ZETIDOLINE IS A DOPAMINE RECEPTOR ANTAGONIST IN THE RAT SUBSTANTIA NIGRA

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Zetidoline (1-(3-chlorophenyl)-3-[2-(3,3-dimethyl-1-azetidinyl)ethyl]imidazolidin-2-one HCl) is a recently introduced drug, having neuroleptic type properties. Thus it inhibits apomorphine-induced emesis and stereotype behaviour, causes catalepsy, increases plasma prolactin levels and increases the levels of striatal dopamine metabolites (Barone et al, 1982). Zetidoline is a fairly potent displacer of (^{3}H)-sulpiride binding in striatal homogenates (Holden-Dye et al, this meeting). However, like sulpiride, zetidoline is virtually inactive as an antagonist of the dopamine-stimulated adenylate cyclase (Barone et al,1982).

In this study we show that zetidoline is a potent antagonist of the dopamine autoreceptors in the substantia nigra zona compacta (SNC).

Male Wistar rats (150-180g) were anaesthetised with chloral hydrate (350 mg/kg). The animals were placed in a stereotaxic frame and anaesthesia was maintained with a 1-2% halothane/O_2 mixture. Conventional extracellular recording techniques were used to record from neurones in the SNC, using either single or standard 8 barrelled protruding tip electrodes. The recording barrels were filled with 2M NaCl containing 2% pontamine sky blue. The SNC cells were identified by their electrophysiological properties and their sensitivity to either apomorphine administered intravenously, or dopamine given iontophoretically (Bunney et al,1973, Guyenet et al, 1978). The position of the recording electrode was later verified histologically. Zetidoline was dissolved in 0.9% NaCl and apomorphine in 0.9% saline containing 8 mM tartaric acid for intravenous administration. For iontophoresis all drugs were made up in distilled water at a concentration of 0.2M, pH 4-5, with the exception of dopamine which was dissolved at a concentration of 0.2M, pH 3-4, in 8 mM tartaric acid.

Apomorphine given intravenously, (1-50 µg/kg) causes a dose-related depression of firing on all SNC cells tested. This depression was rapidly reversed within 20 seconds of administration of zetidoline (100 µg/kg) in 12 out of 12 cells. Apomorphine (1-10 µg/kg) given after zetidoline (100 µg/kg) had no effect on the firing rate, but at higher doses of apomorphine depression of neuronal firing was apparent. Dopamine injected iontophoretically (30-105 nA) depressed neuronal activity in all SNC cells tested. GABA (30-100 nA) depressed neuronal activity in 10 out of 12 cells and glutamate (30-100 nA) excited 8 out of 10 cells. Zetidoline applied iontophoretically (40-50 nA continuously) reversibly blocked the dopamine-induced inhibition in 8 out of 8 cells but had no effect on the GABA or glutamate responses.

These results give direct evidence that zetidoline is a dopamine receptor antagonist on the dopamine autoreceptors in the SNC.

Zetidoline was a gift from Lepetit, Milan.

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A COMPARISON OF (^{3}H)-SULPIRIDE BINDING SITES IN RAT AND PIG STRIATAL MEMBRANES

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A specific binding site for the dopamine antagonist [^{3}H]-sulpiride has been well characterised in rat brain (Woodruff, 1982). The present communication describes a binding site for the ligand in pig brain and compares the characteristics of the two sites.

Brain were removed from sows within 10 min of death, the striata rapidly dissected and frozen on dry ice. All striata were used within 2 months. Partially purified synaptic membranes were prepared as described by Freedman et al (1981) and incubated in Tris-Krebs' buffer, pH 7.4 for 10 min at 37 °C in the presence of 15 nM [^{3}H]-sulpiride (S.A. 26.2 Ci per mmol). Specific binding was defined by the addition of 1 μM S-(-)-sulpiride to half of the tubes and free and bound ligand were separated by filtration through Millipore filters (HAWP0025). Under these conditions specific binding represented 40-50% of total binding.

[^{3}H]-sulpiride bound to the membranes in a specific saturable manner, which was linear with protein concentration. Saturation analysis demonstrated a single binding component having a Kd of 7.3 nM and Bmax of 176 ± 36 fmol bound per mg protein. The values obtained from rat membranes were Kd 9.1 nM and a significantly higher Bmax (426 ± 23 fmol per mg). A series of dopamine agonists and antagonists were tested for their ability to displace the specific binding (Table 1). The order of potency of the drugs tested was similar on both sites. Thus the pig binding site showed stereospecificity, and appeared to represent binding to a dopamine receptor.

Table 1

	IC_{50} (nM)				
Antagonists	Pig	Rat	Agonists	Pig	Rat
Spiroperidol	0.2	0.5	Pergolide	11.2	25.0
Zetidoline	7.5	6.0	ADTN	15.8	11.6
Cis-flupenthixol	4.2	8.9	Apomorphine	63.0	125.0
(+)-butaclamol	7.9	1.6	Dopamine	199.0	398.0
(-)-sulpiride	32.0	31.6			
Trans-flupenthixol	100.0	40.0			
(+)-sulpiride	5620.0	631.0			

(-)-butaclamol, GABA, noradrenaline and nomifensine were inactive (IC_{50} greater than 10^{-5} M). Each value was derived for 1-3 experiments of 6-8 determinations in triplicate.

One important characteristic of rat [^{3}H]-sulpiride binding is that it is totally dependent on Na^+ ions (Woodruff, 1982). Similarly, in pig membrane binding was completely abolished by the omission of Na^+ from the buffer. Furthermore, the pig [^{3}H]-sulpiride binding site was inactivated by preincubation with N-ethyl-maleimide in a dose dependent fashion (IC_{50} 0.4 nM compared with IC_{50} 0.84 nM for rat), while dithiothreitol (10 mM) was ineffective.

The results demonstrate that the [^{3}H]-sulpiride binding site identified in pig striatum is similar to that in rat. Thus pig brain may be used as an economical substitute for further characterisation of the binding sites.

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RESOLUTION OF THE INDIVIDUAL COMPONENTS OF BEHAVIOURAL RESPONSE TO APOMORPHINE AND SKF 38393

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It is now thought that stereotyped behaviour induced by dopamine agonist drugs is not a unitary phenomenon but rather a composite of several individual responses with differing dose dependencies and temporal characteristics in relation to drug challenge; thus, the limitations in the use of conventional stereotypy rating scales have been recognised and the use of behavioural check-lists advocated to better characterise the individual behaviours occurring (Fray et al, 1980). We report here the evaluation of responsivity to two dopaminergic drugs, apomorphine and SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) using the concurrent application of behavioural check-list and stereotypy rating scale to contrast the different information derived from these two procedures. Male Sprague-Dawley rats of 200-300 g were used.

In the first study, groups of 5 animals were injected s.c. with vehicle or 0.125-4.0 mg/kg apomorphine HCl. At 10 min intervals thereafter they were evaluated with the behavioural check-list of Fray et al (1980) over 1 min observation periods and stereotypy assessed by rating scale (Waddington et al, 1982). At 20 min after apomorphine challenge, stereotypy ratings indicated a steep rise in score over 0.0-0.25 mg/kg, a plateau over 0.25-2.0 mg/kg and some further increase with 4.0mg/kg. At 60 min after challenge, stereotypy scores appeared to increase monotonically over 0.5-4.0 mg/kg. For individual behaviours, at the 20 min point the prevalence of sniffing was maximal at 0.25 mg/kg, with locomotion and rearing becoming more prevalent over 0.25-2.0 mg/kg; little licking or gnawing and no grooming were seen at any dose. Thus, the plateau in stereotypy scores represented the insensitivity of the rating scale to the further promotion of locomotion and rearing over the middle of the dose range. At the 60 min point, the apparent monotonic increase in stereotypy score with dose was an artefact of declining sniffing, locomotion and rearing to 0.125-0.5 mg/kg, prolonged responsivity 1.0-2.0 mg/kg and the emergence of prominent licking with 4.0 mg/kg; no grooming was seen.

In a second study with SKF 38393, this procedure was modified such that rats were assessed by the check-list once every minute for 5 consecutive minutes and then rated by the stereotypy scale, this cycle being repeated at 10 min intervals. In agreement with previous reports (Setler et al, 1978; Waddington et al, 1982), no stereotyped behaviour was observed. However, the prevalence of sniffing and grooming was notably and dose-dependently promoted (2.5-40.0 mg/kg s.c.) in a non-stereotyped manner.

Without concurrent evaluation of individual behaviours, the use of stereotypy rating scales can mislead the quantitative assessment of dopaminergic function and can fail to detect behavioural activation not emitted in a stereotyped manner.

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MIANSERIN ENHANCES DOPAMINE METABOLITE FORMATION, IN COMMON WITH CYPROHEPTADINE AND YOHIMBINE, BUT UNLIKE OTHER ANTIDEPRESSANTS

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The antidepressant drug mianserin is atypical because it does not inhibit monoamine reuptake in vivo, but possesses marked α_2-adrenoceptor blocking properties (Baumann & Maître, 1977) and is a potent central serotonin (5-HT) antagonist (Maj et al, 1978). In the rat mianserin increases the rate of noradrenaline (NA) turnover, but not that of 5-HT or dopamine (DA) after acute or chronic administration (Sugrue, 1980).

We have developed a liquid chromatographic method with electrochemical detection (HPLC-ECD) to permit simultaneous direct measurement of NA, DA, 5-HT and their metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in mouse brain, to which we have also applied the probenecid method of elevating metabolite levels to reflect DA and 5-HT turnover.

Mice were dosed orally with mianserin 30 min before probenecid (200 mg/kg i.p.) and 90 min later brains were rapidly dissected, frozen and homogenized in 0.4M perchloric acid prior to HPLC-ECD. After mianserin (50 mg/kg) marked elevations in MHPG (246%), DOPAC (118%), HVA (100%) and 5-HIAA (28%) were found, together with a decrease in NA (44%) and no change in DA or 5-HT levels. The effects were dose-dependent from 12.5 to 100 mg/kg. Enhanced DA and 5-HT metabolite formation was no longer evident in the probenecid model after subchronic oral treatment of mice once daily for 14 days with mianserin (50 mg/kg). Another 5-HT receptor antagonist, cyproheptadine (50 mg/kg orally) also elevated DOPAC (122%) and HVA (23%) but not 5-HIAA levels. The α_2-adrenoceptor antagonist yohimbine (2.5 mg/kg orally) only elevated DOPAC (80%) significantly. These findings suggest that both the 5-HT and α_2-adrenoceptor antagonist actions of mianserin may contribute to its efficacy in elevating DA metabolite levels after acute administration. The specific α_1-adrenoceptor blocker prazosin (3 mg/kg i.p.) did not antagonize the increased DA turnover due to mianserin (50 mg/kg orally), unlike that following yohimbine (Andén et al, 1982), indicating that only presynaptic adrenoceptors are involved in this effect of mianserin.

The relevance of these findings to the antidepressant action of mianserin has yet to be clarified, but in mice, using the probenecid method, DA metabolite levels were not changed after acute treatment with the tricyclics dothiepin (100 mg/kg), desipramine (20 mg/kg), amitriptyline (20 mg/kg) or with another atypical antidepressant iprindole (100 mg/kg). It is possible, since DA turnover in mouse brain is regulated partly by the activity of NA neurons (Andén & Grabowska, 1976), that the enhancement of DA turnover by mianserin is amplified in this species.

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ALTERED DOPAMINE D_2 RECEPTOR FUNCTION IN THE CAROTID BODY OF RABBITS TREATED CHRONICALLY WITH DOMPERIDONE

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Previous studies on the rabbit carotid body have provided evidence for a dopamine D_2-receptor which, upon activation, causes depression of chemosensory discharge (Mir et al, 1983). The present study was undertaken to investigate the effect of chronic treatment with the selective dopamine D_2 antagonist domperidone on rabbit carotid body dopamine D_2 receptors, using a combination of biochemical and neuropharmacological techniques.

Rabbits (New Zealand White) were given domperidone orally (2-5 mg/kg/day in drinking water) for 8 weeks and were used for experiments 4-9 days after withdrawal of the drug.

For biochemical studies, carotid bodies were removed from animals under anaesthesia and washed membranes were prepared for D_2 receptor binding assays (Lazareno & Nahorski, 1982). Assays were performed at a single saturating concentration of ^{3}H-domperidone (0.45 nM) and the non-specific binding was defined by 5 μM d-butaclamol. Tissue amine concentrations were also determined using HPLC/EC, as previously described (Mir et al, 1982). Plasma domperidone levels were monitored after oral dosing by modification of the method of Creese & Snyder (1977). Carotid body content (pmoles/carotid body) of noradrenaline 102 ± 28, dopamine 323 ± 57 and 5-hydroxytryptamine 122 ± 38 (n = 5) in domperidone-treated animals were not significantly different from vehicle-treated controls, 85 ± 19, 287 ± 48 and 103 ± 25 (n = 4) respectively, but binding of ^{3}H-domperidone to carotid body membranes was increased by 63% (5.9 ± 1.1 fmoles/carotid body) compared to the control value of 3.6 ± 0.55 fmoles/carotid body ($P < 0.05$). For neuropharmacological experiments, rabbits were anaesthetised with pentobarbitone (40 mg/kg i.v.), artificially ventilated with air and paralysed by gallamine (3 mg/kg i.v.). Electrical activity was recorded from the peripheral end of a sectioned sinus nerve and the depression of chemosensory discharge associated with intra-carotid injection of dopamine was studied (see Docherty & McQueen, 1979). In untreated animals the ID_{50} (injected dose of dopamine causing a 50% reduction in 'spontaneous' discharge averaged over the 5 s post-injection period) was 3.8 ± 0.9 (n = 13) nmoles, whereas in domperidone-treated rabbits the ID_{50} was 0.59 ± 0.26 (n = 3) nmoles ($P < 0.01$). The ID_{50} for the dopamine D_2 receptor agonist LY 141865 (Tsuruta et al, 1981) was 7.3 ± 1.9 (n = 4) nmoles in controls and 2.8 ± 2.1 (n = 3) in the chronically-treated animals.

These results show that following a period of chronic treatment with the dopamine receptor antagonist domperidone, there is an increase in the number of dopamine D_2 receptor binding sites in the carotid body, and this is accompanied by an increase in the chemodepressant effect of injected dopamine. The responsiveness of the chronically-treated animals to physiological stimuli e.g. hypoxia, are being investigated in detail.

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GABA AND BENZODIAZEPINE BINDING IN RAT CEREBRAL CORTEX DURING FOUR WEEKS ADMINISTRATION OF ETHANOLAMINE O-SULPHATE

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A deficit of GABA-mediated neurotransmission has been implicated in several neurological disorders. Increasing cerebral GABA concentration with inhibitors of GABA-transaminase (GABA-T), the principle enzyme catalysing GABA catabolism, has been proposed as a potential therapeutic strategy in the treatment of epilepsy and Huntington's chorea (Palfreyman et al, 1981). Chronic oral administration to rats of ethanolamine O-sulphate (EOS), an active-site directed irreversible inhibitor of GABA-T, produces marked and sustained increases in brain GABA concentration (Fletcher & Fowler, 1980, 1982). We now report the effects on GABA and benzodiazepine (BZ) binding in rat cerebral cortex in vitro following up to 4 weeks administration of EOS.

