HISTAMINE POTENTIATES THE EFFECTS OF EXCITATORY AMINO ACIDS ON QUIESCENT NEURONES IN THE RAT MEDULLA

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We have previously demonstrated that microiontophoretically applied histamine can excite spontaneously active neurones in the rat caudal medulla (Jones *et al.*, 1983). We now report an indirect, possibly modulatory, action of histamine on medullary neurones, the characteristics of which are different to those already described.

Male Wistar rats (280-320g) were anaesthetised with urethane (1.75g/kg, i.p.) and prepared for electrophysiological recording and microiontophoresis as described previously (Bradley & Dray, 1974). Conventional 7-barrelled micropipettes (tip diameter 6-9 μm) were used, the centre recording barrel contained 4M Na Cl. One barrel, which was used for current balancing and marking the position of cells, contained pontamine sky blue (2.5% in Na acetate, pH 5.6). The remaining barrels contained a combination of the following solutions; histamine (HA) (0.2M pH 4.5), N-telemethylhistamine (TMH) (0.2M pH 4.5), L-glutamic acid (G) (0.5M pH 8), D,L-homocysteic acid (DLH) (0.2M pH 8), acetylcholine (ACH) (0.3M pH 4.5). Agents were applied with positive ejection currents between 0 and 40nA, except DLH and G which were applied with negative currents. Retaining currents of opposite polarity to ejection currents (20-30nA) were employed as was current balancing.

The neurones in the population studied were normally quiescent, i.e. were without spontaneous firing and were located by evoking activity with the excitatory amino acids DLH or G. Neurones were then activated with regular applications of DLH or G at currents of 0 to 40nA for 5 or 10s, every minute. When consistent responses were seen the effect of histamine on the firing induced by the excitatory amino acid was examined by applying HA for up to 15 min. at 10 to 40nA. Although HA did not excite most quiescent neurones (27 neurones unaffected, 5 excited), it caused a potentiation of the effects of both DLH (responses on 15/22 neurones potentiated, 7 not affected) and G (15/19 potentiated, 4 not affected). HA did not potentiate the effects of DLH or G on spontaneously active neurones, which it excited (8/8 neurones). The maximum extent of potentiation was usually seen during the application of HA and varied from 50% to 500% above control responses, while the effect lasted from 5 min. to over 30 min. after cessation of the HA application. Histological analysis of the distribution of pontamine sky blue spots showed that the quiescent neurones were located throughout the reticular formation of the rat medulla. TMH did not mimic the effect of HA on 5/5 quiescent neurones and ACh was without effect on 4/4 quiescent neurones.

These results are consistent with the intracellular studies of Haas on the hippocampal slice preparation (Haas, 1983) and support the hypothesis that histamine, like noradrenaline (Rogawski & Aghajanian, 1980), can increase amino acid-mediated excitation in the CNS.

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EFFECTS OF PSYCHOTOMIMETIC BENZOMORPHANS ON RESPONSES OF CAT SPINAL NEURONES TO EXCITATORY AMINO ACIDS AND ACETYLCHOLINE

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We have recently shown that some psychotomimetic benzomorphans (Anis et al., 1983a; Allan et al., 1984), as well as some dissociative anaesthetics (Anis et al., 1983b), selectively reduce the actions of N-methylaspartate (NMA) on spinal neurones. On Renshaw cells, ketamine and cyclazocine also reduce responses to acetylcholine but to a less extent than those of NMA. Because cyclazocine has effects at mu, kappa and sigma opiate receptors (Zukin and Zukin, 1981) and because neither morphine nor naloxone had any selective effect on NMA actions, we investigated four other benzomorphans with known actions at sigma and/or kappa opiate receptors:- i) the (+) and ii) the (-) isomers of SKF10,047 (N-allyl-normetazocine), iii) MR2033 (N-tetrahydrofuranylmethyl-normetazocine) and, iv) bremazocine which appear to activate i) sigma, ii) sigma and kappa, iii) and iv) kappa receptors respectively.

Using the technique of microelectrophoresis in pentobarbitone-anaesthetised cats, we have tested the effects of these benzomorphans (all 25 mM in 175 mM NaCl) on the sensitivity of spinal neurones to excitatory amino acids and to acetylcholine.

On 13 spinal neurones, (+) SKF10,047 (10 $\pm$ 6 nA; mean $\pm$ S.D.) and (-) SKF10,047 (12 $\pm$ 7 nA) reduced responses to NMA by 67 $\pm$ 15% and 54 $\pm$ 15% respectively with only small and variable effects on responses to kainate and to quisqualate. On Renshaw cells, although (-) SKF10,047 had no clear effect on responses to ACh, the (+) isomer enhanced responses to ACh by 42 $\pm$ 32%.

MR2033 was tested on 14 neurones but with the majority caused reductions in action potential amplitude which made the results on excitants impossible to interpret. On the 4 cells where technically satisfactory results were obtained, MR2033 produced a small and non-selective reduction of the responses to all excitants tested.

Bremazocine (13 $\pm$ 8 nA) reduced responses of 18 neurones to NMA by 70 $\pm$ 17% and those to kainate by 24 $\pm$ 16% whereas ketamine (14 $\pm$ 5 nA) tested on 9 of these cells, reduced responses to NMA by 70 $\pm$ 29% and those to kainate by 2 $\pm$ 7%.

With the apparent exception of these latter results with bremazocine, NMA antagonism seems to be a property of those benzomorphans and dissociative anaesthetics, which have actions at sigma opiate receptors (Zukin and Zukin, 1981; Shannon, 1983). Shearman and Herz (1982) have shown behavioural properties of bremazocine similar to those of SKF10,047 and ketamine. We suggest that opiate binding studies will also show that bremazocine can interact with the sigma receptor.

Our results suggest that reduced transmission at synapses utilising NMA receptors may in part explain the psychotomimetic effects of sigma opioids and may need to be considered in the aetiology of some psychotic diseases.

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ACTIONS OF PIPERIDINE ON SINGLE NEURONES IN THE RAT PONS

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Piperidine is a secondary amine which is distributed widely throughout the mammalian central nervous system, the highest concentrations being found in the striatum, pituitary and pineal of rats and rabbits (Miyata, et al., 1979, 1980). Perfusion of piperidine into brainstem areas reduces the onset latency of both rapid eye movement sleep (REMS) and slow wave sleep (SWS) and significantly increases the duration of REMS (Drucker Colin and Giacobini, 1975).

We have compared the actions of piperidine with those of several putative neurotransmitters on neurones in the pons of the urethane-anaesthetised rat using the technique of microiontophoresis.

The following solutions were used during the course of these experiments (in distilled water unless otherwise stated): acetylcholine chloride (500mM, pH5.0), γ-amino butyric acid (GABA) (200mM, pH8.9), atropine methonitrate (200mM, pH4.5), bicuculline methiodide (200mM, pH3.0, in 0.9% NaCl), glutamic acid diethyl ester hydrochloride (GDEE) (200mM, pH3.5), glycine hydrochloride (200mM, pH4.5), hexamethonium bromide (Sigma, 150mM, pH5.2), α-methylnoradrenalin hydrochloride (α MeNA) (100mM, pH5.0), noradrenalin hydrochloride (50mM, pH5.0), piperidine hydrochloride (50mM, pH4.5), sodium 2-amino-5-phosphonovalerate (APV) (200mM, pH7.0), sodium N-methyl-D-aspartate (NMDA) (200mM, pH7.0), sodium quisqualate (200mM, pH8.0 in 0.9% NaCl), strychnine sulphate (10mM, pH4.5).

