Nature, in press.

EVIDENCE FOR ELEVATED NATRIURETIC HORMONE LEVELS IN SPONTANEOUSLY HYPERTENSIVE RATS

Jackie Cross & M.G. Wyllie, Biochemical Pharmacology, Wyeth Institute of Medical Research, Taplow, Maidenhead, Berks, SL6 OPH.

There is much evidence to suggest that abnormal sodium metabolism is an important factor in the aetiology of hypertension. This has led to the suggestion that there is an increase in levels of a natriuretic hormone which controls sodium excretion and influences Na^+,K^+-ATPase activity (inter alia, McGregor et al, 1981; Hamlyn et al, 1982). Although these and other groups have provided evidence that natriuretic hormone levels are raised in experimental and clinical hypertensive states, a reduction in tissue Na^+,K^+-ATPase is not consistently observed. Indeed, an elevation of enzyme activity may occur in certain models (Friedman & Nakashima, 1978; Songu-Mize et al, 1982). In an attempt to rationalise these anomalies, we have examined Na^+,K^+-ATPase activity in subcellular fractions prepared from spontaneously hypertensive rats (SHR's) and their normotensive controls.

Male rats were used in all studies. Homogenates (10% w/v in 0.32M sucrose) of kidney or cerebral cortex were prepared. These were either assayed "neat" or layered onto 0.5M sucrose and centrifuged at $100,000G_{AV}$ for 60 min. Pellets of this "washed" homogenate were resuspended in 0.32M sucrose (10% w/v). The cytoplasm was collected above the 0.5M sucrose. In other experiments, the homogenate was centrifuged ($20,000G_{AV}$, 20 min) and the supernatant recentrifuged at $100,000G_{AV}$ for 60 min. The resultant microsomal material was resuspended in 0.32M sucrose (10% w/v, initial cortex). Na^+,K^+-ATPase activity was measured spectrophotometrically (Gilbert & Wyllie, 1975).

Na^+,K^+-ATPase activity in both brain and kidney homogenates from SHR's was consistently lower than that of the appropriate controls. The enzyme activity in the microsomal fractions, however, was slightly higher in control material than that prepared from SHR's. In washed homogenates, i.e. where cytoplasm/soluble protein had been removed from particulate material, Na^+,K^+-ATPase activity was similar in both groups. The difference could be partially restored by readdition of cytoplasm.

The observation that Na^+,K^+-ATPase activity was lower in homogenates prepared from SHR's than in those from controls is consistent with a hypertension-induced elevation of natriuretic hormone levels. The finding that this putative hormone influence could be removed easily by "washing" the homogenate might account for inconclusive results in studies involving lengthy preparative procedures.

Preliminary results with the antihypertensive agent, indoramin, indicate that the implied high levels of natriuretic hormone in hypertensive animals may be reduced by drug treatment. Thus, SHR's treated with indoramin ($100mg.kg^{-1}$ p.o., 14 days) displayed only slightly lower homogenate Na^+,K^+-ATPase activity than controls $|3.8 \pm 0.3(6):4.9 \pm 0.2(6)$ $\mu mol.Pi.mg\ protein^{-1}h^{-1}|$. The precise mechanism responsible for this effect of indoramin has yet to be elucidated.

Table 1 Rat brain Na^+,K^+-ATPase activity ($\mu mol\ Pi.mg\ protein^{-1}h^{-1}$)

	Normal Homogenate	Washed Homogenate	Microsomal
Control	4.7 ± 0.3(6)	6.1 ± 0.4(6)	16.4 ± 1.3(6)
SHR	2.1 ± 0.2(6)	6.6 ± 1.6(6)	19.7 ± 0.8(6)

Friedman, S.M. & Nakashima, M. (1978) Can.J.Physiol.Pharmac. 56, 1029
Gilbert, J.C. & Wyllie, M.G. (1975) Biochem.Pharmac. 24, 551
Hamlyn, J.M. et al (1982) Nature (Lond), 300, 650
McGregor, G.A. et al (1981) Br.Med.J. 283, 1335
Songu-Mize, E. et al (1982) Hypertension, 4, 575