Male Wistar rats (mean wt 210g) received tap water (controls) or a 5 mg/ml solution of EOS ad libatum. 1, 2 and 4 weeks after the start of treatment, cortical GABA concentration and glutamic acid decarboxylase (GAD) activity were determined and membrane fractions prepared from control and EOS-treated rats. Membranes were stored at -20°C, subsequently repeatedly washed and assayed for $GABA_A$, $GABA_B$ and BZ binding sites (Horton et al, 1982, Horton & Sykes, 1982).

The mean ($\pm$ s.e.m.) daily dose of EOS was 642$\pm$28, 622$\pm$9 and 618$\pm$8 mg/kg at 1, 2 and 4 weeks respectively. There were no gross behavioural differences between control and EOS-treated rats. Cortical GABA concentration was increased 3.2-4.6 fold over the period studied (range of means; control 1.44$\pm$0.09 - 1.90$\pm$0.09 µmoles/g, EOS-treated 5.59$\pm$0.25 - 7.01$\pm$0.61 µmoles/g).

The binding of ^3H GABA to $GABA_A$ and $GABA_B$ sites was greater in EOS-treated rats than controls. The K_D (equilibrium dissociation constant) values for high and low affinity $GABA_A$ binding sites did not differ between treatments. There was an apparent increase in the number of high affinity $GABA_A$ sites at 2 weeks (40%) and 4 weeks (23%) and low affinity $GABA_A$ sites at 1 week (40%), 2 weeks (38%) and 4 weeks (47%) of EOS-treatment. The K_D values for $GABA_B$ binding did not differ between treatments, but the number of $GABA_B$ sites was greater (22-25%) in EOS-treated rats. There was no significant difference in the K_D or number of BZ binding sites between treatments.

The increase in the number of $GABA_A$ and $GABA_B$ sites was unexpected. Prolonged exposure of receptors to agonist is usually associated with a reduction in receptor number or affinity. Despite the increased GABA concentration, GABA receptors may not be exposed to high agonist concentrations after EOS-treatment. Indeed, the reverse may be true. In agreement with Fletcher and Fowler (1980, 1982) we found a progressive reduction of GAD activity measured in vitro (30% at 1 week, 36% at 2 weeks and 42% at 4 weeks) during EOS-treatment. If this reduction in GAD activity is reflected by reduced GABA synthesis and release in vivo then reduced stimulation of GABA receptors, may be the stimulus for the increase in $GABA_A$ and $GABA_B$ binding sites. Alternatively, EOS may have a direct, but weak, antagonistic action at GABA receptors.

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RECEPTORS FOR GABA AND ACETYLCHOLINE IN THE CENTRAL NERVOUS SYSTEM OF AN INSECT

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The cockroach Periplaneta americana is well suited to investigations of the receptor/ion channel complexes of insect neurotransmitters, since radiolabelled ligand binding studies on CNS extracts can be performed in parallel with electrophysiological experiments on identified neurones (Sattelle, 1980).

Gamma-aminobutyric acid (GABA) receptors

In vertebrate tissues two classes of GABA receptors [bicuculline-sensitive $GABA_A$ receptors (Olsen et al, 1981), and baclofen-sensitive $GABA_B$ receptors (Bowery, et al, 1981)] have been identified and characterized, but there has been comparatively little work to date on GABA receptors in insect CNS preparations. We have demonstrated a saturable component of (^{3}H)GABA binding, representing a putative bicuculline-sensitive GABA receptor. Scatchard analysis yields K_D and B_{max} values of 5µM and 12 pmol/mg protein respectively. Voltage-clamp experiments on an identified motoneurone (D_f) revealed GABA-induced chloride currents. GABA and bicuculline inhibited (^{3}H)GABA binding to CNS extracts, whereas picrotoxin did not. However, both antagonists were membrane-potential dependent in their blocking actions, indicating that picrotoxin interacts with the channel component of the insect GABA-receptor/ion channel complex, whilst bicuculline may act on both the channel component and the recognition site. Preliminary experiments with (^{3}H)flunitrazepam suggest that there are benzodiazepine binding sites in this preparation which may be associated with GABA receptors.

Acetylcholine (ACh) receptors

The biochemical and pharmacological properties of putative nicotinic and muscarinic cholinergic receptors were investigated using the ligands N-[propionyl-^{3}H]propionylated ∝-bungarotoxin (∝-BGTX) and (^{3}H)quinuclidinyl benzilate (QNB). A saturable component of (^{3}H)∝-BGTX binding was detected and Scatchard analysis yielded a K_D of 5nM and a B_{max} of 910 fmol/mg. The order of effectiveness of cholinergic ligands in inhibiting (^{3}H)∝-BGTX binding broadly paralleled their relative effectiveness on D_f. A saturable component of (^{3}H)QNB binding was also demonstrated. As was the case for the (^{3}H)∝-BGTX binding component, the pharmacological profile for this putative insect receptor site was observed to closely correspond to that of its vertebrate counterpart. However, the (^{3}H)∝- BGTX binding sites were found to be more abundant, by a factor of 10, than the (^{3}H)QNB binding sites, in direct contrast to vertebrate CNS studies (Yamamura & Snyder, 1974; Segal et al, 1978).

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ACTIONS OF THE BENZODIAZEPINE RECEPTOR 'INVERSE AGONIST', DMCM, IN THE PRIMATE, PAPIO PAPIO

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Agents competing for high affinity binding sites for benzodiazepines within the brain (Squires & Braestrup, 1977) can be classified into 3 groups: 1) agonists e.g. diazepam, lorazepam, which show anticonvulsant and anxiolytic actions, 2) antagonists e.g. RO 15-1788, which have no intrinsic pharmacological activity but block the actions of other benzodiazepine ligands, and 3) inverse agonists e.g. DMCM, B-CCM. This latter class of compounds facilitate audiogenic seizures in DBA/2 mice stimulated with subconvulsive sound intensities (Jensen et al, 1983). Additionally, inverse agonists possessing high efficacies induce spontaneous convulsions in rodents following systemic administration (Braestrup et al, 1982). We report here the activity of a potent inverse agonist, DMCM, in a primate model of epilepsy, the photosensitive baboon Papio papio.

A group of adolescent baboons (n = 3) were selected for their low sensitivity to photic stimulation, control tests inducing only intermittent eyelid myoclonus. Little change in photosensitivity or behavioural activity was observed following intravenous administration of DMCM, 0.125 mg/kg. After DMCM, 0.25 mg/kg however, photosensitivity was dramatically increased (Table 1).

Table 1 Facilitation by DMCM of photically-induced seizures in the baboon, Papio papio. (Myoclonic response to stroboscopic stimulation graded:- 1 = eyelids; 2 = face & neck; 3 = limbs; 4 = self-sustaining myoclonus).

	Mean myoclonic response (n = 3)			
DMCM (mg/kg, iv)	Control	1 min	60 min	120 min
0.125	1.2	1.7	0.7	0.3
0.25	0.7	3.2	0.5	0.2
	Control	5 min	60 min	120 min
0.125	0.7	1.2	0.5	-
0.25	1.3	2.3	1.0	0

All animals exhibited a diffuse myoclonic response to photic stimulation involving both limbs and trunk, whilst in one animal myoclonus was self-sustaining beyond the end of stimulation. This activity was manifest 1 min after drug administration, had diminished 4 min later, and was no longer evident after 1 h. Additionally, these animals displayed an anxiety syndrome consisting of expressions of expectancy and apprehension accompanied by continuous vigilant eye-movements.

DMCM, 0.5 mg/kg, induced spontaneous tonic-clonic seizures in all animals within 10-90 sec of administration. These seizures could be abolished by prior intravenous administration of the potent NMDA receptor antagonist 2-amino-7-phosphonoheptanoic acid suggesting that the NMDA receptor plays an important role in the generation of seizures by inverse agonists.

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ROLE OF OPIATES IN BAROREFLEX CONTROL OF HEART RATE IN CONSCIOUS RABBITS

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We have recently demonstrated the existence of genetic differences in the sensitivity of baroreflex control of heart rate in response to a vasoconstrictor agent in normotensive rabbits (Weinstock & Rosin, 1983). This difference was only detected when baroreflex sensitivity (BRS) was assessed by the "steady state" method (Korner et al. 1974), in which BP and heart period (HP) are measured at the peak of a series of increasing pressor stimuli. This method detects both increases in vagal activity and sympathetic withdrawal, while the more commonly used "ramp method" (Smyth et al. 1969) detects almost exclusively changes in vagal activity. Thus the degree of sympathetic inhibition in response to a pressor stimulus appears to vary widely in normotensive rabbits.

Enkephalins and opiate receptors are present in the n. tractus solitarius and may play a role in cardiovascular regulation (Petty & Reid, 1982). This study reports the influence of opiate receptor activation on BRS in the two groups of rabbits. BRS was assessed in response to i.v. phenylephrine (2.5-20μg/kg) in conscious rabbits before, and 15 min. after i.v. morphine (2mg/kg) or i.v. naloxone (0.1mg/kg), by both "steady-state" and "ramp" methods.

Naloxone had no significant effect on resting heart rate or BP in either group of rabbits, but it decreased the slope of the BP-HP relationship in group I rabbits only, when measured by the "steady-state" method. Morphine, caused a significant increase in slope by this method in group II rabbits, which was antagonised by naloxone. Neither drug had any effect when the ramp method was used in the same rabbits.

Table 1 - Effect of morphine and naloxone on baroreflex control of heart rate in the rabbit

Dose of drug (mg/kg)	Rabbit group	BP mmHg ±s.e.m.	Heart rate beats/min. ±s.e.m.	Baroreflex slope msmmHg^{-1} Steady State method ±s.e.m.	Ramp method ±s.e.m.
Control	I (16)	85.9±1.8	247± 7	23.2±1.5	6.58±1.08
	II (16)	90.4±2.0	255± 5	7.68±0.75 [a]	5.64±0.68
Morphine 2.0	I (8)	81.5±3.9	200±21	26.0±1.0	7.12±1.14
	II (8)	83.2±3.0	207±15 *	15.5±1.79 **	6.77±1.44
Naloxone 0.1	I (8)	82.5±2.8	233±10	9.94±1.23 **	5.10±0.78
	II (8)	86.9±2.6	271±9	10.3±1.4	5.92±1.6

a P< 0.01 cf GpI * P< 0.05 cf Control ** P < 0.01 cf Control.

It is concluded that sympathetic withdrawal in the baroreflex response to phenylephrine is prevented by naloxone and may therefore involve the activation of opiate receptors. The observed differences in BRS may reflect the degree of opiate receptor stimulation.

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SECRETION OF CORTICOTROPHIN AND ITS RELEASING FACTOR IN THE MORPHINE-TREATED RAT

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Although it is well known that the activity of the hypothalamo-pituitary-adrenocortical (HPA) system is influenced by drugs which interact with opiate receptors, their site and mode of action is not known. Accordingly, we have compared the effects of acute and chronic treatment with opiate drugs on the secretion in vivo and in vitro of corticosterone, corticotrophin (ACTH) and corticotrophin releasing factor (CRF) in the rat.

A single injection of morphine (2mg/100g i.p.) stimulated the release of ACTH (Alaghband-Zadeh, et al (1974)) and corticosterone (Al-Dujaili, et al (1981)) and caused an exaggeration of the HPA response to stress (laparotomy). None of the opioid substances tested influenced directly the secretion in vitro of ACTH by the pituitary gland. However, morphine (10^{-10}M-10^{-6}M), leu-enkephalin (10^{-11}M-10^{-5}M), met-enkephalin (10^{-8}M-10^{-6}M) and β-endorphin (10^{-12}-10^{-10}) caused dose-related increases in the CRF (Buckingham & Hodges, 1977) contents of both the hypothalami and the medium in which they were incubated. Their actions were competitively antagonized by naloxone and the pA_2 values suggested that their stimulatory effects on CRF release and CRF content were mediated by the same receptors. In complete contrast, rats rendered tolerant to morphine (2mg/100g i.p. daily for 9 days) failed to secrete ACTH in response either to a further injection of the opiate or to stress. The capacities of hypothalami and pituitary glands, removed from morphine-tolerant rats, to secrete CRF and ACTH in response to trophic stimuli were reduced with a striking inability of the hypothalami to respond to morphine (10^{-10}-10^{-6}M) or acetylcholine (10^{-9}-10^{-6}M). The development of the inhibitory effect paralleled the development of tolerance to the analgesic action of the opiate.

The results suggest that morphine, given acutely, stimulates HPA activity by acting on specific receptors in the hypothalamus and supports the hypothesis that opiate receptors are physiologically important in the control of CRF secretion. The mechanisms responsible for the inhibition of HPA function in morphine-tolerant rats are being investigated.

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FACILITATION OF THE PITUITARY ADRENOCORTICOTROPHIC RESPONSE TO CRF-41 BY ARGININE VASOPRESSIN

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Studies in rats with inherited diabetes insipidus (Brattleboro strain) suggest that the activity of the physiological corticotrophin releasing factor(s) (CRF) is facilitated by arginine vasopressin (AVP) (Buckingham, 1981). This hypothesis has now been examined further using the recently isolated hypothalamic peptide, CRF-41 (Vale et al, 1981).

CRF-41 (0.1-1µg/100g body weight), injected intravenously, caused dose-related increases in the plasma corticotrophin (ACTH) concentrations in rats in which endogenous 'CRF' secretion had been blocked by treatment with pentobarbitone and chlorpromazine (Lammers & de Wied, 1964). The slopes of its dose-response lines were parallel with those of rat hypothalamic extracts (0.2-0.8 hypothalamic equivalents (HE)/100g body weight) but greater than those of AVP (100-200ng/100g body weight). CRF-41 (10-200ng/ml) also stimulated the secretion of ACTH by segments of adenohypophysial tissue in vitro but, in contrast to the situation in vivo, the slopes of its dose-response lines were significantly ($P < 0.01$) less than those of hypothalamic extracts (0.1-0.8 HE/ml) although still greater than those of AVP (50-400ng/ml). Simultaneous addition of AVP to the incubation medium, in concentrations (10-50pg/ml) well below those required to evoke ACTH secretion directly, potentiated the activity of CRF-41 and increased significantly the slope of its dose-response lines. The adrenocorticotrophic response to CRF-41 was enhanced similarly by pre-treatment of the pituitary segments with AVP (10-50 pg/ml). This effect appeared to be specific to vasopressin for pre-treatment of the tissue with CRF-41 (0.1-100ng/ml) depressed the subsequent response to CRF-41 (10-100ng/ml) but did not affect that to AVP (50-100ng/ml).