In the pons, 134 out of 149 neurones tested were responsive to piperidine and also cholinoceptive. The great majority of cells were excited by these two substances with rapid-onset excitations being the most frequent response observed (69% of all cells). Almost one fifth of the cells exhibited excitations with long latencies of onset and 12% were inhibited. Hexamethonium and atropine antagonised the excitatory responses to piperidine and acetylcholine in 94/97 and 19/20 cells, respectively. Inhibitory responses in 17 cells were unaffected by either atropine or hexamethonium.

In a further 112 neurones, piperidine responses were unaffected by the iontophoretically-applied antagonists α-MeNA, APV, GDEE, strychnine and bicuculline at currents which successfully antagonised the effects of noradrenaline, NMDA, quisqualate, glycine and GABA, respectively.

Our results suggest that piperidine exerts its central actions by acting at cholinergic receptors and not at the receptors for noradrenaline, glutamate or aspartate. Moreover, the inhibitory actions of piperidine are not mediated by the receptors for either glycine or GABA. Thus, the putative role of piperidine as a sleep substance maybe due to its interaction with brainstem cholinergic receptors located in structures which are important in sleep regulation like the gigantocellular tegmental field (McCarley and Hobson, 1975).

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INTERACTIONS OF THE BENZODIAZEPINE Ro5-4864 WITH THE $GABA_A$ RECEPTOR COMPLEX

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Two major types of benzodiazepine binding site were originally characterised by Braestrup & Squires (1977); the high affinity site in brain that is part of the $GABA_A$ receptor complex (Olsen, 1982) and the peripheral site that is now known also to occur in the brain but appears to be unrelated to the $GABA_A$ receptor complex (Marangos et al., 1982). The peripheral-type sites are characterised by high affinity binding of ^3H Ro5-4864, a benzodiazepine that has very low affinity for the brain-type sites linked to the $GABA_A$ receptor. There are recent reports, however, that Ro5-4864 can have anxiogenic and convulsant effects in rats and mice, respectively (File & Lister, 1983; File & Mabbutt, 1983). This prompted the present electrophysiological investigation of the effects of Ro5-4864 on the $GABA_A$ receptor complex.

Depolarizing responses to the GABA analogue muscimol were obtained on slices of rat cuneate nucleus at room temperature (Simmonds, 1980). Ro 5-4864 was dissolved in acetone and diluted into the Krebs medium. The maximum concentration of acetone resulting was 0.1% and this did not itself influence the responses. At 1 µM, Ro5-4864 did not affect responses to muscimol but, at 30 µM, a small and significant ($P < 0.05$, Student's t-test) antagonism of muscimol was observed (log dose ratio 0.053 ± 0.020 s.e.m., $n = 12$). Three other aspects of the $GABA_A$ receptor complex were also studied. At 30 µM but not at 1 µM, Ro5-4864 significantly enhanced the potency of picrotoxin as an antagonist of muscimol (Schild plot shifted by 0.190 ± 0.039 log units, $P < 0.05$ paired t-test). At 30 µM but not at 1 µM, Ro5-4864 completely abolished the potentiation of muscimol by 10 µM pentobarbitone. At both 30 µM and 1 µM, Ro5-4864 completely abolished the potentiation of muscimol by 1 µM flurazepam and at 0.1 µM Ro5-4864 there was still a significant 66% reduction in flurazepam effect ($P < 0.05$, paired t-test).

These results could provide an explanation for the reduction by Ro5-4864 of evoked inhibition *in vivo* (Polc & Schaffner, 1983) and suggest that Ro5-4864 can interact with the $GABA_A$ receptor complex via site(s) that have so far not been characterised in binding studies.

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QUANTITATIVE ANALYSIS OF GABA AGONISTS AND ANTAGONISTS USING THE RAT HIPPOCAMPAL SLICE PREPARATION

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It has previously been shown that GABA mimetics depress the synaptically-induced population spike recorded in the CA1 region of the hippocampal slice following stimulation of the Schaffer collateral-commissural pathway (Ault & Nadler, 1983). We have used this preparation as a CNS bioassay to obtain quantitative data on the activity of several GABA agonists and antagonists.

Rat hippocampal slices of 350μm thickness were cut using an Oxford vibratome. Individual slices were placed in a recording chamber and superfused with oxygenated artificial cerebrospinal fluid at a rate of 1.5mls/min. Glass micropipettes filled with 3M NaCl were used to stimulate the Schaffer collateral commissural pathway at a frequency of 2/min, and to record the CA1 population spike. Drugs were perfused for a period of 5 min to ensure a maximum effect was achieved. Cumulative dose response curves were generated by plotting drug concentration against inhibition of the population spike.

GABA and the $GABA_A$ agonists muscimol and isoguvacine depressed the population spike in a dose-dependent manner with EC_{50} values of 460±80, 1.7±0.3 and 13±1μM respectively (mean±S.E., $n>16$). Baclofen also depressed the population spike with an EC_{50} of 3.8±0.6μM (n=10). Bicuculline methochloride (1-100μM) produced parallel shifts to the right of the isoguvacine and muscimol dose response curves. Linear regression analysis of Schild plots of these data gave slopes of 1.08 and 1.03 for isoguvacine and muscimol respectively and estimated pA_2 values for bicuculline methochloride of 6.18 and 6.10. In agreement with previous studies (Ault & Nadler, 1983), bicuculline produced only a small shift to the right of the GABA dose response curve, which was not further increased with higher antagonist concentrations. Bicuculline had no effect on the responses to Baclofen. Picrotoxin (0.1-10μM) also produced parallel shifts to the right of the isoguvacine dose response curve; the Schild plot of these data had a slope of 0.82 and gave a pA_2 value of 6.94.

These results with bicuculline and picrotoxin are in broad agreement with those of Simmonds (1980) using the rat cuneate nucleus slice, although both antagonists were slightly more potent in the present study. The lack of antagonism of the GABA response suggests that GABA can also inhibit the CA1 population spike by an action at a bicuculline-insensitive, probably $GABA_B$, site.

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AGING AND THE SENSITIVITY OF NEURONES IN THE RAT CINGULATE CORTEX TO SUBSTANCE P AND ACETYLCHOLINE

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Excitatory responses of cortical neurones to iontophoretically applied substance P(SP) can be reduced by iontophoretic noradrenaline (NA) or activation of the noradrenergic pathway to the cortex (Jones and Olpe, I983a, I984).Also, cortical cell responses to acetylcholine (ACh) are reportedly increased by iontophoretic NA (Waterhouse et al., I98I). We have demonstrated a reduced ability of NA to depress the firing of cortical cells in very old rats (Jones and Olpe, I983b). It is of interest therefore to determine if there is a change in responsiveness to SP or ACh in the cortex of aging rats.

Experiments were conducted on spontaneously active cells in the anterior cingulate cortex of young (3-4 months) and old(24-30 months) rats anaesthetised with chloral hydrate. Conventional techniques were used to record action potentials extracellularly and to apply substances by iontophoresis. Standard applications of NA (40nA, 60s), SP (80nA, 60s), ACh (30nA, 20s) and GABA (20nA, 20s) were made to 6-8 cells in the cortex of a young rat and, using the same micropippette, to the same number in an old rat on the same day.At least 4 pairs were used to test each substance. Responses were quantified by measuring their area and converting to the number of action potentials below (depression, NA and GABA) or above (excitation, SP and ACh) the baseline firing rate.