The results suggest that arginine vasopressin facilitates the activity of CRF-41 on the pituitary gland and hence may be essential for the full expression of the physiological action of the releasing factor.

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SK&F 93319: A SPECIFIC ANTAGONIST OF HISTAMINE AT H_1 - AND H_2-RECEPTORS.

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Two types of histamine receptor, H_1 and H_2, have been characterised by use of anta-gonists differing widely in chemical structure and physicochemical properties.

We now report that SK&F 93319 (2-{4-(3-methoxypyrid-2-yl)butylamino}-5-{(6-methyl-pyrid-3-yl)methyl}-pyrimidin-4-one} has approximately equipotent antagonist activi-ties at both types of histamine receptor. It differs chemically from most of the described H_1-receptor antagonists in that it is not a tertiary aliphatic amine and is not cationic at physiological pH (pK_a values at 25°C ca 3.6, 5.7 and 6.1).

It differs inter alia from reported pyrimidone H_2-receptor antagonists (oxmetidine and SK&F 93479) in that the imidazole or furan rings are replaced by a 3-methoxy-pyridine ring and the side-chain S is replaced by CH_2.

On the isolated guinea-pig atrium (H_2) SK&F 93319 antagonised the positive chrono-tropic action of histamine. Full development of antagonism was slow so that al-though after 60 minutes incubation the pA_2 value was 7.49 (7.00-8.50) with a Schild plot slope of 0.89±0.33 (mean ±95% confidence limits), after 8 minutes the pA_2 value was only 6.66 (6.48-6.88) with a slope of 0.95±0.17. Concentrations up to 160 µM did not cause parallel displacement of the isoprenaline dose-response curve but did depress the maximal response.

SK&F 93319 inhibited H_1 histamine-induced contractions of the isolated guinea-pig ileum in a concentration dependent manner. Antagonism rapidly reached equilibrium with pA_2 values after 2 and 8 minute incubations of 7.67 (7.20-8.50) and 7.77 (6.85-8.39) respectively; slopes of the Schild plots were not significantly differ-ent from unity suggesting competitive antagonism. SK&F 93319 antagonised the res-ponse to carbachol at high concentrations giving an approximate pA_2 value of 4.8.

In vivo SK&F 93319 inhibited histamine-stimulated gastric acid secretion in the perfused stomach preparation of the anaesthetised rat (H_2), 50% peak inhibition being obtained at an intravenous dose of 0.21 µmol/kg (c.f. 1.37 µmol/kg for cimetidine). In the conscious Heidenhain pouch dog, doses of 0.5 and 1.0 µmol/kg i.v. gave mean peak inhibitions of 42% and 86% respectively (c.f. cimetidine 70% inhibition at 4 µmol/kg).

The in vivo H_1-receptor antagonistic action was measured using histamine-induced bronchoconstriction in the anaesthetised guinea-pig. At doses of 0.125 and 1.25 µmol/kg i.v. SK&F 93319 produced a parallel displacement of the histamine dose-response curve with dose ratios of 1.6 and 6.3 respectively (c.f. mepyramine dose ratio = 5.2 at 12.5 nmol/kg i.v.)

Previously, H_1 and H_2 receptors have been differentiated by use of chemically distinct specific antagonists from which it has been inferred that the receptors may differ in their molecular architecture. The discovery that both types of anta-gonism can be combined in one molecule raises the question of the extent to which the receptors may be similar.

For some effects of histamine, blockade requires antagonism at both H_1 and H_2 receptors (e.g. on the cardiovascular system) but use of a combination of conven-tional antagonists may be problematical since their differing physicochemical properties can lead to widely differing pharmacodynamics. In such circumstances there may be a considerable advantage in using a compound which combines the two activities, and potential uses of SK&F 93319 are being explored in human studies.

INHIBITION OF VASCULAR RESPONSES TO HISTAMINE BY SK&F 93319, A HISTAMINE ANTAGONIST AT H_1- AND H_2-RECEPTORS

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Histamine causes dose-dependent falls in blood pressure in most species due to dilatation of peripheral resistance vessels. Studies using histamine receptor agonists and antagonists have clearly demonstrated the involvement of both H_1- and H_2-receptors on peripheral resistance vessels in these responses; both H_1- and H_2-receptor antagonists are needed to inhibit depressor and vasodilator responses to histamine (Black et al, 1975; Flynn & Owen, 1975).

SK&F 93319 is a combined antagonist of histamine at H_1- and H_2-receptors (Blakemore et al, 1983).

We have studied the inhibition of vascular responses to histamine and selective histamine receptor agonists by SK&F 93319.

Experiments have been made in cats, anaesthetised with sodium pentobarbitone, 60 mg/kg i.p. Antagonism of depressor responses to intravenous injections of histamine, 2-(2-aminoethyl)-pyridine (an H_1-receptor agonist), dimaprit and impromidine (H_2-receptor agonists) by SK&F 93319 were assessed by measurement of displacement of dose-response curves to each agonist. Similarly, the antagonism of vasodilator responses to intra-arterial injections of histamine and histamine receptor agonists were made in the acutely denervated femoral vasculature (Flynn & Owen, 1975).

SK&F 93319 caused dose-dependent displacement to the right for the depressor and vasodilator responses to histamine, 2-(2-aminoethyl)pyridine, dimaprit and impromidine (depressor responses only studied) and, for a given dose of SK&F 93319, the dose-ratios were similar for both depressor and vasodilator responses. SK&F 93319 had similar antagonist activity against histamine and 2-(2-aminoethyl)-pyridine but was more effective as an antagonist of dimaprit and impromidine responses, which contrasts with its similar activity at H_1- and H_2-receptors measured in vitro (Blakemore et al, 1983).

Dose of SK&F 93319 μmol/kg/min	Mean dose-ratio (95% confidence limits, n=4) Histamine	2-(2-aminoethyl) pyridine	Dimaprit	Impromidine
		Depressor responses		
1.6×10^{-8}	1.1(1.02-1.1)	-	9.7(9.7-10.3)	9.9(9.4-10.5)
8×10^{-8}	3.4(2.4-4.9	5.5(3.3-10.5)	200(111-500)	-
4×10^{-7}	32.3(19.2-52.6)	55.6(9.6-500)	>1000	-
		Vasodilator responses		
8×10^{-8}	2.8(1.5-5.2)	5.3(2.8-11.5)	500	-
4×10^{-7}	47.6(19.6-143)	53.5(19.3-208)	>2000	-

Thus, SK&F 93319 possesses antagonist activity against histamine at both H_1- and H_2-receptors on peripheral vasculature. SK&F 93319 displaces histamine dose-response curves to an extent that can only be achieved by a combination of H_1- and H_2-receptor antagonists e.g. mepyramine plus metiamide or cimetidine (Black et al, 1975; Flynn & Owen, 1975). SK&F 93319 might have therapeutic utility in conditions requiring simultaneous antagonism of histamine at H_1- and H_2-receptors e.g. inflammatory skin diseases.

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PHARMACOLOGICAL MODULATION OF PLATELET-ACTIVATING FACTOR (PAF-ACETHER) INDUCED BRONCHOSPASM AND PLATELET AGGREGATION

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PAF-acether is a potential mediator of asthma and inflammation (Vargaftig et al, 1981). Recently, the local administration of disodium cromoglycate (DSCG) was shown to inhibit the cutaneous wheal and flare response elicited by PAF-acether (Basran et al, 1982). This prompted the suggestion that inhibition of PAF-acether by DSCG may be partly responsible for the effectiveness of DSCG in asthma. We have attempted to extend these observations using a variety of bronchodilator and antiallergic drugs using PAF-acether induced bronchospasm in guinea pigs and platelet aggregation in rabbits. The role of lipoxygenase products in the former response has also been investigated.

PAF-acether (40-80 ng/kg i.v.) produces submaximal bronchospasm in pentobarbital-anesthetised male Hartley guinea pigs as measured by the Konzett-Rossler overflow technique (Lewis et al, 1982). DSCG administered by aerosol (1%) and i.v. (1-10 mg/kg)60 s prior to PAF-acether failed to influence the bronchospasm. Wy-41,195 (10 mg/kg i.v.), an antiallergic agent more potent than DSCG (Carlson et al, 1982), ipratropium bromide (1%), promethazine (1%), ketotifen (1%) and FPL 55712 (1%) also failed to alter this response. In contrast, thiazinamium chloride (1%) and FPL 55712 (10 mg/kg, i.v.) both administered 1 min prior to the agonist, and phenidone, BW 755C and indomethacin (all at 10 mg/kg i.v.) administered between 2 and 10 min prior to the agonist, significantly inhibited the bronchospasm.

Platelet aggregation was monitored using a Chrono-log aggregometer containing platelet-rich plasma prepared from fresh citrated (1 part 3.8% sodium citrate to 9 parts blood) rabbit blood. When platelet aggregation was induced with PAF (5×10^{-9} M), thiazinamium chloride inhibited the aggregation in a dose-related manner ($IC_{50} = 8 \times 10^{-6}$ M). In contrast, DSCG and FPL 55712 had no effect on PAF-induced aggregation even at a dose as high as 10^{-4} M. Neither thiazinamium nor DSCG had any effect on platelet aggregation induced by either arachidonic acid or ADP.

The failure of antiallergic drugs to inhibit bronchospasm and platelet aggregation induced by PAF-acether is in marked contrast to the effects observed in the skin (Basran et al, 1982). The inhibitory effects observed with thiazinamium cannot be explained by its anticholinergic, antihistaminic and mast cell stabilizing properties (Lewis et al, 1982). Other anticholinergics, antihistamines and antiallergic drugs failed to modify the bronchospasm so that another mechanism(s) needs to be invoked to explain the activity of thiazinamium. Both lipoxygenase and cyclooxygenase products appear to be involved in the PAF-acether response since a leukotriene antagonist, lipoxygenase inhibitors and cyclooxygenase inhibitor independently suppressed the bronchospasm. This suggests that thiazinamium may be capable of modulating the metabolism of arachidonic acid, or antagonizing the activity of the end products.

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THE ROLE OF CYCLO-OXYGENASE METABOLITES OF ARACHIDONIC ACID IN FELINE ANAPHYLACTIC BRONCHOSPASM

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There have been relatively few studies using cats as a model of anaphylaxis in vivo. It has been shown that cats can be actively sensitised to foreign proteins and subsequently react to antigen challenge with bronchoconstriction (Barch and Talbott, 1976; Mitchell and Sparrow, 1977). We have confirmed and extended these observations by determining the nature of the mediators involved in feline anaphylactic bronchospasm.

Female mongrel cats (1.8 - 2kg) were sensitised to ovalbumen (OA) by the intraperitoneal injection of 50 mg of this antigen in 1ml saline on three consecutive days. Between 6-8 weeks later randomly selected animals were anaesthetised (induction with 5% halothane; maintained with a mixture of 1% chloralose/sagatal 9:1, i.v.), and the cervical trachea cannulated. Animals were pump-ventilated with 27 strokes/min of 15 ml of laboratory air/kg body weight. The pleural cavity was opened by bilateral intercostal incisions and intrapulmonary pressure measured with an Ether UP1 differential pressure transducer. Airflow was measured with a heated Fleisch size 00 pneumotachograph and a Validyne MP45 differential pressure transducer. Pulmonary airways resistance (R_A) and dynamic lung compliance (C_D) were calculated on a breath by breath basis by an on line analogue computer (Buxco Electronics, Model 6). Femoral arterial blood pressure and heart rate were also recorded. All administrations of OA, test drugs etc., were made via an indwelling catheter in the left femoral vein. In some experiments blood samples (1ml) were taken from the aortic arch for radioimmunoassay of plasma thromboxane B_2, TXB_2 (Salmon, 1978) and leukotriene B_4, LTB_4 (Salmon et al 1982) at various times during antigen challenge.

Intravenous challenge with OA (1-160 mg/kg) provoked bronchoconstriction which increased progressively up to a maximum fall in C_D of 31.3 ± 2.9% (n=15) and rise in R_A of 91.8 ± 33% (n=15). Intravenous administration, 5 mins before OA challenge, of a mixture of mepyramine (5 mg/kg), methysergide (1 mg/kg), atropine (2 mg/kg) and the slow reacting substance of anaphylaxis antagonist FPL55712 (10 mg/kg) had no effect on the bronchoconstriction provoked by O.A. (1-160 mg/kg i.v., n=4).

TXB_2, but not LTB_4, was detected in cat plasma during O.A. challenge (1-160 mg/kg i.v.). The levels of TXB_2 found also increased progressively (up to 10.9 ± 3.1 ng/ml, n=6) in relation to both the amount of OA and the resulting degree of bronchoconstriction. In addition the time course of TXB_2 formation coincided with the time course of development of bronchoconstriction during any one challenge. The cyclo-oxygenase inhibitor indomethacin (0.1 - 1.0 mg/kg i.v., n=6) and the dual cyclo-oxygenase/lipoxygenase inhibitor of arachidonic acid metabolism BW755C (10 mg/kg i.v., n=3) significantly inhibited both the bronchoconstriction and TXB_2 formation associated with OA challenge.

We can conclude, therefore, that in cats OA-induced bronchospasm following i.v. challenge is due to the formation and release of bronchoconstrictor cyclo-oxygenase products of arachidonic acid metabolism, possibly TxA_2. Inhibition of bronchoconstriction by BW755C is likely to be due to its effectiveness as a cyclo-oxygenase inhibitor.

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PGE_2-INDUCED RENAL VASODILATATION IN THE GENETICALLY HYPERTENSIVE NEW ZEALAND RAT

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Recently, we have been able to demonstrate PGE_2-induced renal vasodilatation in the normotensive Wistar rat using an electromagnetic flow probe to measure renal blood flow (Haylor & Towers 1982). This result was in marked contrast to experiments performed in the isolated perfused rat kidney where PGE_2 is a renal vasoconstrictor (Malik & McGiff 1975). From experiments using isolated perfused rat kidneys, Armstrong et al (1976) proposed that reduced renal catabolism of PGE_2 and an increase in sensitivity to its renal vascular effects may contribute to the elevated blood pressure of the genetically hypertensive New Zealand rat. In the following experiments, the influence of PGE_2 on renal blood flow in the genetically hypertensive New Zealand rat has been studied in vivo, using an electromagnetic flow probe.