The results, summarized in the table, confirm that the depressant responses to NA were much smaller in the aged animals but without a concurrent reduction in the depression evoked by GABA. Excitatory responses to ACh were also greatly reduced in the old rats but, in direct contrast, excitatory responses to SP were much bigger than in the young rats.

	Response Size (Mean ± S.E.M.)			
	NA	SP	ACh	GABA
YOUNG	I958±346	665±76	64I±67	582±70
OLD	997±I87*	I570±II4**	387±74*	550±42

*p<0.0I **p<0.00I ('t' test)

Thus, advancing age in the rat is associated with multiple changes in the responsiveness of cingulate cortical neurones to putative transmitters.Whether the observed changes in the excitatory effects of SP and ACh are secondary to the the reduced effectiveness of NA is a matter of speculation at present. However, it seems possible that these changes in sensitivity could underlie some of the motor and cognitive deficits which accompany senescence.

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AUTORADIOGRAPHIC VISUALIZATION AND CHARACTERISATION OF BRAIN AND SPINAL TRH RECEPTORS

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Thyrotropin-releasing hormone (TRH) coexists with substance P(SP) and serotonin in raphe-spinal (SC) terminals. Here it potentiates motoneuronal excitability presumably via specific receptors (Sharif and Burt, 1983a). Spinal TRH receptors are modulated in vitro by SP (Sharif and Burt, 1983b) and in the rat exhibit supersensitivity after chemical deafferentation (Sharif et al., 1983). Similar receptors prevail in the brain whose distribution has been previously determined in membrane preparations (Burt and Sharif, 1983). New methods and results of auto-radiographic (ARG) localization of TRH receptors in rabbit SC and rat brain sections will now be described.

Adult animals were anesthetised, perfused (5% DMSO/NaCl) and killed. Sections (20μm) were cut from frozen tissues, thaw-mounted on subbed slides and stored (-20^{o}C). After thawing, washing (1min/0.9% NaCl), drying (3min/80^{o}C) and equilibration at 5^{o}C, each of 3 SC sections received 20μl of 2nM [^{3}H]MeTRH (NaP + bacitracin) $\pm$ 10μM TRH. Following the 1h/5^{o}C incubation slides were rinsed in saline (5min) and drained. Radiolabelled sections were wiped-off with damp filter paper and counted. For ARG, the wiping step was replaced with drying and cooling. This was followed by apposition of tritium-sensitive film against the labelled sections and storage (2 months/5^{o}C) and subsequent development of images.

Specific [^{3}H]MeTRH binding (63-80% of total) equilibrated within 90 min/ 5^{o}C and was enhanced by 67% by washing and warming (3min/80^{o}C) of slides before assay. Scatchard plots of competition data revealed high-affinity binding (K_d = 7.5 $\pm$ 0.6 (7) nM; B_{max} = 69 $\pm$ 5 fmol/mg protein in 3 SC sections). The pharmacological potency of analogs in displacing [^{3}H]MeTRH (MeTRH>TRH>TRH-GlyNH$_2$, etc.) and these equilibrium data corroborate our studies with membranes (Sharif and Burt, 1983a) and support TRH receptor identification. In SC autoradiograms TRH receptors had this distribution: substantia gelatinosa (SG) = central canal area > ventral gray >> white matter. In rat forebrain sections the labelling was: amygdaloid nuclei = rhinal sulcus > ventral and dorsal medial hypothalamus > nucleus accumbens > hippocampus >> basal ganglia and cerebral and cerebellar cortices. This distribution of visualized TRH receptors is also in excellent agreement with our test-tube results.

The predominance of TRH receptors (by ARG) in SG of SC, amygdaloid nuclei and hippocampus indicates that TRH may also have a role in pain modulation, personality behaviours and memory processes. However, the anti-spasticity action of TRH (Engel et al., 1983) mediated via motoneurons, remains the better known therapeutic use of this peptide in motor dysfunction.

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VISUALISATION OF MULTIPLE SEROTONIN RECEPTORS IN THE RAT BRAIN BY AUTORADIOGRAPHY

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There is evidence for the presence of multiple serotonin receptors in the mammalian brain. Binding assays have revealed the presence of at least two different subpopulations of serotonin (5-HT) recognition sites (Peroutka and Snyder, 1979). We have used quantitative autoradiographic techniques (Palacios, 1983) to study the anatomical distribution and the pharmacological characteristics of the recognition sites for several serotoninergic ligands in the rat brain.

5-HT-2 sites as defined by Peroutka and Snyder 1979, were visualised using ^{3}H-ketanserine, ^{3}H-mesulergine, ^{3}H-spiperone and ^{3}H-LSD. The same distribution of 5-HT-2 sites was observed with the four ^{3}H-ligands, although none of the four ligands labeled exclusively 5-HT-2 sites. For example ^{3}H-ketanserine recognises sites in the nucleus caudatus that were insensitive to other 5-HT-2 drugs. These sites were concentrated in the neocortex and sensitive to nanomolar concentrations of the drug pirenperone but required micromolar concentrations of serotonin to be blocked.

5-HT-1 sites were localised using ^{3}H-serotonin, ^{3}H-LSD and ^{3}H-DPAT (8-OH-2-di-n--propylaminotetraline) (Hjorth et al., 1982). Three subpopulations of 5-HT-1-sites were characterised by their anatomical localisation and drug sensitivity. 5-HT-1A sites were concentrated in the hippocampal formation, cerebral cortex and nucleus raphé dorsalis. These sites were recognised with nanomolar affinities by ^{3}H-5-HT, ^{3}H-LSD and ^{3}H-DPAT. 5-HT-1B sites were labeled by ^{3}H-5-HT and highly concentrated in the globus pallidus, subiculum and substantia nigra and sensitive to nanomolar concentrations of the agonist RU 24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H-indole) (Euvrard, C. and Boissier, 1980) and some beta adrenergic blockers (Middlemiss et al., 1977). Finally 5-HT-1C sites were extremely enriched in the choroid plexus of the brain ventricles and labeled with nanomolar affinities by ^{3}H-mesulergine, ^{3}H-5-HT and ^{3}H-LSD. However, RU 24969, DPAT, the beta adrenergic blockers and 5-HT-2 antagonists such as ketanserine and pirenperone presented very low affinities for these sites.

Data on the behavioural effects of drugs recognising differentially one or the other recognition sites strongly suggest that these recognition sites represent different types of brain serotonin receptors. These observations could provide experimental basis for the further characterisation of these serotonin receptors and the development of more specific drugs.