Normotensive and genetically hypertensive New Zealand Wistar rats (300-400g) were anaesthetised with sodium thiobutabarbitone (100-180mg/kg). PGE_2 was infused locally into the left kidney through a cannula which had been placed in the femoral artery and advanced up the aorta so that its tip lay adjacent to the left renal artery. Blood pressure was recorded from the carotid artery and NaCl (0.153M) was infused into the jugular vein at 50µl/min. A small electromagnetic flow probe (0.48-0.64mm diameter) was placed over the left renal artery using a retro-peritoneal approach. Following carotid arterial cannulation, the mean systemic arterial blood pressure was significantly higher ($P<0.05$) in the hypertensive group (180 ± 18mmHg n = 6) than in the normotensive controls (132 ± 14mmHg n = 6), although these values were influenced by both anaesthetic and surgical procedures. In the 15 minute period before PGE_2 dose response experiments were performed, the hypertensive group had a lower ($P<0.01$) renal blood flow (2.79 ± 0.53 compared to 5.58 ± 0.42 ml/min/g wet weight) a higher ($P<0.01$) mean arterial blood pressure (132 ± 7 compared to 104 ± 4mmHg) and a higher calculated renal vascular resistance (52.9 ± 5.8 compared to 19.0 ± 1.6 mmHg/ml/min/g wet weight). In the hypertensive rat PGE_2 1µg/min increased ($P<0.05$) renal blood flow by 12 ± 2% and reduced ($P<0.05$) systemic arterial blood pressure by 9.5 ± 1.2% and calculated renal vascular resistance by 20 ± 3.9%. Normotensive rats appeared to be more sensitive to the actions of PGE_2 which, at 1µg/min, increased ($P<0.01$) renal blood flow by 20 ± 4% and decreased ($P<0.01$) both mean arterial blood pressure by 14 ± 2.6% and calculated renal vascular resistance by 32 ± 4.5%.

It is concluded that PGE_2 has the ability to increase renal blood flow in both normotensive and genetically hypertensive New Zealand Wistar rats. A decrease in the renal catabolism of PGE_2 would therefore be unlikely to promote a prohypertensive response in the New Zealand genetically hypertensive rat.

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SPONTANEOUS RELEASE OF A 6 KETO PROSTAGLANDIN E_1-LIKE SUBSTANCE FROM ISOLATED PERFUSED RAT LUNGS

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6 keto prostaglandin E_1 (6 keto PGE_1) is a biologically active metabolite of prostacyclin (PGI_2) formed by the action of the enzyme prostaglandin 9-hydroxy dehydrogenase. To date this enzyme has not been demonstrated in lungs. We now report the conversion of PGI_2 to 6 keto PGE_1 by homogenates of rat lung in vitro and the spontaneous release of a 6 keto PGE_1 like substance from perfused rat lungs ex vivo.

High speed (100,000g) supernatants (HSSN) of lungs from male, Wistar rats (200-400g) were prepared as previously described (Hoult & Moore, 1977) and incubated with PGI_2 (1µg/ml) containing 0.05µCi/ml 9β ^{3}H PGI_2 methyl ester (sp. act. 12.0 Ci/mmol). After timed intervals at 37°C 0.2ml aliquots were removed, acidified and extracted into ethyl acetate. Dried residues were reconstituted in methanol and the radioactivity determined by liquid scintillation counting. Conversion to non radioactive 6 keto PGE_1 was measured by comparison with radioactivity in samples extracted at zero time on ice. Some incubations contained naringenin to inhibit 6 keto PGE_1 synthesis (Griffiths et al 1983). In separate experiments rats were killed by cervical dislocation, a cannula inserted into the pulmonary artery and the lung perfused with warmed, oxygenated Krebs' solution (5ml min^{-1}). At 30min intervals 50ml fractions were collected, acidified and extracted by column chromatography using Sep Pack C18 columns (Waters Ass.). Prostaglandins were eluted into ethyl acetate and bioassayed against ADP induced human platelet aggregation and on the field stimulated guinea pig vas deferens (Griffiths & Moore, 1983). Some extracts were subjected to thin layer chromatography in solvent F6 (ethyl acetate:acetone:acetic acid, 90/10/1 v/v). After development for 40min the chromatogram was cut into 1cm strips from the origin to the solvent front, eluted with 2ml methanol and bioassayed against ADP induced human platelet aggregation.

Incubation of 9β ^{3}H PGI_2 with rat lung HSSN resulted in a time dependent loss of extractable radioactivity which reached a maximum between 60-120min (34.9±9.0%, n=8, T=120min). Naringenin (3-300uM) dose dependently inhibited the loss of extractable radioactivity (IC_{50}=10.3±0.8µM, n=8).

Perfused lungs released a stable, anti-aggregatory substance at a constant rate over the period 30-120min (0.77±0.07 ng 6 keto PGE_1 equivs./min, n=76). Indomethacin (20µM) and naringenin (100µM) reduced release of this substance by 68.8±8.5%, n=11 and 66.2±13.8%, n=8, respectively. There was no significant difference in the amount of bioassayable activity extracted from 50ml fractions assayed on the field stimulated guinea pig vas deferens and against ADP induced human platelet aggregation (0.41±0.10, n=12 and 0.36±0.06, n=15, ng 6 keto PGE_1 equivs/min respectively). After extraction and chromatography of 50ml fractions of perfusate all anti-aggregatory activity co-chromatographed with authentic 6 keto PGE_1 (3.2±0.48 ng 6 keto PGE_1 equivs, n=13).

These results indicate that rat lung 100,000g HSSN converts PGI_2 to 6 keto PGE_1 and that isolated perfused rat lungs spontaneously release a 6 keto PGE_1 like substance.

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THE TRANSFER OF LITHIUM ACROSS EVERTED SACS OF RAT SMALL INTESTINE

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1 in 2000 of the U.K. population receives lithium orally for the treatment of manic-depressive psychoses (Birch, 1982). However, the site and mechanisms of lithium absorption across the small intestine and its subsequent effects on intestinal function have not been fully characterised. The present study investigates the transfer of lithium across everted sacs of small intestine and examines the effect of lithium on intestinal potential difference, glucose transfer and fluid transfer.

Everted sacs were prepared as described by Wilson and Wiseman (1954). Potential difference was measured by the methods of Barry et al (1964) and glucose assayed by glucose oxidase method (Bernt and Lachenicht 1974). Serosal, mucosal and tissue levels of lithium were determined by Atomic Absorption Spectroscopy (Birch and Jenner, 1973).

In the presence of clinically relevant concentrations of lithium (20mM), a highly significant increase in potential was observed (20-30%; $P < 0.001$) which may reflect an increase in ion movement across the small intestine. A concomitant increase in glucose transfer was not statistically significant. At higher lithium concentrations (100mM) a significant decrease in fluid transfer was observed although this may be due to sodium depletion in the bathing medium rather than a specific effect of lithium. In presence of 20mM and 100mM choline chloride fluid transport was not significantly different from the control value; the reduction in fluid uptake appears to be a lithium effect rather than due to sodium depletion.

The movement of lithium from the mucosal solution to the serosal compartment was similar for all regions of the intestine (Duodenum, 76.6; Jejunum, 104; and Ileum, 103.6 nmoles/g final wet weight/min.) and proceed in a time and concentration-dependent linear fashion. Uptake of lithium by intestinal tissue followed by a similar pattern over the range of lithium concentrations used therefore indicating the mechanism involved may well be passive. However, time-based uptake demonstrated that equilibration occurred after 15 minutes incubation, which suggests that the initial mechanism of uptake is a surface adsorption process.

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COMPARISON OF THE ACTIVITY PROFILE OF INDOMETHACIN AND PREDNISOLONE IN THE KOCH MODEL

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In the Koch model, the inflammatory response induced by the subplantar inoculation of Freund's complete adjuvant (FCA) requires the presence of T lymphocytes and results in the formation and persistence of both extra-vascular (e-v) fibrin deposits and oedema (Bullock, et al., 1983).

The ability of indomethacin and prednisolone to inhibit e-v fibrin formation, fibrinolysis, exudation, and oedema formation was investigated in the present study. Foot-pad inflammation was induced in male COB Wistar rats (200-250 g) previously immunised to FCA (Bullock, et al., 1983). Fibrin formation and removal was quantified using ^{125}I-human fibrinogen, B.P., (2-4 µCi; Amersham International PLC) administered (i.v.) 6 h after challenge. Exudation was measured using ^{131}I human serum albumin, B.P., (3-6 µCi; CIS U.K. Ltd.) administered (i.v.) 1.5 h before measurement. Oedema was measured as the increase in fresh weight of the challenged compared with unchallenged paws. Drugs were administered (p.o.) dissolved in polyethylene glycol, (PEG 400; 1 ml/kg), followed by 5% (w/v) dextrose (3 ml). Control animals received PEG 400 and dextrose only.

At 24 h post-challenge, 18 h after a single drug dose, both prednisolone and indomethacin inhibited fibrin formation (Figure 1a) and oedema. Inhibition using prednisolone was reproducibly dose-related and reached 80-90%. Inhibition using non-ulcerogenic doses of indomethacin was not reproducibly dose-related and did not exceed 30%.

At 96 h post-challenge, 18 h after the last of four once daily doses, inhibition of oedema by prednisolone reached 80-90%, and by indomethacin reached 40%. Prednisolone was marginally anti-fibrinolytic at its highest dose only (Figure 1b). By comparison, indomethacin was potently anti-fibrinolytic in a dose-related manner.

At 24 and 96 h post-challenge, indomethacin inhibited exudation but prednisolone did not.

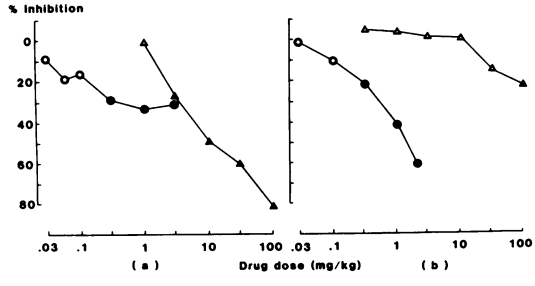

Figure 1. Mean per cent inhibition of fibrin formation after a single dose (a) and fibrinolysis after four once daily doses (b) of indomethacin (O-O) and prednisolone (Δ-Δ) in FCA-challenged rats. Filled symbols indicate $p < 0.05$ (Student's t-test), n = 6.

The significance of these findings to the management of chronic immunological oedema, and the possible mechanisms underlying the anti-fibrinolytic effect exerted by indomethacin and six other analgesic NSAIDs investigated, remain to be elucidated.

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THE INVOLVEMENT OF THE ENDOTHELIUM IN THE ACTION OF SUBSTANCES WHICH RELAX THE RAT AORTA

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Acetylcholine (ACH) relaxes pre-contracted strips of rabbit and dog aortae and this relaxant effect is abolished by removing the endothlium or exposing to the dual lipoxygenase/cyclo-oxygenase inhibitor eicosatetraynoic acid (ETYA) (Furchgott & Zawadski, 1980; De Mey & Vanhoutte, 1980). The present experiments were carried out to determine whether other dilators acted in a similar fashion and whether an arachidonic acid (AA) metabolite was implicated.

Male Wistar rats (300-500g) were killed and the thoracic aortae carefully dissected out. A ring 5mm in width was cut off and opened by longitudinal cut. Fine mounting needles were pushed through the cut edges and the preparation attached to an isometric transducer to record tension changes in the circular muscle. The preparation was placed under 1g initial tension and bathed with Krebs' solution at 37°C bubbled with 95% O_2/5% CO_2. Each strip was allowed to equilibrate for 45 min.

A cumulative dose-response curve to noradrenaline (NA) was then constructed (0.3-358 x 10^{-9}M). A dose producing approximately 80% of maximum was then used as the standard dose to pre-contract the strip prior to addition of doses of ACh to produce cumulative relaxant responses (0.85-437 x 10^{-8}M). Relaxant removal of the endothelial cells by rubbing with a metal probe abolished the relaxant effects of ACh (without affecting sensitivity to NA) but did not affect the response to papaverine. Other dilators tested on the NA-contracted aortic strip were: vasoactive intestinal polypeptide (VIP) (0.38-48 x 10^{-10}M); and histamine (0.7-184 x 10^{-7}M). The relaxant effects of VIP and histamine were abolished after de-endothelialisation. AA when added to the NA-contracted strip in a low dose (0.17-0.68 x 10^{-6}M) caused a relaxant response. Higher doses (0.34-1.36 x 10^{-5}M) when added in the absence of NA gave a graded increase in tension followed by a relaxation. This relaxation (and that noted in the presence of NA) was abolished by de-endothelialisation but the contractile action of AA was unaltered.

Several drugs which affect AA metabolism were also studied; indomethacin (2.8 x 10^{-5}M), ETYA (3.4 x 10^{-5}M) and BW755C (1.5 x 10^{-4}M). Each drug was allowed to equilibrate with the aortic strip for 15 min. All these drugs reduced sensitivity to NA and new dose-response curves were constructed. Indomethacin did not affect the relaxant actions of ACh, VIP, histamine or AA in precontracted strips. However the stimulant action of AA was abolished revealing a weak relaxant effect. ETYA and BW755C blocked the relaxant effects of ACh, VIP, histamine and AA.

These studies indicate that ACh, VIP and histamine mediate their relaxant effects on the rat aorta via the endothelial cell which appears to involve the formation of a lipoxygenase product. AA exerts a stimulant effect via cyclo-oxygenase product formation in the vessel wall and a relaxant action which again involves an endothelial lipoxygenase metabolite.

This work forms part of a collaborative project with the Centre International de Recherches Dermatologiques (C.I.R.D.). We thank Roche Ltd and Wellcome Research Ltd., for gifts of drugs.

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EFFECT OF QUININE, INDOMETHACIN AND FPL55712 ON CONTRACTIONS OF GUINEA PIG AIRWAY SMOOTH MUSCLE

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Recent reports suggest that histamine-induced contraction of airway smooth muscle can be augmented by a lipoxygenase-dependent mechanism (Adcock & Garland, 1982; Mitchell, 1982). We were interested in establishing whether contractions mediated through other than classical receptor mechanisms were also augmented. In this study, therefore, we have compared the effects of quinine, indomethacin and FPL55712 on contractions of airway smooth muscle induced by KCl, calcimycin (A23187), histamine and methacholine.

Spirally cut sections of guinea pig tracheae were suspended in Krebs-Henseleit solution at 37°C and bubbled with 5% CO_2 in O_2. Contractions to equi-effective concentrations (Emax90) of KCl, calcimycin, histamine and methacholine were recorded by conventional methods. The results are summarised in Table 1.

Table 1 Effects of quinine, indomethacin and FPL55712 on contractions of guinea pig tracheae. (mean ± S.E. mean; n = 4 to 7)

Agonist (Emax90)	% Control Response			
	Quinine (2×10^{-4}M)	Indomethacin (1×10^{-6}M)	FPL55712 (1×10^{-6}M)	Indomethacin + FPL55712
Methacholine (1×10^{-4}M)	68 ± 6**	104 ± 3	91 ± 9	109 ± 5
Histamine (4×10^{-5}M)	65 ± 6**	135 ± 9**	126 ± 4**	141 ± 13*
KCl (9×10^{-2}M)	0**	115 ± 6*	106 ± 5	120 ± 5*
Calcimycin (5×10^{-6}M)	35 ± 6**	68 ± 7**	86 ± 10	28 ± 8**

*P < 0.05; **P < 0.01 compared with control response

Indomethacin and to a lesser extent FPL55712 reduced basal tone by 36 ± 6% and 12 ± 5% respectively. After taking account of these reductions, contractions to histamine and KCl were still significantly augmented by indomethacin and indomethacin + FPL55712. Contractions to histamine were significantly augmented by FPL55712 (see table). However contractions to calcimycin were significantly reduced by indomethacin. Methacholine contractions were unaffected. The indomethacin-augmented KCl and histamine contractions were not inhibited by simultaneous addition of FPL55712, whereas calcimycin contractions were further reduced. Quinine reduced contractions to all agonists, KCl being most affected.

These results are consistent with KCl possessing a mechanism of action that in part involves the generation of lipoxygenase products such as had been described for histamine (see references). The sensitivity of the calcimycin contractions to inhibition by indomethacin and FPL55712 suggests that they are largely dependent upon the generation of constrictor eicosanoids.

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We are grateful to Fisons for the gift of FPL55712.

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THE DOSE-DEPENDENCY OF THE DISPOSITION AND ENZYME INDUCING PROPERTIES OF CINNAMYL ANTHRANILATE IN THE C3B6F1 MOUSE

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Cinnamyl anthranilate (CA) is a synthetic food flavour and fragrance agent. There is currently some concern over the potential risk to man from its use as it produces liver and lung tumours in mice following administration of very large doses. However, it is not a carcinogen in rats, nor is it a mutagen in a variety of tests. We now report on studies designed to examine the disposition and hepatic effects of CA in the C3B6F1 mouse, in relation to dose, to provide a pharmacokinetic critique of the toxicity tests of CA in this species.

Following oral administration of CA (500mg/kg) to C3B6F1 mice, peak plasma levels of unchanged CA were reached in 30 min and were higher in males (5.7µg/ml) than in females (1.5µg/ml). Unchanged CA in urine accounted for 0.3-0.4% of dose. Anthranilic acid (ca. 17%) and hippuric acid (ca. 35%; the major metabolite of cinnamyl alcohol) were present in urine, with higher recoveries in females.

Groups of male and female C3B6F1 mice were given 0, 10, 100, 1000, 5000, 15000 and 30000 ppm CA in the diet. After 4 days, the diet was removed and urine collected. This contained in increasing concentration with dose CA (more in males) and hippuric and anthranilic acids (more in females). Other animals were given these diets for 19days and then killed. Relative liver weight and microsomal cytochrome P450 increased with increasing dose above 1000 ppm, more markedly in females than males although the maximal response (2-fold) was the same in both sexes.

Microsomal protein content was unchanged. SDS-PAGE of the microsomes revealed the dose-dependent induction of a protein of 72kd (possibly NADPH-cytochrome P-450 reductase) and a cytochrome P-450 isozyme of 53kd. Aniline hydroxylase activity in the 9000xg supernatant was unaltered by CA dosing, as was p-nitroanisole O-methylation activity in female mice. In males, this latter is significantly reduced at doses of 5000 ppm and above.

In these studies, CA has been shown to cause liver hypertrophy and microsomal enzyme induction. These effects are seen at the higher doses, above 1000 ppm, and are not detected at lower doses, suggesting that they may be mediated by unchanged CA, whose hydrolysis has been shown to be saturated at high doses. The association of these hepatic effects and enhancement of liver tumours in mice suggests that CA resembles the so-called promoting agents e.g. phenobarbitone, rather than alkylating agents such as the nitrosamines.

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EFFECT OF CIMETIDINE ON PHENOBARBITONE-INDUCED CHANGES IN HEPATIC CYTOCHROME P450 AND δ-AMINOLAEVULINIC ACID SYNTHASE

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Induction of the haemoprotein cytochrome (Cyt) P450 by phenobarbitone (PB) requires increased haem synthesis and this is reflected by increased activity of the rate-controlling enzyme of haem synthesis delta-aminolaevulinic acid (ALA) synthase (Rajamanickam et al, 1975). We have observed in Sprague-Dawley rats that cimetidine (50 mg/kg/24 h i.p.) administered 45 min prior to PB (40 mg/kg/24 h i.p.) diminishes the rise in ALA synthase activity whilst enhancing the rise in Cyt P450. Following PB alone ALA synthase activity increased from a baseline of 52 ± 13 (mean ± SD) pmol ALA/mg prot/h, reaching a peak of 172 ± 67 at 8½ h after the first injection and remained significantly elevated at 17 h (120 ± 83), 23 h (81 ± 69) and 29 h (74 ± 11) compared to placebo-treated rats. When cimetidine was injected 45 min prior to the PB, ALA synthase activity increased reaching a maximum activity of 155 ± 85 at 8½ h, but at 17 h (48 ± 29), 23 h (55 ± 47) and 29 h (56 ± 4) it was similar to placebo-trated rats and signficantly lower ($p < 0.05$) than with PB alone. In contrast to its effects on ALA synthase, cimetidine pre-treatment enhanced the PB-induced rise in hepatic Cyt P450 concentration. At 25 h following commencement of PB alone, Cyt P450 was 1.02 ± 0.18 nmol/mg protein compared to 1.46 ± 0.2 with PB + cimetidine ($p < 0.05$) and at 42 h after PB alone 1.39 ± 0.28 compared to 1.63 ± 0.25 with PB + cimetidine ($p < 0.05$). The plasma concentrations of PB were not altered by cimetidine pre-treatment. Cimetidine given alone did not affect ALA synthase activity or Cyt P450 concentration.

The combination of increased rate of rise of Cyt P450 concentration and diminished rate of haem synthesis as reflected by ALA synthase activity suggested that cimetidine was reducing the rate of degradation of PB-induced Cyt P450 haem. In order to study this, PB-induced Cyt P450 haem was labelled by administering 3H-ALA to PB-induced rats and at frequent time intervals thereafter the rats were sacrificed and hepatic Cyt P450 concentration and specific radioactivity of isolated Cyt P450 haem measured. At 24 h following commencement of cimetidine (50 mg/kg every 8 hours) the specific radioactivity of Cyt P450 haem (cimetidine = 11,518 dpm x 10^3/nmol haem, placebo = 5,595 $p < 0.0001$) and hepatic cytochrome P450 concentrations (cimetidine = 1.79 ± 0.20, placebo = 1.61 ± 0.21 $p < 0.05$) were significantly increased in the cimetidine treated animals.

Cimetidine treatment did not alter the activity of the microsomal enzyme haem oxygenase which catalyses the oxidative degradation of haem to bilirubin.

Our results indicate that cimetidine reduces the rate of turnover of PB-induced Cyt P450 haem and consequently the demand for new haem synthesis during induction of this haemoprotein. Cimetidine is known to bind to the haem moiety of Cyt P450 (Knodell et al, 1982) and we propose that this may protect the haem from oxidative degradation. This ability of cimetidine to reduce the rate of haem degradation and consequently the demand for haem synthesis may be of therapeutic value in the management of patients with acute porphyria who have a hereditary impairment of haem synthesis.

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EFFECTS OF ENVIRONMENTAL AND SOCIAL SEPARATION ON THE AMPHETAMINE INDUCED BEHAVIOUR IN RATS

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Most conventional methods of testing exploratory behaviour, usually based on placement in a novel environment, involve separation from familiar environmental stimuli and separation from social companions. As separation leads to distress behaviour and searching in humans (Bowlby, 1969, 1973) and animals (Salzen, 1979; Gallup and Suarez, 1980), that behaviour can, therefore, be seen as partly a response to the separation experience. If separation and changes in exploratory drive and behaviour are central to human affective disorders (Ashcroft, 1972), studies of the effects of environmental and social separation on exploration (EX) and stereotyped behaviour (ST) could provide an animal model for the study of depression and its treatment.

150 Albino Sprague Dawley rats (Charles River U.K. Ltd., London) were divided into five groups for the five testing conditions: in their home cage either singly (HS) or with companion (HC); singly in a 35 x 24 cms. open field (OFS); and in a 50 x 50 cms. holeboard either singly (HBS) or with companion (HBC). Twenty minutes before the behavioural observations they received one i.p. dose of amphetamine (0, 1, 2, 4 or 8 mg/Kg) and the amount of locomotion (LOC), exploration (EX), or stereotyped behaviour (ST) was recorded for four minutes every twenty minutes during eighty minutes. For this report we pooled the activity of the last three periods when the rat is used to the test situation. The results in Table 1 clearly show the differences between the typical test situation (HBS) and the familiar one (HC). EX and LOC are increased in HBS ($p<0.02$) whereas ST is reduced ($p<0.005$). This is not due to the different size and the presence of holes as the same happens with group OFS ($p<0.001$) which does not differ from HBS. The group HS comes between groups HC and HBS and the highest levels of EX appear in the group HBC ($p<0.01$, data not shown).

Table 1: Amphetamine dose response curves for groups HC and HBS. $\bar{x} \pm$ S.E. of mean

		0 mg/Kg	1 mg/Kg	2 mg/Kg	4 mg/Kg	8 mg/Kg
H.C.	LOC	0.4 ± 0.3	8.1 ± 2.5	11.7 ± 3.1	11.9 ± 5.7	3.4 ± 2.1
	EX	0.8 ± 0.5	16.1 ± 4.6	34.0 ± 8.2	21.1 ± 8.1	8.3 ± 4.6
	ST	0 ± 0	0.6 ± 0.6	11.6 ± 5.9	124.2 ± 28.6	210.7 ± 20.3
H.B.S	LOC	8.1 ± 2.5	34.5 ± 4.3	46.6 ± 13.3	77.3 ± 16.3	6.2 ± 3.6
	EX	9.9 ± 3.1	27.8 ± 4.7	43.6 ± 12.9	52.0 ± 7.9	11.0 ± 9.0
	ST	1.1 ± 0.6	4.5 ± 1.9	1.9 ± 1.2	5.7 ± 3.1	173.7 ± 42.6

In summary the above data show important differences in the amphetamine induced EX, LOC and ST in the different environmental conditions. This could provide a useful animal model for clinical conditions such as depression and separation anxiety in which a change in exploratory behaviour may be essential to their pathology.

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THE EFFECT OF RESERPINE AND PENTYLENETETRAZOLE IN VIVO ON SODIUM-DEPENDENT CHOLINE UPTAKE IN FINE SLICES OF RAT HIPPOCAMPUS

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The Na-dependent uptake of ^{3}H-choline in rat hippocampus in vitro is thought to reflect cholinergic neuronal activity in vivo. Thus pentylenetetrazole (PTZ) given by i.p. injection to rats increased choline uptake in synaptosomes prepared from hippocampus, an effect abolished by prior (1 h) lesion of the septum (Simon et al, 1976). Reserpine or selective antagonists of noradrenaline (i.p. injections) had no effect on ACh concentrations in rat hippocampus, but blocked oxotremorine-induced increases. The septal-hippocampal cholinergic neurones were thought to be subject to inhibitory feedback control by noradrenaline (Ladinsky et al, 1980). We therefore measured ^{3}H-choline uptake in fine slices of hippocampus from reserpinised rats treated with PTZ.

Male Wistar rats (groups of 5, 120-150 g) were treated with reserpine (5 mg/kg, i.p.) and then PTZ (75 mg/kg, i.p.) 16-22 h and 2 min respectively, before use. Some groups were treated only with reserpine, others only with PTZ, vehicle-treated groups served as controls. Hippocampi from individual rats were chopped (0.1 x 0.1 x approx 1 mm) washed twice, preincubated (10 mg, 2.5 ml, 37°C, 5 min) and incubated (^{3}H-choline, 0.5 µM, 1 µCi, 4 min) in 2.5 ml Tris-buffered Krebs' solution, then filtered, (GF/C discs). Na-dependent uptake was calculated by correcting for uptake in sodium-free medium.

PTZ caused tonic extension approx. 1 min after administration to rats pretreated with reserpine and generalised asynchronous movements in those not pretreated. Na-dependent uptake in reserpinised rats that had also received PTZ was 49% greater than in vehicle-treated controls (Table 1). PTZ or reserpine administered separately caused 21% and 32% increases respectively. The increase caused by the combined treatment was significantly greater (two tailed t-test, $P < 0.05$) than those caused by either single treatment.

Table 1 Na-dependent ^{3}H-choline uptake+ in rat hippocampus

	reserpine (5 mg/kg)	PTZ (75 mg/kg)	reserpine + PTZ (5 + 75 mg/kg)
drug treated	6868 (349, 20)‡	7662 (398, 25)‡	10363 (292, 33)‡
vehicle treated	5187 (237, 18)	6305 (288, 25)	6966 (244, 34)

+c.p.m./10 mg tissue/4 min (assay specific radioactivity in exogenous choline 800 Ci/mole, counting efficiency 35%) mean values; in parentheses - between animal variation as s.e. mean, number of animals; quadruplicate determinations per animal, assay s.e. mean not greater than 5%; ‡significantly different from control ($P < 0.02$) by 2-way analysis of variance.

Thus a reserpine-sensitive tonic inhibitory mechanism regulates septal-hippocampal cholinergic neurones. This is not apparent from assays of ACh in hippocampus (Ladinsky et al, 1980). Na-dependent choline uptake therefore seems a more sensitive index of cholinergic neuronal activity where increases in uptake tend to maintain ACh concentrations. That uptake in the hippocampus is more markedly increased in rats given both reserpine and PTZ than in those given either drug seperately (Table 1) is consistent with an inhibitory feedback mechanism of the kind described by Ladinsky et al, although summation from independent actions cannot be excluded.

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TRYPTAMINE AND SEROTONIN INHIBIT COMPETITIVELY (^{3}H)-IMIPRAMINE BINDING IN HUMAN PLATELETS AND MODIFY THE RATE OF DISSOCIATION

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The experimental evidence available so far supports the view that the ^{3}H-IMI binding sites are associated with the neuronal uptake for serotonin (Langer et al., 1980). We have recently found that ^{3}H-IMI binding in rat cortex is displaced by tryptamine with a IC_{50} value (5000 nM) which is very close to that of serotonin (IC_{50} : 2000 nM). In addition it was reported that tryptamine inhibits 5HT uptake in hypothalamic synaptosomes (Horn, 1973) and that it is taken up by platelets (Costa et al., 1977).

In the present study we demonstrate that tryptamine and serotonin are equipotent to inhibit ^{3}H-IMI binding in platelets. In addition we present evidence in support of a modulatory role for tryptamine and serotonin at the level of the dissociation of ^{3}H-IMI bound to human platelets.

^{3}H-IMI binding in outdated human platelets was measured by the method described by Langer et al., 1980. ^{3}H-IMI binding was saturable and of high affinity (Kd=0.6 nM and Bmax=450 fmoles/mg protein). The displacement of ^{3}H-IMI binding in platelets by imipramine (IC_{50} = 5 nM), desipramine (IC_{50} = 50 nM), fluoxetine (IC_{50} = 9 nM), serotonin (IC_{50} = 4560 nM and tryptamine (IC_{50} = 3300 nM) closely resembled the pharmacological profile obtained in the rat cortex (Sette et al., 1983). The Hill coefficients for all the drugs tested were close to unity, indicating a competitive interaction.