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LESION OF THE DORSAL RAPHE NUCLEUS INCREASES THE NIGRAL CONCENTRATION OF 5-HT_1 RECEPTORS

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Recent radiolabelled binding studies have indicated that 5-HT receptors on synaptic membranes can be subdivided into 5-HT_1 and 5-HT_2 sites (Peroutka and Snyder, 1979: Leysen et al. 1981). Behavioural changes induced by 5-HT or its analogues have been generally associated with the 5-HT_2 site. Administration of 5-HT-mimetics in rats with a unilateral 5,7-dihydroxytryptamine (5,7DHT) lesion of the dorsal raphe nucleus (DRN) induce contralateral rotational behaviour apparently due to a denervation supersensitivity in the nigra (Blackburn et al. 1981). In the present study we have attempted to characterise the 5-HT receptor subtype involved in this turning behaviour using the selective 5-HT_1 agonist, 8-hydroxy-2-(di-n-propyl-amino) tetralin (8-OHDPAT, Arvidsson et al. 1981) and 5-methoxy-3-(1,2,3,6 tetrahydropyridin-4-yl) 1H indole (RU24969, Hunt et al. 1981). In addition we have performed an autoradiographical analysis of the nigral 5-HT receptors to provide further evidence for denervation supersensitivity.
The rotational behaviour studies were performed as described previously (Blackburn et al. 1980). Radioligand binding was performed according to the method of Biegon et al. (1982) with some minor modifications. Briefly, 10µm transverse sections of frozen rat brains were thawed and washed for 45 min in tris-HCl (50 mM, pH 7.6) solution and then allowed to dry. Sections were then incubated for 60 min at 20°C in tris-HCl solution containing [^{3}H]-5-HT (2.5 nM), fluoxetine (1 µM) and ascorbate (0.01%). Under these conditions 5-HT_1 sites are preferentially labelled. Non-specific binding was determined by the addition of 1 µM unlabelled 5-HT or 8-OHDPAT. Sections were then rinsed for 40 s at 20°C and allowed to air-dry before placing in close apposition to LKB Ultrafilm or emulsion-coated (K5 Ilford) cover slips. After 10 weeks contact, the films or coverslips were developed.

Intraperitoneal injection of the 5-HT_1 agonists 8-OHDPAT (0.1 - 7.5 mg/kg) or RU 24969 (0.1 - 7.5 mg/kg) into 5,7DHT lesioned rats induced dose-related rotational behaviour whereas the 5-HT_2 agonist quipazine (10 - 30 mg/kg) and MK212 (6-chloro-2-1-piperazinyl pyrazine 2-5 mg/kg) were inactive. Autoradiography of 5-HT binding sites indicated a greater density in the SN on the 5,7DHT lesioned side (4 rats, 4 sections/animal). Densitometric measurement of the autoradiograms showed > 2 fold increase on the lesioned side supporting the >2-fold increase in Bmax previously reported in binding studies on homogenates of SN prepared from 5,7DHT lesioned rats (Blackburn et al. 1981). In summary, the 5-HT receptor supersensitivity arising in the SN after 5,7DHT lesion of the DRN appears to be due to an increase in the concentration of 5-HT_1 receptor subtypes. This would suggest that this receptor subtype receives a functional innervation from the DRN.

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CENTRAL 5-HT RECEPTOR SUBTYPES AND THE BEHAVIOURAL RESPONSE TO 5-METHOXY-N,N-DIMETHYLTRYPTAMINE

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Centrally acting 5-HT receptor agonists, such as 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) induce a similar complex behavioural syndrome in the rat in which reciprocal forepaw treading is a major feature (Grahame-Smith, 1971; Hjorth et al, 1982). For 8-OH-DPAT, forepaw treading persists unchanged in rats depleted of monoamines with reserpine and is suggested to reflect activation of the 5-HT_{1A} subtype of the putative 5-HT_1 receptor (Tricklebank, 1984). I now describe a similar pharmacological analysis of the behavioural response to 5-MeODMT in the reserpinised rat. The results are compared with those from intact animals in which the response to 5-MeODMT is suggested to reflect stimulation of the 5-HT_2 receptor (see for example, Green et al, 1983).

Male Sprague-Dawley rats (200-300 g) were observed 3-18 min after injection (s.c.) of a submaximal dose of 5-MeODMT (1 mg/kg) and behaviour scored as previously described (Tricklebank, 1984). Antagonists were injected (s.c.) 30 min before 5-MeODMT.

Ambulation and head weaving induced by 5-MeODMT were abolished by reserpine (1 mg/kg s.c., 18h beforehand), but reciprocal forepaw treading was qualitatively and quantitatively similar to that of non-reserpinised rats. In reserpinised animals, forepaw treading was not altered by ketanserin or haloperidol (Table 1), suggesting that 5-HT_2, α_1-adrenoceptors and dopamine receptors are not directly involved. In contrast, it was antagonised by (-), but not (+), -pindolol and by spiperone, consistent with direct interaction of 5-MeODMT with the 5-HT_{1A} recognition site (Tricklebank, 1984). Forepaw treading was similarly antagonised by (-)-pindolol and spiperone in non-reserpinised rats. However, in these animals, blockade by ketanserin and haloperidol was also seen.

Table 1 Forepaw treading induced by 5-MeODMT after treatment with reserpine

	Dose (mg/kg)	Behavioural Score Reserpinised	Non-reserpinised
Control		8.6 ± 0.8	8.7 ± 0.5*
Ketanserin	5	8.5 ± 0.8	1.7 ± 0.7*
Haloperidol	0.25	8.6 ± 1.1*	1.0 ± 0.5*
Spiperone	0.25	1.2 ± 0.7*	2.8 ± 1.0*
(-)-Pindolol	4	1.7 ± 0.6*	0.5 ± 0.3*
(+)-Pindolol	4	8.0 ± 1.3	9.0 ± 1.3

Control values are the accumulated means±S.E.M of animals given 5-MeODMT only (N= 15-18). Other values are the means±S.E.M. of 6-9 animals per group. Significance of differences from control : * $p < 0.01$.

It is concluded that in reserpinised rats reciprocal forepaw treading may reflect activation of the putative 5-HT_{1A} receptor, and is unlikely to be mediated by 5-HT_2 receptors. In non-reserpinised animals this behaviour may arise from a similar mechanism, although a contribution from other monoamines complicates the pharmacological analysis.

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CHANGES IN 5-HT RECEPTOR-MEDIATED BEHAVIOUR FOLLOWING WITHDRAWAL FROM ACUTE AND CHRONIC AMITRIPTYLINE ADMINISTRATION

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Withdrawal from chronic administration of antidepressant drugs produces a functional supersensitivity of serotonergic neurones (Stolz & Marsden, 1982). Recently it was reported that a single day's treatment with amitriptyline followed by 11 days withdrawal produced a similar supersensitivity as determined by 5-hydroxytryptophan (5HTP)-induced head twitches (Antelman et al, 1983). We have now repeated their experiment using the directly-acting 5HT agonists 5 methoxy-N,N-dimethyltryptamine (5MeODMT) and N,N-dimethyltryptamine (NNDMT) rather than the indirectly-acting 5HTP. In the first experiment male Wistar rats (180 - 200 g) were injected i.p. twice daily with amitriptyline (20 mg/kg/day) or saline (0.3 ml/dose) for one day (ACUTE GROUPS) or 10 days (CHRONIC GROUPS). The acute groups were tested 11 days after drug administration and the chronic groups were tested 3, 6 and 28 days after drug withdrawal. The 5MeODMT-induced behavioural syndrome, scored using a 0-3 rating scale every 2 min for the first 10 min after administration (Stolz & Marsden, 1982) consisted of fore-paw treading, hind-limb abduction, lateral head-weaving and Straub tail. Total behavioural scores for each group are shown in Table 1.

Table 1. Behavioural scores following 5MeODMT administration

Treatment	Days drug free	5MeODMT dose (mg/kg)	Behaviour score mean (range)
Saline (acute)	11	5	33.3 (6-52)
Amitriptyline (acute)	11	5	40.8 (24-55)
Saline (chronic)	3	5	29 (7-43)
Amitriptyline (chronic)	3	5	8.5 (3-13)*
Saline (chronic)	6	3	29 (7-43)
Amitriptyline (chronic)	6	3	13 (5-34)
Saline (chronic)	28	3	36.3 (23-45)
Amitriptyline (chronic)	28	3	43.6 (25-50)

*P<0.05 compared to control (Mann Whitney U-test). n=6 in each group.