Membranes from human platelets incubated with 0.6 nM ^{3}H-IMI at 0°C for 90 min are at equilibrium and the addition of an excess buffer (50 vol) results in the rapid loss of bound ^{3}H-IMI. Under these conditions at 0°C the $t_{1/2}$ for dissociation was calculated to be 46 min. Under these experimental conditions the $t_{1/2}$ values obtained by the addition of drugs were as follows : 10 µM DMI (75 min), 10 µM fluoxetine (75 min), 1000 µM serotonin ($t_{1/2}$ > 300 min), 1000 µM tryptamine ($t_{1/2}$ >300 min).

In separate experiments 1000 µM serotonin or 1000 µM tryptamine was added together with 10 µM DMI and this resulted in a pronounced delay in the dissociation times for ^{3}H-IMI, while the $t_{1/2}$ for 10 µM DMI alone was 75 min, the $t_{1/2}$ for 10 µM DMI plus 1000 µM serotonin or 1000 µM tryptamine exceeded the value of 300 min). The substitution of 10 µM DMI by 10 µM fluoxetine in these experiments produced essentially the same dissociation time when tested in the presence of tryptamine or serotonin (>300 min).

These results are compatible with the view that serotonin and tryptamine exert an inhibitory effect on the rate of dissociation of ^{3}H-IMI binding through an allosteric interaction as already suggested in the brain (Sette et al., 1983).

The present results provide further evidence for the view that the ^{3}H-IMI binding sites and the substrate recognition site of the serotonin transporter are closely associated but not identical. In addition the fact that tryptamine is equipotent with serotonin at inhibiting ^{3}H-IMI binding may be of considerable interest because of the recent report that there is a high affinity binding site for ^{3}H-tryptamine in the rat brain (Kellar et al., 1982) which differs from the serotonin receptor binding sites.

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DEPRESSANT AND EXCITATORY EFFECTS OF ADRENOCEPTOR AGONISTS ON MEDULLARY NEURONES IN THE RAT

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The existence of noradrenergic terminals in the caudal trigeminal nucleus suggests that noradrenaline may play a modulatory role in sensory processing in this area. In addition, noradrenaline has been proposed as an excitatory neurotransmitter in the mesencephalic reticular formation (Haigler & Spring, 1981). In a previous study on trigeminal sensory neurones we showed that iontophoretically applied noradrenaline depressed the firing of a high proportion of the neurones sampled, although some were excited (Cahusac & Hill, 1982). In the present study selective α-adrenoceptor agonists and antagonists were employed in an attempt to characterise the receptors at which endogenous noradrenaline may act.

The extracellular action potentials of 55 neurones in the caudal medulla of 25 urethane-anaesthetised rats were recorded using the 4M NaCl filled centre barrel of seven-barrelled micropipettes. Drug barrels contained Na L-glutamate (0.5M, pH 8.5), gamma-amino-n-butyric acid (GABA) (0.5M, pH 3.5), noradrenaline HCl (0.2M, pH 4), phenylephrine HCl (0.2M, pH 4), piperoxane HCl (0.1M, pH 4.6), RX 781094 (2-(2-(1,4-benzodioxanyl))-2-imidazoline) HCl (0.2M, pH 3.3), WB 4101 (2-((2',6'-dimethoxy)phenoxyethylaminomethyl)-1,4-benzodioxane (0.1M, pH 3.2). Recording sites were identified by deposition of pontamine sky blue (2% in 0.5M Na acetate). Systemic drugs were made up in physiological saline.

Iontophoretic application of the α_2 agonist clonidine (35 - 100 nA) depressed all 9 neurones studied. In contrast, phenylephrine had excitatory effects (13 of 21 neurones studied) or no effect (6 of 21), with only 2 neurones showing depression. Iontophoretic piperoxane was able to reverse depressions induced by iontophoretic noradrenaline in 5 out of 12 neurones. The more selective α_2 antagonist, RX 781094 applied iontophoretically was also able to reverse these noradrenaline-induced depressions (11 of 12). These antagonists had no effect on the depression of the same neurones by GABA. Iontophoresis of the α_1 antagonist, WB 4101, had no effect on these noradrenaline-induced depressions but did reverse 3 out of 5 phenylephrine-induced excitations. Systemic administration of clonidine (10 - 250 μg/kg) and RX 781094 (0.1 - 1.5 mg/kg) was carried out in 16 animals. Clonidine depressed the firing in 15 out of 16 neurones and lateral reticular neurones were particularly sensitive to this effect. In 4 out of 5 neurones tested, systemic RX 781094 reversed the clonidine-induced depression of firing.

This study suggests that on rat medullary neurones activation of α_2 adrenoceptors produces neuronal depression and activation of α_1 receptors produces neuronal excitation.

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SPECIFIC HIGH-AFFINITY BINDING SITES FOR (^{3}H)-IMIPRAMINE ARE PRESENT IN THE RAT LUNG

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It was demonstrated by Junod (1972) that 5HT uptake by lungs is saturable and sodium dependent and it was suggested that this uptake takes place essentially in capillary endothelial cells (Strum and Junod, 1972).

The kinetic parameters for 5HT-uptake in the lungs are similar to those reported in platelets and in the brain and imipramine markedly inhibits the pulmonary uptake of 5HT. On the other hand, the lungs unlike the other tissues do not store 5HT which is rapidly metabolized once taken up (Junod, 1972).

The available experimental evidence supports the view that ^{3}H-imipramine binding labels a site associated with the mechanism for serotonin uptake (Langer et al., 1980). It was therefore considered of interest to determine if high affinity ^{3}H-imipramine binding sites were present in the rat lung. The binding of ^{3}H-imipramine was measured by incubating washed rat lung membranes at a final concentration of 1.2 mg/ml with ^{3}H-imipramine (21 Ci/mmole) for 60 min at 0°C as described earlier by Raisman et al., 1979.

The high affinity binding of ^{3}H-imipramine in the rat lung was specific and saturable giving a linear Scatchard plot. The mean dissociation affinity constant Kd, calculated from 6 such Scatchard plots was 2.7 $\pm$ 0.49 nM (mean $\pm$ SEM). The maximal binding Bmax was 650 $\pm$ 69 fmoles/mg protein.

The binding of ^{3}H-imipramine in the lung was competitively inhibited by tricyclic antidepressants such as imipramine (IC_{50} = 13 nM), desmethylimipramine (IC_{50} = 120 nM), chlorimipramine (IC_{50} = 20 nM) and amitryptiline (IC_{50} = 40 nM), the Hill coefficients for these tricyclic drugs were close to unity.

Non tricyclic 5HT uptake inhibitors like citalopram (IC_{50} = 50 nM) and fluoxetine (IC_{50} = 40 nM) displaced ^{3}H-imipramine binding from membranes of the rat lung in a complex manner with a Hill coefficient below unity. Serotonin (IC_{50} = 5500 nM) also inhibited ^{3}H-imipramine binding in the lung in a complex manner.

It is concluded that the specific high-affinity ^{3}H-imipramine binding sites in the rat lung are similar to those described in the brain and platelets. In addition our results support the view that the ^{3}H-imipramine binding sites and the transporter for serotonin are closely associated regardless of whether the uptake of 5HT is neuronal or is present in non-neuronal structures.

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PUTATIVE CALCIUM CHANNEL (PCC) MOLECULAR WEIGHT DETERMINATION WITH TARGET SIZE ANALYSIS

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Target size analysis with 10 MeV electrons (generated by a linear accelerator) was used to determine the molecular weight of the PCC in guinea-pig brain membranes (prepared according to Ferry and Glossmann, 1982). Irradiation was performed at -110°C at dose rates of 1.5-2.5 Mrad/min with doses of up to 25 Mrad; dosimetry was performed with radiochromic dye films.

Following irradiation membranes were assayed for the PCC with [^{3}H]-nimodipine (158 Ci/mmol) at a protein concentration of 0.2-0.4 mg/ml in 50 mM TRIS HCl, 0.1 mM phenylmethylsulfonylfluoride, pH 7.4 at 37°C, as previously described (Ferry and Glossmann, 1982). [^{3}H]-Flunitrazepam (72 Ci/mmol) labelling of benzodiazepine receptors (BZR) was performed in 50 mM TRIS HCl/1 mM EDTA, pH 7.4, allowing 60 minutes at 30°C prior to the separation of bound and free [^{3}H]-ligand by filtration through GF/C filters. Blanks were defined by 1 µM diazepam. Generally six points saturation isotherms were generated for each radioligand for control and irradiated membranes. The dissociation constants (K_D) of [^{3}H]-nimodipine and [^{3}H]-flunitrazepam in non-irradiated membranes were 0.5± 0.06 nM (n = 10, mean ± s.e.) and 4.5 ± 0.5 nM (n = 11, mean ± s.e.) respectively.

Irradiation reduced the density of PCCs and BZRs without altering the K_D of the respective label for the residual binding sites. The decay of PCC and BZR when plotted against dose of radiation displayed a monoexponential decay indicating homogeneity of both targets. Molecular weights are calculated from the empirical formula (Kepner and Macey, 1968):

$$\text{Molecuar weight} = f \frac{6.4 \times 10^5}{D_{37}}$$

D_{37} is the dose of radiation in Mrad at which 37% of starting activity remains; f is a temperature correction factor which is 2.8 at liquid nitrogen temperatures (Kempner and Haigler, 1982). We found a D_{37} of 10.3 ± 1.3 Mrad (n = 3, mean ± s.e.) for the PCC, corresponding to a Mr of 185,000 ± 26,800. The BZR had a D_{37} of 24.2 ±2.3 Mrad (n = 3, mean ± s.e.) corresponding to a molecular weight of 76,500 ±7,800. This value for the BZR is close to that reported by Paul et al (1982) in rat cortex membranes.

Taking the density of protein to be 1.35 g/cm^3 and assuming that the PCC is a sphere, then the diameter would be 74 ±10 Å. This structure could span the plasma membrane and is similar in size to PCCs identified in presynaptic terminals by freeze-fracture methods which have a diameter of 92 ±20 Å (Pumplin et al, 1981). In conclusion, target size analysis of PCCs has demonstrated that the binding site of [^{3}H]-nimodipine is a large structure and further underlines the probability that such binding sites are indeed on calcium channels.

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OXYPERTINE: BEHAVIOURAL EVIDENCE FOR A DEPLETER MODE OF ACTION

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Oxypertine is an antipsychotic agent that belongs to the group of amine depleter drugs (Back & Hassler 1968; Anden & Fuxe1971). However its pharmacology is still poorly understood and some reports suggest a possible blocker type of action for both dopamine and 5HT receptors(Nakahara et al 1980). Although its action differs with the dose (Hassler et al 1970), most of the animal studies are based on very high doses ranging from 35 to 100 mg/kg. We have recently reported that 1 or 4, but not 16 , mg/ kg doses of Oxypertine have a differential effect on amphetamine induced activity in that it enhances exploration (EX) while blocking stereotyped activity (ST), whereas Haloperidol blocks both EX and ST (Palomo & Russell 1983). If this action of Oxypertine is of a depleter type it should be prevented by pretreatment with monoamine oxidase inhibitors.

30 Albino Sprague-Dawley rats (Charles River U.K. London) were used in these experiments. Four hours after 50 mg/kg Pargyline (M.A.O.I.) or saline, i.p., they received a second injection of either 4mg/kg Oxypertine, 1mg/kg Haloperidol or saline. They were placed in a hole-board apparatus and 90 minutes after the second injection they all received 8mg/kg d-amphetamine , i.p. The rat motor behaviour was recorded for 4 minutes immediately before the amphetamine (pre-amph) and 40 minutes after(post-amph). Results in table 1 show clearly that Pargyline, at a dose that inhibits both A and B types of M.A.O., prevents the effect of Oxypertine (but not that of Haloperidol).

Table 1: Rat motor activity before and after amphetamine 8 mg/kg. $\bar{x} \pm$ S.E. of mean, n = 5.

		SALINE		M.A.O.I.	
		Pre-amph	Post-amph	Pre-amph	Post-amph
Saline :	LOC	4.4 ± 3.0	13.6 ± 11.2	3.8 ± 1.8	0.0 ± 0.0
	EX	6.6 ± 4.6	9.4 ± 7.8	2.8 ± 0.9	0.0 ± 0.0
	ST	0.0 ± 0.0	159.0 ± 35.0*	0.6 ± 0.4	201.8 ± 26.9*
Oxypertine:	LOC	6.2 ± 2.9	103.8 ± 24.6*	0.0 ± 0.0	5.0 ± 5.0
	EX	5.2 ± 2.5	60.0 ± 14.2*	0.0 ± 0.0	6.2 ± 5.0
	ST	5.0 ± 5.0	2.4 ± 2.5	0.0 ± 0.0	180.6 ± 58.2*
Haloperidol:	LOC	0.4 ± 0.4	0.0 ± 0.0	1.8 ± 1.8	0.6 ± 0.6
	EX	0.8 ± 0.8	0.0 ± 0.0	1.2 ± 1.2	1.0 ± 0.8
	ST	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

*, $p<0.01$ compared with pre-amph.

The above data suggest that the reported action of 4mg/kg Oxypertine on amphetamine induced behaviour is of a depleter type affecting the amphetamine releasable pool of amines.

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DOSE RELATED EFFECTS OF PREJUNCTIONAL MODULATORS OF SYMPATHETIC TRANSMISSION IN THE MOUSE VAS DEFERENS

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The secretion of transmitter from one or a few release sites of the sympathetic innervation of the vas deferens may be followed by observing transient accelerations in the rate of depolarisation of excitatory junction potentials (e.j.p.s) termed Discrete Events (D.Es). D.Es. occur intermittently at fixed latencies and vary discontinuously in amplitude between a few preferred values (Blakeley & Cunnane, 1979).

In the guinea pig vas deferens α_2-adrenoreceptor agonists such as clonidine depress facilitated e.j.p. amplitude by an action on pre-junctional α-adrenoreceptors whilst α_2-adrenoreceptor antagonists such as Yohimbine elevate it (Blakeley, Cunnane & Petersen, 1981). We have examined the effect of varying doses of clonidine and yohimbine on facilitated D.E. amplitude and probability of occurrence at different external calcium concentrations in the mouse vas deferens. Thus pre-junctional modulation of individual release sites can be assessed in a tissue where the nature of the transmitter is less in doubt than in the guinea pig vas deferens (Ambache & Zar, 1971; Sneddon, Westfall & Fedan, 1982).