Components of the syndrome considered to be $5HT_{1A}$ receptor responses (forepaw treading, hind-limb abduction, Tricklebank, 1984) were the only ones significantly altered by amitriptyline. Although there was a 33% increase in the behavioural score obtained in the acute amitriptyline group, it was not significant (Table 1). We therefore repeated this part of the experiment but used NNDMT (10 mg/kg i.p.) following pargyline pretreatment (75 mg/kg i.p.) 1 hour before. A syndrome lasting 60-90 min was elicited (hyperactivity, flat body posture, hind-limb abduction and Straub tail) which was significantly increased ($p<0.01$) in rats given amitriptyline twice, eleven days previously.

These results show that chronic amitriptyline treatment causes prolonged inhibition of 5HT receptor-mediated behaviour, while acute amitriptyline can induce long-term changes in 5HT receptor sensitivity. The mechanism by which this latter effect is mediated remains to be determined.

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SUPPRESSION OF 5-HT RECEPTOR MEDIATED INOSITOL PHOSPHOLIPID BREAKDOWN IN BRAIN BY CHRONIC ANTIDEPRESSANT TREATMENT

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There is now evidence that chronic but not acute treatment of rats with antidepressant drugs lead to a down-regulation of beta-adrenoceptors and $5HT_2$ receptors in the cerebral cortex (Peroutka & Snyder, 1980). Support for a functional deficit associated with the beta-adrenoceptor decline has come from evidence that chronic antidepressant treatment also induces a reduction in the responsiveness of the beta-adrenoceptor mediated cyclic AMP production in cerebral slices (Vetulani & Sulser, 1975). However, until recently, no appropriate biochemical effector system has been associated with the $5HT_2$ receptor. We provide evidence here that 5HT may stimulate inositol phospholipid breakdown by interacting with a $5HT_2$ receptor and that chronic treatment with antidepressants markedly suppresses this response.

Male Sprague-Dawley rats, 200-250 g, were treated with imipramine hydrochloride or iprindole hydrochloride 10 mg/kg i.p. or with an equivalent volume of normal saline, and were sacrificed 48 h after a single dose or 20 consecutive daily doses. Cerebral cortex slices (350 x 350 μM) were preincubated for 60 min at 37°C in Krebs/Henseleit buffer, then transferred to tubes containing 0.2 μM ^{3}H-myoinositol + 5 mM lithium chloride for 30 min, after which concentrations of 5HT from 1 μM to 3 mM were added for a further 45 min. Total ^{3}H-inositol phosphates (IP), the products of ^{3}H-phosphoinositide hydrolysis, were extracted and separated from ^{3}H-inositol by ion exchange chromatography. $5HT_2$ receptor binding was measured in membranes prepared from cortical slices using ^{3}H-ketanserin with the methodology as described by Leysen et al (1982).

Preliminary in vitro experiments were undertaken using slices prepared from untreated rats to elucidate the nature of the receptor mediating the 5HT stimulated ^{3}H-IP accumulation. 5HT log dose/response curves were clearly biphasic, the higher affinity portion having an EC_{50} of 30 μM with the lower affinity portion being apparently unsaturable at the concentrations of 5HT employed. The $5HT_2$ selective antagonist ketanserin competitively shifted the 5HT dose/response curves to the right, generating an apparent K_i value of 16 nM. In contrast, (-)-pindolol which shows high affinity and stereoselectivity for the $5HT_1$ site (Nahorski & Willcocks, 1983) was without effect at concentrations upto 10 μM. The putative 5HT antagonists methysergide, spiperone, metergoline and mianserin, all had K_i values in the nM range, while iprindole, prazosin, atropine and mepyramine were inactive at 10 μM. The non-indole containing 5HT agonist quipazine also stimulated phosphoinositide breakdown with an EC_{50} of 5 μM, and this response was also potently antagonised by ketanserin.

20 d but not 1 d treatment with the antidepressants imipramine and iprindole reduced the B_{max} of ^{3}H-ketanserin binding by 20% and 43% respectively, without a change in K_D. Furthermore, the maximum 5HT induced ^{3}H-IP accumulation was reduced by 54% and 66% respectively after 20 d but was not influenced by 1 d treatment with imipramine or iprindole.

These observations suggest that $5HT_2$ receptors appear to be linked to inositol phospholipid breakdown in brain and that antidepressant-induced down-regulation of $5HT_2$ receptors is accompanied by marked loss in 5HT responsiveness.

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CHRONIC CORTICOSTERONE DECREASES 5-HT DEPENDENT RESPONSES IN THE RAT

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Repeated immobilisation of rats (2 h/day, 7 days) increases some behavioural responses to the 5HT releaser, p-chloroamphetamine (PCA) and the 5HT agonist, 5-methoxy-N,N-dimethyltryptamine (5MeODMT) given 24 h after the final immobilisation (Curzon et al., 1984). As immobilisation increases plasma corticosterone and as there are reports of decreased (Nausieda et al., 1982) and increased (Buckett et al., 1984; Nausieda et al., 1982) 5HT function after various chronic corticoid treatments we have examined the effect of chronic corticosterone treatment on responses to PCA and 5MeODMT.

Male Sprague-Dawley rats, 200-260 g were injected 2 x daily for up to 4 days with corticosterone 50 mg/kg i.p. in 0.005% BRIJ saline. This dose is reported to elevate plasma corticosterone to levels comparable to those observed during immobilisation (Hodges & Jones, 1963). 24 h after the last injection, rats were put singly in observation cages and 60 min later given i.p. saline, d-amphetamine, PCA or 5MeODMT. 5HT and DA dependent responses were recorded by a blind observer as described by Dickinson & Curzon (1983).

Table 1: Behavioural responses, mean (range) induced by PCA and 5MeODMT.

Drug (mg/kg)	n	Tremor	Head Weaving	Limb Abduction	Forepaw Treading	Backward Movement
PCA (4)	(10)V	1.4(0-3)	7.7(1-13)	10.0(4-13)	9.7(3-14)	0.1(0-1)
" "	(10)CS	0.4(0-2)	1.8(0-6)*	4.2(3-7)**	4.8(0-9)*	0(0-0)
5MeODMT(5)	(9)V	1.9(0-3)	0.7(0-2)	4.8(3-7)	4.5(1-7)	0(0-0)
" "	(9)CS	0.4(0-2)**	0(0-0)	2.1(1-4)**	2.1(1-4)*	0.1(0-1)

V = vehicle, CS = corticosterone pretreated. *$p < 0.01$, **$p < 0.001$ by 2 tailed Mann-Whitney U test.

Rats treated with corticosterone for 4 days, given either saline or amphetamine 3 mg/kg i.p. 24 h later exhibited no significant behavioural differences from vehicle treated controls. However major 5HT-dependent behavioural effects of PCA 4 mg/kg i.p. were significantly reduced (Table 1). This effect could not be explained by altered PCA disposition as brain PCA levels measured fluorometrically (adapted from Axelrod, 1954) 30 min after PCA injection i.p. were comparable in corticosterone and vehicle treated rats. Behavioural responses to 5MeODMT 5 mg/kg i.p. (Table 1) were also significaly reduced which suggests that 4 days corticosterone treatment reduced sensitivity to 5HT at, or distal to the postsynaptic site. Behavioural responses to PCA (4 mg/kg, i.p.) were not reduced 24 h after 1 day of the standard corticosterone treatment or 24 h after 14 days treatment with a lower dose (2 x 10 mg/kg s.c. daily). The results suggest that corticosterone changes may oppose rather than cause the increase in 5HT dependent responses induced by repeated immobilisation.