Clonidine (10^{-9}-10^{-7}M) depresses facilitated e.j.p. amplitude in a dose dependent manner and this is associated with a similar dose dependent decrease in mean amplitude and probability of observation of D.Es. The preferred value of D.E. amplitude are unchanged by clonidine; however large D.Es are observed less frequently and small ones more frequently.

Yohimbine (10^{-8}-10^{-6}M) produces a dose dependent increase in facilitated e.j.p. amplitude associated with an increase in mean D.E. amplitude and probability of observation. As with clonidine only the shape and not the modes of the amplitude distribution is altered.

D.E. amplitude can be increased by raising $[Ca^{++}]_{EXT}$ or decreased by lowering $[Ca^{++}]_{EXT}$. When $[Ca^{++}]_{EXT}$ is high and transmitter release is increased, the effects of yohimbine are found to be decreased and the effects of clonidine increased. These observations do not accord with the α-feedback hypothesis.

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KINETICS OF HEXAMETHONIUM ACTION ON ACh RECEPTOR-CHANNELS IN FROG SKELETAL MUSCLE

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The action of the bis-quarternary ammonium compound, hexamethonium, was examined in frog skeletal muscle with the vaseline-gap voltage clamp method. The ends of single muscle fibres were cut in 80 mM K_2EGTA and drugs were bath applied. Relaxations of agonist-induced currents in response to voltage-jumps were measured in frog denervated muscle fibres. Voltage-jumps in the presence of acetylcholine (ACh) or suberyldicholine (SubCh) gave simple exponential relaxations (Fig. 1A). However, on hyperpolarization in the presence of hexamethonium (Fig. 1B) there is initially a rapid decrease in current followed by opening of channels that is slower than the control rate shown in Fig. 1A. When the membrane potential is returned to -50 mV there is a rapid increase followed by a slow decrease in current. The two kinetic components resolved in the presence of agonists and hexamethonium suggest that hexamethonium can produce a rapid potential-dependent block of open ion channels.

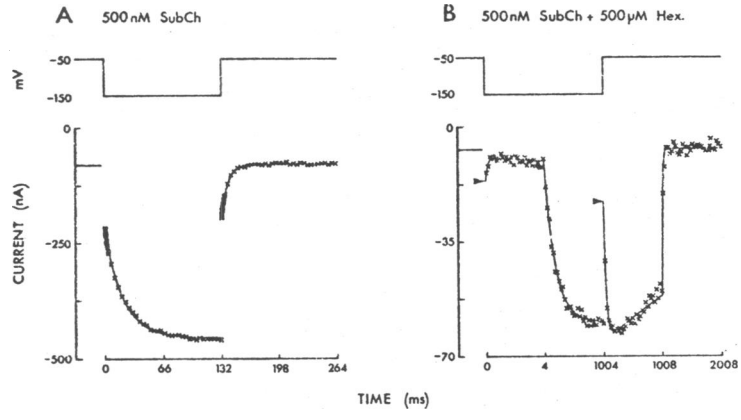

Figure 1 Relaxation of net agonist-induced current following step changes of 100 mV in membrane potential (temperature 10°C). (A) Relaxations in 500 nM SubCh. The relaxations at -150 mV and -50 mV are fitted with single exponentials (τ_{-150mV} =20.6 ms and τ_{-50mV} =7.0 ms). (B) Relaxations in 500 nM SubCh and 500 μM hexamethonium. Note the expanded time scale for the first 4 ms of each jump. The relaxations at -150 and -50mV are fitted by the sum of two exponentials (τ_{-150mV} =0.12 ms and 173ms; τ_{-50mV} =0.15ms and 14ms). The arrowheads indicate the (ohmic) instantaneous currents.

Hexamethonium reduced neurally evoked end-plate current (e.p.c.) amplitudes in a voltage-dependent manner while e.p.c. decays followed a single exponential time course. Current fluctuation measurements in the presence of 10 μM ACh and 500 μM hexamethonium gave noise spectra which were fitted by a single Lorentzian curve with a mean single channel lifetime (τ) of 2.45 ms at -70 mV (10°C) which is similar to the value with ACh alone (τ =2.33 ms). In contrast, at -100 mV 500 μM hexamethonium increased τ by 30%. The rate and voltage dependence of hexamethonium binding to the open channel, measured by voltage-jump experiments, can at least in part account for the reduction of e.p.c. amplitude that is observed with hyperpolarization.

EFFECT OF α-ADRENOCEPTOR AGONISTS ON THE FRACTIONAL DISTRIBUTION OF RADIOACTIVE MICROSPHERES IN THE PITHED RAT

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Although vasoconstriction in the rat can be induced by activation of either post-junctional α_1 or α_2-adrenoceptors, (Timmermans & Van Zweiten 1981) the location of the post-junctional α_2-adrenoceptor remains uncertain. This has prompted us to examine the effect of selective α_1 and α_2-adrenoceptor agonists upon the fractional distribution of radioactive microspheres in the pithed rat.

Male Sprague Dawley rats (300-350g) were anaesthetised with pentobarbitone (60 mg/kg i.p.), the jugular vein and femoral artery were cannulated for the continuous infusion and measurement of diastolic blood pressure respectively. The left cardiac ventricle was also cannulated for the administration of 15μM Cr^{51} labelled microspheres (New England Nuclear), rats were then pithed, respired with room air and allowed to stablise for 15 minutes.

Three groups of rats were used. In the control group (n=15) no infusion of agonist was given before the administration of the microspheres. In the second group (n=12), an infusion of the selective α_1-adrenoceptor agonist methoxamine (21-25 μg/kg/min) was started, so that a constant pressor response was maintained (68.5±4.8 mmHg mean ±SEM) before the injection of the microspheres. In the final group (n=12) an infusion of the selective α_2-adrenoceptor agonist UK 14304 (Cambridge 1981)(28-33 μg/kg/min) was started, so that again a constant pressor response was maintained (74±5.8 mmHg mean±SEM), before injection of the microspheres. The pressor responses induced by these two agonists were not significantly different. 10 minutes after the administration of the microspheres the respirator was disconnected, and the following tissues (table 1) were removed, weighed, and counted in a gamma counter (Packard). The results were expressed as a percentage of the fractional distribution of microspheres per gram wet weight of the organ (table 1).

Table 1

TISSUE	CONTROL	METHOXAMINE	UK 14304
HEART	4.13±0.25	7.29±0.82*	6.22±0.48**
L KIDNEY	5.55±0.46	3.81±0.16*	5.11±0.27
R KIDNEY	5.36±0.45	3.78±0.28*	5.01±0.23.
LIVER	0.128±0.016	0.141±0.02	0.113±0.014
STOMACH	0.612±0.042	0.562±0.034	0.611±0.0306
SMALL INTESTINE	1.267±0.11	1.218±0.092	1.325±0.066
MESENTERY	0.596±0.083	0.235±0.036**	0.299±0.0376*
LARGE INTESTINE	0.949±0.11	1.03±0.106	0.849±0.084
ABDOM MUSCLE	0.077±0.012	0.107±0.023	0.104±0.016
ABDOM SKIN	0.0517±0.011	0.094±0.0163	0.078±0.018
TAIL	0.051±0.006	0.054±0.007	0.023±0.002*
SKELETAL MUSCLE	0.196±0.009	0.146±0.018*	0.246±0.028
SPLEEN	1.57±0.22	0.761±0.117*	1.38±0.167
LUNGS	1.08±0.26	4.43±1.8	3.05±0.554**

* P<0.05 Mann Whitney U test; ** P<0.01 Mann Whitney U test.

These results suggest that in the pithed rat, α_1-adrenoceptor mediated vasoconstriction may occur in the mesenteric, renal, splenic and skeletal muscle vascular beds, whereas α_2-adrenoceptor mediated vasoconstriction may only occur in the vascular beds of the mesentery and tail.

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β_1 SELECTIVE AFFINITY OF PRENALTEROL AND CORWIN: DIRECT COMPARISON BY RADIOLIGAND BINDING

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Prenalterol (P) and Corwin (C) have been developed as potentially useful cardiac inotropic agents. In whole animal experiments, both P and C have been characterised as β_1 selective partial agonists with approximately 80% and 45% of the intrinsic activity of isoprenaline respectively (Carlsson et al, 1977; Nuttal et al, 1982). However, data from isolated tissues has yielded conflicting results, suggesting that P is of low intrinsic activity and may also exhibit β_2 agonist properties in some tissues (Kenakin and Beek, 1982).

We have directly compared the ability of P and C to displace ^{3}H-dihydroalprenolol (DHA) binding from the β-adrenoceptors in rat and rabbit lung membranes. These tissues contain a heterogeneous population of β-adrenoceptors - rabbit 70% β_1, rat 80% β_2 (Rugg et al, 1978). Lung membranes were prepared and binding assays carried out as previously described (Rugg et al, 1978). All incubations were for 30 minutes at room temperature in the presence of a final concentration of 100 μM GTP. Displacement of ^{3}H-DHA binding by P and C was assessed in competition experiments performed in parallel on three different sets of rat and rabbit lung membranes for both drugs.

P and C were both more potent in competing for ^{3}H-DHA binding in rabbit (predominant β_1) than rat lung membranes, leading to a separation between the displacement curves in the two species for each drug which was greater for C (20 fold) than P (4 fold). All displacement curves exhibited Hill slopes (nH) less than unity suggesting interaction with a heterogeneous population of binding sites. Therefore, similar competition experiments were performed as previously described (Nahorski et al, 1979), in the presence of highly selective β-adrenoceptor antagonists in appropriate concentrations to occupy > 90% of the β_2-adrenoceptors in rabbit lung (using 50 nM ICI 118,551) or the β_1-adrenoceptors in rat lung (using 7.5 μM atenolol). Under these conditions displacement of ^{3}H-DHA binding to the remaining homogenous β-adrenoceptor populations by P and C produced steep monophasic curves (nH = 1), with greater separation between the species. IC_{50}'s for P and C respectively were - rabbit lung (homogenous β_1) $2.1 \pm 0.3 \times 10^{-7}$ M (mean ± SEM) and $1.2 \pm 0.1 \times 10^{-7}$ M; and rat lung (homogenous β_2) $1.9 \pm 0.1 \times 10^{-6}$ M and $5.4 \pm 0.5 \times 10^{-6}$ M. Therefore, both drugs exhibited selective affinity for the β_1-adrenoceptor with an overall selectivity ratio compared to the β_2 subtype of approximately 10 fold and 40 fold for P and C respectively.

The relevance of these results to the clinical use of the drugs is uncertain, but they suggest that in vivo P in high dosage may exert some of its effects via the β_2-adrenoceptor.

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EFFECT OF BLOOD LOSS ON PLATELET α_2-ADRENOCEPTORS

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An investigation of the platelet α_2 adrenoceptor binding characteristics of women in the perinatal period showed a decrease in binding post-partum (Metz et al, 1983). This decrease may be related to fluctuating oestrogen levels at this time; however an increase in blood platelet count was also noted. A preliminary investigation of a small group of female blood donors showed an increase in platelet count but no decrease in α_2 adrenoceptor binding capacity. We have therefore investigated a larger number of both male and female blood donors to further study the effects of blood loss on platelet α_2 adrenoceptor binding characteristics. Blood samples (30 ml) were taken from a group of 21 blood donors (12 male and 9 female) firstly at the time of donation of 500 ml of blood and again 8-10 days later. Platelet suspensions were prepared according to the method of Boon et al (1982) and α_2 adrenoceptor binding was investigated using $\{^3H\}$ yohimbine. For each sample, binding was studied at six concentrations of $\{^3H\}$ yohimbine (range 1-15nM) and binding characteristics calculated by Scatchard analysis. For 15 of the volunteers the whole blood platelet count and mean platelet volume were also estimated.

A significant increase in both the α_2 adrenoceptor binding capacity (paired t-test, $p < 0.01$) and platelet count (paired t-test, $p < 0.001$) were observed 8-10 days after blood loss. No change in α_2 adrenoceptor affinity was noted. There was a trend for the mean platelet volume to be increased 8-10 days after blood loss but this was not statistically significant. Analysis of the male and female groups separately showed an increase in binding capacity 8-10 days after 500 ml blood loss in both cases (see Table 1). However this reached statistical significance for the female group only. The platelet count was also increased in the separate groups.

Table 1: Effect of blood loss (500 ml) on platelet α_2 adrenoceptor binding characteristics and whole blood platelet count.

	Kd (nM)	Bmax (fmoles/ 10^8 plts.)	Platelet count ($x10^9/l$)	Mean platelet Volume (fl)
Female (n=9)				
At time of donation:	3.44 ± 0.34	40.62 ± 2.83	248.33 ± 26.74 (n=3)	8.43 ± 0.28 (n=3)
8-10 days later:	4.09 ± 0.21	46.42 ± 2.72*	290.00 ± 26.27	8.57 ± 0.33
Male (n=12)				
At time of donation:	3.51 ± 0.17	50.60 ± 3.36	244.33 ± 9.31	8.88 ± 0.2
8-10 days later:	3.77 ± 0.17	54.86 ± 3.70	275.5 ± 11.4**	9.07 ± 0.27

Values ± s.e. mean; * $p < 0.01$; ** $p < 0.005$; Student's paired t-test

We conclude that 8-10 days following donation of 500 ml of blood there is an increase in platelet α_2 adrenoceptor binding capacity together with an increase in whole blood platelet count. Reports suggest that young platelets may be larger and more metabolically active than old platelets (Karpatkin S. 1969). It may be possible therefore that this increase in binding is related to the increased number of new platelets released into the circulation following blood loss.

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(^{125}I)-A55453: A NEW AFFINITY RADIOIODINATED PROBE FOR α_1-ADRENOCEPTORS

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The development of specific high affinity ligands has greatly advanced our knowledge of the molecular properties of adrenergic receptor systems. We report here the pharmacological characteristics of ^{125}I-A55453 (4-amino-6,7-dimethoxy-2{4{5-(4-aminophenyl)-pentanoyl}-1-piperazinyl}-quinazoline), a novel radioiodinated prazosin analog which binds reversibly and with high affinity to hepatic α_1-adrenoceptors. Moreover, this ligand can be covalently photo-incorporated into the hepatic α_1-adrenoceptor using the bifunctional photoactive crosslinker N-succinidyl-6-(4'-azido-2'-nitrophenylamino)-hexanoate (SANAH). The major labelled peptides appear at M_r = 82,000 and 53,000, and the covalent incorporation is inhibited by adrenergic drugs with an α_1-adrenoceptor specificity.

Rat hepatic plasma membranes were prepared according to the method of Clarke et al (1978). Membranes (10 µg protein) were incubated with 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, and ^{125}I-A55453 for 90 min. at 25°C. Bound ligand was separated from free by vacuum filtration and washing, and filters were counted at 75% efficiency. Covalent labelling of receptors was performed by incubating membranes (1 mg protein) with 50-100 pM radioligand in a total volume of 10 ml containing competing drugs, 150 mM NaCl, 50 mM Tris-HCl, and 5 mM EDTA. Incubation proceeded for 90 min. at 25°C and, following removal of free radioligand by centrifuging and washing, the membranes (in 5 ml total volume) were reacted with SANAH and photolyzed. SDS-PAGE was performed as described by Shorr et al (1982). Autoradiography was performed on the dried gels.