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ARTERIOVENOUS ANASTOMOTIC CONSTRICTION AND ARTERIOLAR DILATATION BY BEA 1654, A PUTATIVE 5-HT_1 AGONIST

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Studies with radioactive microspheres have revealed that intracarotid infusions of 5-HT, which may or may not decrease total carotid blood flow, increase the perfusion of extracerebral tissues and reduce the blood flow via arteriovenous anastomoses (AVAs). A blockade of 5-HT_2 receptors by cyprohetadine (Saxena & Verdouw, 1982), methysergide (Saxena & Verdouw, 1984), ketanserin or WAL 1307 (Verdouw, Jennewein, Heiligers, Duncker & Saxena, 1984) completely reverses the 5-HT-induced decrease in total carotid flow, enhances the dilatation of extracerebral vascular bed but only slightly attenuates the constriction of the AVAs. Thus, while 5-HT_2 receptors constrict large arteries and arterioles, 'atypical' 5-HT receptors, possibly of the 5-HT_1 type, are involved in the constriction of AVAs and in the dilatation of arterioles (Saxena & Verdouw, 1982; 1984; Verdouw *et al.*, 1984).

To further elucidate the pharmacological role of the 5-HT_1 receptors we studied the vascular effects of a new piperazine derivative, BEA 1654 (n-3-acetylamino-phenyl) piperazine hydrochloride). Using appropriate ligands - [^{3}H]5-HT, [^{3}H]spiperone, [^{3}H]prazosin and [^{3}H]clonidine for, respectively, the 5-HT_1, 5-HT_2, α_1 and α_2 receptors - it was found that BEA 1654 has a high and selective affinity for the 5-HT_1 sites. The IC_{50} values for the different binding sites were: 5-HT_1 (1.9×10^{-8} M), 5-HT_2 ($>10^{-4}$ M), α_1 adrenoceptors (1.9×10^{-5} M) and α_2 adrenoceptors (1.5×10^{-6} M). The compound had no acetylcholine, histamine or dopamine receptor activity.

The effects of four cumulative intracarotid infusions of BEA 1654 (0.1-1.0 $mg \cdot kg^{-1} \cdot min^{-1}$) were investigated on the distribution of the common carotid artery blood flow in two groups (n=6 each) of pentobarbital anaesthetised (Saxena & Verdouw, 1982) pigs, without or after blockade of 5-HT_2 and α adrenergic receptors with ketanserin (0.3 $mg \cdot kg^{-1}$) and phentolamine (1 $mg \cdot kg^{-1}$). BEA 1654 exerted no effects on heart rate and arterial blood pressure was only slightly affected. The compound, however, caused a dose-dependent decrease in ipsilateral carotid flow from 248 ± 15 to 145 ± 24 $ml \cdot min^{-1}$ (mean ± SEM, $P<0.05$), which was due to a decrease in AVA flow from 210 ± 17 to 38 ± 16 $ml \cdot min^{-1}$ as extracerebral flow increased from 28 ± 5 to 113 ± 13 $ml \cdot min^{-1}$. In particular, the skin and ears contributed to this increase in extracerebral flow. The responses to BEA 1654 were not modified by pretreatment of the animals with ketanserin and phentolamine. In view of the binding profile of BEA 1654 and the similarity of pharmacological responses to 5-HT and BEA 1654, we conclude that 5-HT_1 receptors appear to mediate the constriction of AVAs and the dilatation of arterioles. However, the lower pharmacological potency of the compound, than could be expected from its binding affinity, suggests that BEA 1654 is not a full agonist of 5-HT_1 receptors.

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5-CARBOXAMIDO-TRYPTAMINE - A POTENT AGONIST AT 5-HYDROXYTRYPTAMINE RECEPTORS MEDIATING RELAXATION

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5-Carboxamido-tryptamine has been shown to be a selective agonist at pre- and post-junctional 5-HT receptors in the dog saphenous vein (Feniuk et al., 1981). However, in ganglion-blocked anaesthetised dogs 5-carboxamide tryptamine (0.1 - 30 µg/kg i.v.) produces vasodilatation and vasodepression (unpublished observations), suggesting that this compound has direct vasodilator action. We therefore investigated its actions in the cat saphenous vein and guinea-pig ileum, preparations thought to contain specific 5-HT receptors mediating smooth muscle relaxation (Feniuk et al., 1983).

Cat isolated saphenous veins and guinea-pig ileums were prepared as previously described (Feniuk et al., 1983). In the methoxamine contracted cat saphenous vein and the histamine contracted guinea-pig ileum, 5-carboxamido-tryptamine was 30 - 100 times more potent than 5-HT at causing relaxation with EC_{50} values of 7.2 (5.9 - 8.5) x 10^{-9} and 4.4 (3.3 - 5.5) x 10^{-8} mol/l respectively (geometric mean and 95% confidence limits, n = 4 observations). Methysergide was a specific and competitive antagonist of these relaxant effects of both 5-HT and 5-carboxamide tryptamine (Table 1).

Table 1: Antagonistic activity of methysergide against 5-HT, 5-carboxamido tryptamine and isoprenaline-induced relaxation of cat isolated saphenous vein and guinea-pig ileum

Agonist		Cat saphenous vein	Guinea-pig ileum
5-HT*	pA_2	6.75 (6.50 - 7.00)	7.37 (7.02 - 7.73)
	Slope	1.09 (0.90 - 1.27)	0.88 (0.71 - 1.09)
5-carboxamido tryptamine	pA_2	7.75 (7.05 - 8.45)	7.35 (7.10 - 7.60)
	Slope	1.10 (0.73 - 1.47)	0.95 (0.77 - 1.05)
Isoprenaline	pA_2	<6	<6

Values are mean (95% confidence limits) from at least 4 experiments. *Results taken from Feniuk et al. (1981). pA_2 values calculated according to the method of Arunlakshana & Schild (1959).

The findings show that 5-carboxamido-tryptamine is a potent smooth muscle relaxant and suggest that this relaxant action is mediated via specific 5-HT receptors.

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MONOAMINE OXIDASE ACTIVITY AFFECTS ANTAGONISM OF RESPONSES TO TRYPTAMINE BUT NOT TO 5-HYDROXYTRYPTAMINE IN RAT CAUDAL ARTERY

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Several workers have suggested that tryptamine can stimulate specific receptors distinct from those for 5-hydroxytryptamine (5-HT) (e.g. Cox et al., 1981). However, in the isolated fundus of the rat stomach the relative differences in antagonist potency against the two agonists may be accounted for by the differential accessibility of tryptamine and 5-HT to intracellular monoamine oxidase enzymes (Handschumacher & Vane, 1967) while in the rabbit isolated aorta, an additional α-adrenoceptor-mediated component in the response to tryptamine but not to 5-HT may be involved (Stollak & Furchgott, 1983). We have examined the effects of monoamine oxidase inhibition on the potency of the 5-HT_2 ligand, ketanserin, in antagonising contractions to tryptamine and 5-HT in the isolated caudal artery of the rat, and have investigated the extent of α-adrenoceptor stimulation in the response to tryptamine.