Specific binding of ^{125}I-A55453 to hepatic membranes (defined as that binding displaceable by 10^{-5} M prazosin) was reversible, of high affinity K_D = 80 ± 12 pM (n = 4), and represented 80-90% of the total binding. The B_{max} determined with this ligand (405 ± 20 fmol/mg protein) agreed well with values determined using ^{3}H-prazosin and ^{125}I-BE2254. Adrenergic drugs competed for the specific sites with potencies expected for labelling of an α_1-adrenoceptor system: prazosin >> phentolamine > yohimbine ≡ rauwolscine and (-)-adrenaline ≡ (-)-noradrenaline > (+)-adrenaline > (-)-isoprenaline. A55453 was 1100 times more potent at hepatic α_1-adrenoceptors than at platelet α_2-adrenoceptors (K_i = 0.9 nM and 1 µM respectively). These data suggest that ^{125}I-A55453 is an α_1-adrenoceptor selective ligand.

The major peptides covalently labelled in hepatic membranes by this probe appeared at 82,000 and 53,000 with minor labelled peptides at 44,000 and 32,000. The presence of multiple bands may result from proteolysis of the parent 82,000 subunit to smaller peptides which do nonetheless contain the ligand binding site of the α_1-adrenoceptor. Hence, the incorporation of radioactivity into the major bands is blocked by adrenergic agents with the same α_1-adrenoceptor specificity as above. ^{125}I-A55453 appears to be a useful probe for molecular studies of α_1-adrenoceptors.

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THE EFFECT OF WY 26392 AND COCAINE ON HEART RATE AND BLOOD PRESSURE IN THE PITHED RAT

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Inhibition of neuronal uptake of noradrenaline (NA) and blockade of prejunctional $alpha_2$ adrenoceptors, which inhibit NA release, both theoretically, increase the synaptic concentration of NA. We have compared the effects of raising the NA levels by these two mechanisms in vivo, using cocaine (NA uptake blocker) and Wy 26392 a selective $alpha_2$ adrenoceptor antagonist (Pierce and Waterfall, 1982).

Diastolic blood pressure (DBP) and heart rate (HR) were recorded from pithed, vagotomised and adrenalectomised rats. The spinal sympathetic outflow to the heart or splanchnic regions was electrically stimulated via the pithing rod (Gillespie et al 1970). All drugs were administered via a jugular vein.

In the unstimulated preparation, cocaine (0.1-10mg kg^{-1}) and Wy 26392 (0.1-10 mg kg^{-1}) had no significant effect on HR. Both compounds evoked small pressor responses at 3 and 10 mg kg^{-1} (DBP=36 ± 2mmHg, maximum increase = 26 ± 6mmHg for cocaine and 18 ± 4 for Wy 26392). Cocaine, but not Wy 26392, enhanced the tachycardia evoked by intravenous doses of NA. (mean ± s.e. mean; n=4)

Cardiac nerve stimulation (0.25 - 4Hz, 1 ms 20v) evoked a frequency related tachycardia (55 ± 4 to 169 ± 12b.min^{-1}). The $alpha_2$ agonist, clonidine 30µgkg^{-1} inhibited this response in a frequency dependent manner, (93% at 0.25Hz to 8% at 4Hz). Stimulation of the splanchnic nerves (1-16Hz) evoked a frequency related pressor response. (8.8 ± 2 to 80 ± 7 mmHg). Clonidine (30µg kg^{-1}) had no significant effect on this response. These results support the suggestion that the cardiac, but not the vascular innervation, is modulated by presynaptic $alpha_2$ adrenoceptors in the rat (Cavero 1982).

When the cardiac nerve ($alpha_2$ modulated) was stimulated (1Hz, 10s every 45s) cocaine (1mg kg^{-1}) enhanced the basal HR by 70 ± 8 b. min^{-1}, and inhibited the evoked tachycardia (58 ± 3b. min^{-1}) by 38%. Wy 26392 (1.0mgkg^{-1}) enhanced the basal HR (29 ± 7 b. min^{-1} max) but had no significant effect on the evoked tachycardia. In contrast, during splanchnic stimulation (4Hz, 10s every 45s.no $alpha_2$ modulation), cocaine (1mg kg^{-1}) increased both the basal DBP (by 44 ± 2 mmHg) and the evoked pressor response (from 34 ± 2 to 48 ± 1 mm Hg). Wy 26392 (0.1-10mg kg^{-1}) had no effect on DBP, and inhibited the evoked response at 1-10mg kg^{-1} (27 - 50%).

In conclusion, under conditions in which $alpha_2$ modulation exists, both NA uptake and $alpha_2$ blockade enhance the basal response in the presence of intermittent nerve stimulation. This enhancement may be an indication of elevated NA concentrations in the neuroeffector junction. The elevated NA levels result in an inhibition of the stimulation evoked response unless the $alpha_2$ receptors are blocked. Blockade of $alpha_2$ adrenoceptors per se had no effect on the evoked response.

In the absence of $alpha_2$ modulation, uptake blockade enhanced the basal and evoked responses. Predictably, under these conditions $alpha_2$ blockade did not enhance either parameter. These results suggest that the ability of uptake blockers to improve sympathetic transmission may be limited by presynaptic $alpha_2$ adrenoceptors. The $alpha_2$ adrenoceptor blockers are not limited in this way and may thus be a more effective substitute in conditions which indicate the therapeutic use of uptake blockers.

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REVERSAL OF CLONIDINE INDUCED HYPOTENSION FOLLOWING PERIPHERAL ADMINISTRATION OF Wy 26392 AND Wy 26703

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Two members of a novel series of benzoquinolizines, Wy 26392 and Wy 26703 have been shown to be selective α_2 adrenoceptor antagonists in peripheral structures both in vivo (Pierce and Waterfall 1982) and in vitro (Lattimer et al 1981). We now report that these compounds readily penetrate the CNS, and are potent blockers of the central α_2 adrenoceptors mediating the hypotensive response to clonidine. (Timmermans et al 1981).

Groups of 4 female Sprague-Dawley rats (250-300g) were anaesthetised with pentobarbitone (50 mg kg^{-1} i.p.), and blood pressure recorded from the left femoral artery. Clonidine (500 ng or 5 µg) was administered into a lateral cerebral ventricle (10µl i.c.v.) and 30 min. later α_2 adrenoceptor antagonists were administered via a femoral vein (iv). Control groups were given either saline i.c.v. followed by the antagonists i.v., clonidine i.c.v. followed by saline i.v. or chlorisondamine (1mg kg^{-1}) i.v. followed by the antagonists iv. CNS penetration and central α_2 adrenoceptor blockade has previously been demonstrated for the selective α_2 adrenoceptor antagonist RX 781094, (Berridge et al, 1982). We have therefore compared the results obtained for Wy 26392 and Wy 26703 with this compound.

I.C.V. administration of saline did not significantly alter the diastolic blood pressure (DBP) of the preparation. Subsequent administration of each of the α_2 adrenoceptor antagonists (1µg-10 mg kg^{-1}) evoked a dose dependent fall in DBP up to a maximum of 40 mmHg over the dose range 30µg to 10 mg kg^{-1}. Following i.c.v. administration of 500 ng or 5µg clonidine, DBP fell by 45 ± 4 and 63 ± 2 mm Hg respectively. Throughout the duration of the experiment DBP remained constant following 5 µg clonidine when saline was given i.v. (63 ± 4 mm Hg below pre-clonidine levels at the end of the experiment). After 500 ng clonidine DBP rose slightly during this period, being 41 ± 8 mmHg below preclonidine levels at the end of the experiment.

The administration of Wy 26392 or Wy 26703 (1-100 µg kg^{-1}iv) dose dependently reversed the hypotension evoked by either dose of clonidine. Following 500µg of clonidine the threshold effective dose was 1µg kg^{-1} for both compounds and following 5µg of clonidine the threshold was raised to 3µg kg^{-1} for Wy 26703 and 10µg kg^{-1} for Wy 26392. RX 781094 also reversed the clonidine hypotension, but was slightly less potent, the threshold effective dose being 10µg kg^{-1} following 500 ng of clonidine and 30µg kg^{-1} following 5µg clonidine.

Chlorisondamine lowered DBP by 66 ± 5 mm Hg. Subsequent administration of Wy 26703 or Wy 26392 (1µg-10mg kg^{-1} iv) had either no significant effect, or evoked slight falls in DBP following chlorisondamine. In contrast, RX 781094 evoked a dose dependent pressor response over the dose range 0.3-10mg kg^{-1} (maximum response of 29 ± 1 mmHg at 10mg kg^{-1}).

In conclusion, peripherally administered Wy 26392 and Wy 26703 both reverse clonidine induced hypotension in the anaesthetised rat. This reversal is obtained over a dose range which has no significant effect on either normal DBP, or pressure lowered by a mechanism not involving α_2 adrenoceptors. These results indicate that Wy 26703 and Wy 26392 readily penetrate into the CNS and block central α_2 adrenoceptors.

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AGE-RELATED CHANGES IN PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS IN RAT VAS DEFERENS

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Peripheral alpha-adrenoceptors have been subdivided into $alpha_1$- and $alpha_2$-subtypes independent of receptor location, based on the relative potencies of agonists and antagonists (Starke & Langer, 1979). In the rat vas deferens there are postsynaptic $alpha_1$-adrenoceptors, and both $alpha_2$- and $alpha_1$-adrenoceptors presynaptically (Docherty, 1983). The purpose of this investigation is to compare the responsiveness of alpha-receptors in vasa deferentia in tissues taken from young (2-3 months) and old (24 months) male Sprague-Dawley rats.

Vasa deferentia were bisected into prostatic and epididymal portions and isometric contractions were obtained to single pulse field stimulation (supramaximal voltage, 0.5ms) at intervals of 5 min. The effects of the $alpha_1$-agonist amidephrine and the $alpha_2$-agonist xylazine were assessed against stimulation-evoked contractions in the absence of any prior drug in the prostatic portion, but in the presence of nifedipine (10 μM) in epididymal portions.

In prostatic portions, xylazine produced a concentration-dependent inhibition of the isometric contraction to a single stimulus, and was significantly more potent in young than in old rats ($P < 0.01$) with IC_{50} values of 7.05 ± 0.16 and 6.40 ± 0.32 in young and old, respectively (mean and 95% confidence limits, -logM). In prostatic portions, amidephrine produced a concentration-dependent potentiation of the isometric contraction to a single stimulus, but there was no significant difference in potency or maximum potentiation between young and old rats.

In epididymal portions in the presence of nifedipine, amidephrine has a two component concentration-response curve for the inhibition of the isometric contraction to a single stimulus: at low concentrations an $alpha_1$-mediated, and at high concentrations an $alpha_2$-mediated, inhibition (see Docherty, 1983). There was no significant difference between young and old rats in the steep $alpha_1$-mediated inhibitory response curve to low concentrations of amidephrine, but amidephrine was less potent in old rats in the $alpha_2$-mediated component to higher concentrations. In epididymal portions in the presence of nifedipine, xylazine was significantly more potent in young than in old rats at inhibiting the isometric contraction to a single stimulus.

In conclusion, there is a reduced sensitivity of presynaptic $alpha_2$-adrenoceptors in old rats, without change in sensitivity of $alpha_1$-adrenoceptors, whether pre- or postsynaptic. Since presynaptic $alpha_2$-adrenoceptors mediate a negative feedback whereby noradrenaline inhibits its own further release (see Starke,1977), a reduced sensitivity of these receptors may result in increased peripheral sympathetic neurotransmission.

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POSTJUNCTIONAL α-ADRENOCEPTOR SUBTYPE IN THE RAT ISOLATED SEMINAL VESICLE

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Based on calculated dissociation constants for clonidine, a selective alpha$_2$-adrenoceptor agonist, Ruffolo et al (1980) concluded that postjunctional alpha-adrenoceptors in the rat aorta, portal vein, spleen, bladder and vas deferens could be divided into three distinct classes. The rat seminal vesicle, a tissue not included in their study, has been shown to possess 'classical' postjunctional alpha-adrenoceptors. However, the receptor subtype remains to be clarified. It was, therefore, of interest to examine the effect of clonidine in this tissue.

Seminal vesicles removed from reserpine pre-treated rats (0.5mg/Kg.i.p. on two successive days) were dissected and mounted as described previously (Gokhale and Sharif, 1982). In a total of 15 experiments, though phenylephrine gave maximal contractile responses at concentrations of 5×10^{-6}-1×10^{-5}M, at no concentration up to 5×10^{-2}M did clonidine elicit a contraction of the tissue. However, subsequent responses to phenylephrine were totally blocked. In view of this finding, the effect of clonidine (added 2 min before exposure to the test agonist) on responses to adrenaline, noradrenaline, phenylephrine, iso-prenaline and acetylcholine was tested in 6 further experiments. At concentrations of 1×10^{-8}M, clonidine potentiated responses to all the test agonists. At concentrations above 5×10^{-7}M, responses to the alpha-adrenoceptor agonists were reduced in a dose-dependent manner but responses to acetylcholine remained unaltered. As all the experiments were performed on tissues removed from reserpine pre-treated rats and in the presence of cocaine (30 μM) normetanephrine (1.0 μM) and propranolol (1.0 μM), it was assumed that all the adrenergic effects observed were the result of a direct interaction of the drugs used with postjunctional alpha-adrenoceptors.

The postjunctional alpha-adrenoceptor blocking effect of clonidine was studied in greater detail using phenylephrine as the test agonist. Exposure to clonidine for 30 min, resulted in a parallel shift of the phenylephrine concentration-response curves to the right. Using paired preparations, corrected dose-ratios for four different concentrations(1×10^{-6}, 5×10^{-6}, 1×10^{-5} & 5×10^{-5}M) of clonidine were determined in triplicate (n=12). Arunlakshana & Schild plots of the data were linear and had slopes (1.06, 0.94-1.38 mean and 95% confidence limits) not significantly different from unity ($P < 0.05$), thus indicating competitive antagonism. For each concentration of clonidine tests pA_2 and K_β values were determined as described by Mackay (1978) and by Furchgott (1972) respectively. The mean values and 95% confidence limits for pA_2 and -log K_β were 6.6 (6.55-6.67) and 6.66 (6.58-6.75) respectively.

Our results indicate that, in the rat seminal vesicle, clonidine behaves as a competitive antagonist at postjunctional alpha-adrenoceptors. Based on a comparison of the -log K_β values obtained in this study with those reported by Ruffolo et al (1980), we conclude that these receptors are similar to the post-junctional alpha-adrenoceptors of the rat portal vein, spleen and bladder but differ from those of the rat aorta and vas deferens.

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