Middle caudal arteries were removed from male Wistar rats (220 - 350g) reserpinised 18h prior to use (5 $mgkg^{-1}$ i.p.) and anaesthetised with sodium pentobarbitone (90 $mgkg^{-1}$ i.p.). Four cylindrical segments of the vessel were prepared for the recording of isometric contractions using a method similar to that of Edvinsson et al. (1974). pA_2 values were determined as described previously in the presence and absence of iproniazid ($5 \times 10^{-5}M$) added to the modified Krebs solution (Apperley et al., 1976).

Phentolamine ($1.0 \times 10^{-6}M$) caused a slight rightward shift of concentration-response curves for tryptamine (concentration ratio of 2.4 ± 0.2, mean ± s.e. mean, n = 4). However, contractile responses to tryptamine were markedly potentiated by iproniazid (concentration ratio of 0.045 ± 0.22, n = 4) while responses to 5-HT and methoxamine were unaffected. The effect of iproniazid ($5 \times 10^{-5}M$) on pA_2 values (30 min) for ketanserin against 5-HT and tryptamine are shown in the table below.

	KETANSERIN		KETANSERIN (+ iproniazid)	
	pA_2	Slope	pA_2	Slope
Tryptamine	9.12 (8.55 - 9.69)	0.47 (0.34 - 0.60)	8.91 (8.63 - 9.19)	0.96 (0.77 - 1.15)
5-hydroxytryptamine	9.08 (8.88 - 9.28)	1.15 (1.04 - 1.26)	9.23 (8.70 - 9.76)	1.08 (0.86 - 1.30)

Each value is the mean (95% confidence limits) of 5 - 6 separate estimates.

High concentrations of ketanserin produced greater antagonism of contractile responses to 5-HT than of those to tryptamine, as shown by the low slope of the Schild plot in the latter case. This difference was not evident when iproniazid was present. These findings suggest that tryptamine and 5-HT both act on a common 5-HT receptor in this preparation and that the selective removal of tryptamine by monoamine oxidase enzymes accounts for their different susceptibilities to the antagonist examined.

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DIFFERENTIAL EFFECTS OF VERAPAMIL ON HYPOXIC AND RECOVERY CONTRACTIONS OF RAT AORTA AFTER EXPOSURE TO CHRONIC HYPOXIA

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Detar and Bohr (1972) proposed that some adaptive phenomena of the contractile responses of vascular muscle, exposed to chronic hypoxia, might be explained by changes in calcium influx. We have examined the adaptive phenomena in rat aorta and have investigated the effects of verapamil (VER).

All preparations were obtained from male Wistar rats (200-280g) sacrificed by cervical dislocation. Circular preparations were mounted under a 3g resting tension in Krebs' solution gassed initially with 5% CO_2 in O_2 maintained at 37°C. In all cases reproducible responses were obtained to 1μM noradrenaline (NA) (Normoxic response, pO_2 = 380 mmHg). Control responses were then obtained following either acute or chronic hypoxia. Acute hypoxia was induced by gassing with 5% CO_2 in N_2 (pO_2<70 mmHg) for 30 min. Chronic hypoxia was maintained by storage of tissues at 10°C under an atmosphere of 5% CO_2 in N_2 for 70hr (pO_2 = 40 mmHg).
In each case responses to NA were re-elicited and peak contractions were measured. Following re-oxygenation peak recovery contractions were recorded. Hypoxic and recovery contractions were re-examined following further 30 min hypoxic periods incorporating VER incubation.

The peak contractile responses to NA (1 μM) during hypoxia (hypoxic contractions), expressed as a percentage of control normoxic contractions, were 70.2 ± 6.4% (n=24) and 60.0 ± 8.8% (n=6) in preparations exposed to acute or chronic hypoxia respectively. Following re-introduction of oxygen the corresponding figures for the recovery contractions were 106.4 ± 11.3% (n=24) and 77.3 ± 11.3% (n=6) respectively. VER in a concentration (10μM) which caused a 50.2 + 6.1% (n=6) reduction of control normoxic contractions caused 55.5 + 6.7% (n=12) and 19.8 ± 1.2% (n=6) reductions of hypoxic contractions in preparations exposed to acute or chronic hypoxia respectively. A 71.2 ± 4.0% (n=12) and 83.0 ± 4.3% (n=6) reduction of the corresponding recovery contractions were seen with this concentration of VER. The effect of VER on a preparation exposed to chronic hypoxia is shown in figure 1. Qualitatively similar results have been obtained using diltiazem, flunarizine and nifedipine.

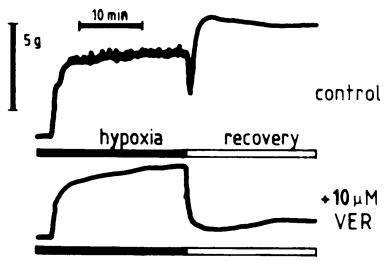

Figure 1. Representative trace showing effects of verapamil (10μM) on NA (1μM) induced hypoxic and recovery contractions in a preparation exposed to chronic hypoxia.

The results suggest that on exposure to chronic hypoxia, the contractile response of the rat aorta becomes adapted such that in comparison with normoxic (control) contractions the hypoxic contractions are less dependent upon extracellular Ca^{2+} for contraction, whereas, recovery contractions are more dependent on extracellular Ca^{2+}.

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POTENT ANTAGONISM BY DILTIAZEM OF α_2-RECEPTOR MEDIATED CONSTRICTION IN SHR, BUT NOT NORMOTENSIVE RAT TAIL ARTERIES IN VITRO

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Ca^{2+} entry blocking drugs can preferentially inhibit the vasoconstriction induced by α_2- rather than α_1-receptor agonists in vivo (Van Meel et al., 1981 , Cavero et al., 1983), although certain partial agonists at α_1-receptors are also susceptible to blockade by Ca^{2+} antagonists (Timmermans et al., 1983). The demonstration of this phenomenon in vitro has however been restricted to some isolated veins eg rat portal vein (Hicks, 1983) or dog saphenous vein (Cavero et al., 1983). We now report on the potent antagonist effects of diltiazem on α_2-receptor mediated responses in tail arteries of spontaneously hypertensive rats (SHR) in vitro, an arterial preparation in which postsynaptic α_2-receptors have been identified pharmacologically (Hicks et al., 1984).

Male SHR, Wistar Kyoto (WKY) or Sprague-Dawley (SD) rats were used (12 - 15 weeks of age). The animals were screened for systolic blood pressure (tail cuff) SHR with blood pressures > 190 mmHg and normotensive WKY and SD rats (< 140 mmHg) were used. The proximal tail artery was removed under pentobarbitone anaesthesia (60 mg/Kg i.p.) perfused and superfused with Krebs' bicarbonate containing propranolol (1 µM). cocaine (4 µM), pargyline (10 µM) and indomethacin (2.5 µM). Vasoconstrictor responses were obtained as increases in back perfusion pressure (mmHg) to the α_2-receptor agonist TL99 (Hicks et al., 1984), the α_1-receptor agonist methoxamine, or to KCl (40 mM). In some experiments, responses were obtained to field stimulation (supramaximal voltage : 0.3 ms , 0.1 - 10 Hz).

Diltiazem progressively antagonised the dose-response curve to TL99 (0.1 - 30 µM), with a depression of the maximum response in all strains of rat. Diltiazem was significantly ($p<0.01$) more potent against TL99, in SHR, compared with normotensive rats. IC_{50} values (concentration causing 50% reduction in the response) for diltiazem were 25 - 90 nM in SHR depending on the dose of TL99. In WKY or SD, the IC_{50} values were between 800 - 900 nM. In SHR diltiazem was less potent against methoxamine (IC_{50} 0.4 - 1.4 µM) compared with TL99. The response induced by KCl (40 mM) in tail arteries consists of a rapid spike, followed by a smaller sustained plateau. The inhibitory effects of diltiazem against the spike (IC_{50} 400 - 500 nM), or plateau (IC_{50} 0.8 - 3 µM) were not significantly different between SHR or normotensive rats. In SHR, the responses to field stimulation were significantly antagonised by diltiazem (30 - 300 nM), but in normotensive arteries these responses remained resistant to blockade with diltiazem (3 - 10 µM).

These results are entirely compatible with the concept that postsynaptic smooth muscle α_2-receptor mediated responses involve influx of Ca^{2+} ions. The fact that the postsynaptic α_2-receptor subtype is readily identified in tail arteries of SHR and that these responses are preferentially blocked by a calcium entry blocking drug like diltiazem is of particular interest.

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PRE- AND POSTSYNAPTIC ACTIONS OF BAYER K8644 IN THE RAT VAS DEFERENS

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Bayer K 8644 is a dihydropyridine derivative which acts to facilitate calcium entry into smooth and cardiac muscle, and this action is antagonised by the calcium entry blocker nifedipine (Schramm et al., 1983). The object of this present investigation was to examine the pre- and postsynaptic effects of this compound on the isolated bisected vas deferens of the rat.

Vasa deferentia were obtained from young adult wistar rats (2-3 months old) and bisected into prostatic and epididymal portions. In isotope experiments, epididymal portions were incubated for 1 hr in medium containing (^{3}H)-noradrenaline (1uM), and then superfused with medium containing cocaine (3uM) and corticosterone (40uM). Tissues were stimulated 4 times (S_1-S_4) for 3 min at 5 Hz (0.5ms, supramaximal voltage) at intervals of 30 min, and stimulation-evoked tritium overflow was measured and taken to indicate transmitter overflow. Test drugs were infused from 20 min before S_3 and S_4. In isometric contraction experiments, contractions were obtained to single pulse field stimulation at intervals of 5 min, and cumulative concentration/response curves were obtained to Bayer K 8644 or to the alpha$_1$-adrenoceptor agonist amidephrine in the absence or presence of prior drugs.

In isotope experiments, Bayer K 8644 (10uM) and nifedipine (10uM) had no significant effect on the stimulation-evoked overflow at 5 Hz, but Bayer K 8644 potentiated, and nifedipine inhibited, the stimulation evoked contraction. Bayer K 8644 reversed the inhibition by nifedipine.

Bayer K 8644 and amidephrine potentiated the early (presumed non-adrenergic) component of the two phase contraction to a single pulse in prostatic and epididymal portions (e.g. Bayer K 8644 produced a maximum potentiation of 204.4±10.4 % of control in prostatic portions, n=4), and prazosin (0.1uM) attenuated the effects only of amidephrine. In the presence of Bayer K 8644 (10uM), amidephrine produced no further potentiation of the contraction to a single stimulus (and indeed inhibited the contraction, see Docherty et al., 1984), but the spontaneous direct contractions to amidephrine were potentiated. Nifedipine (10uM) abolished the spontaneous contractions to amidephrine but also abolished the early component of the contraction to a single pulse (see Docherty & O'Malley, 1983).

In conclusion, Bayer K 8644 had no presynaptic effect on neurotransmission in the epididymal portion of the rat vas deferens. Postsynaptically, Bayer K 8644 potentiated, and nifedipine abolished, the early component of the contraction to a single pulse and the contractions to amidephrine.

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LATE ADMINISTRATION OF NIFEDIPINE FAILS TO PREVENT REPERFUSION-INDUCED VENTRICULAR FIBRILLATION IN ANAESTHETISED GREYHOUNDS

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We have demonstrated previously (Coker & Parratt, 1983) that administration of nifedipine (5μg/kg + 0.67μg kg^{-1} min^{-1} i.v.) prior to coronary artery occlusion reduced the incidence of ventricular fibrillation (VF) resulting from subsequent reperfusion of the myocardium after 40 min of ischaemia. Since nifedipine also had marked antiarrhythmic activity during the occlusion period it is possible that the reduction in reperfusion-induced VF may be related to effects of nifedipine during myocardium ischaemia. Thus, the aim of the present study was to examine the effects of nifedipine on reperfusion arrhythmias, when administered after the onset of myocardial ischaemia.

Greyhounds were anaesthetised with chloralose after induction with sodium thiopentone. Intra-cardiac catheters were positioned under fluoroscopic control and a left thoracotomy was performed at the level of the sixth rib. The pericardium was incised and a ligature was placed around the left anterior descending coronary artery (LAD). After establishing stable control values for haemodynamics and blood gases the LAD was occluded for 40 min. Ectopic activity was evident during the first 30 min of ischaemia and the majority of control dogs died in VF within 2 min of reperfusion. Treatment with nifedipine (5μg/kg + 1μg kg^{-1} min^{-1} i.v.) was started 25 min after occlusion of the LAD, i.e. 15 min prior to reperfusion. Table 1 compares the effects of early and late administration of nifedipine on arrhythmias.

Table 1. The effects of nifedipine on the number of ischaemia-induced ectopic beats (VEBs) and the incidence of reperfusion-induced VF.

	Control	Nifedipine pre-ischaemia	Control	Nifedipine post-ischaemia
	n=9	n=9	n=10	n=10
VEBs	720±136	119±105*	702±188	677±233
% VF	88%	22%†	70%	70%

*P<0.01 independent t-test; †P<0.01 Chi-squared test.

These results indicate that administration of nifedipine 25 min after the onset of myocardial ischaemia does not prevent reperfusion-induced VF. The haemodynamic effects of early and late administration of nifedipine were similar. For example, in those dogs receiving nifedipine after the onset of ischaemia mean arterial pressure was reduced from 139±9 to 112±9 mmHg and peripheral vascular resistance from 61±2 to 41± 3 units. Cardiac output and stroke volume were increased by nifedipine (2.28±0.15 to 2.80±0.27 l/min and 16.3±1.4 to 19.0±1.8 ml respectively). The ischaemia-induced reductions in local coronary venous PO_2 and pH and increases in PCO_2 were similar in all four groups, as was the magnitude of the ST-segment depression. In the group which received nifedipine prior to LAD occlusion there were less ectopic beats during ischaemia and no ischaemia-induced changes in haemodynamics.

This study suggests that VF following reperfusion depends largely on events occurring during myocardial ischaemia.

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DO GUINEA-PIG VENTRICULAR MYOCARDIAL α-ADRENOCEPTORS EXIST?

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Reports exist that both confirm (Wagner & Brodde 1978) and deny (Shibata et al. 1980) the involvement of myocardial α-adrenoceptors in the production of a positive inotropic effect in the guinea-pig ventricle. Another report (Sanchez-Chapula 1981) describes an α-adrenoceptor-mediated response to phenylephrine which could not be confirmed with methoxamine. This study examines the responses of left ventricular papillary muscles of the guinea-pig to various α-adrenoceptor agonists.

Tissues were suspended in Krebs-bicarbonate solution at 32°C, gassed with 5% CO_2: O_2 and containing metanephrine (10μM) to inhibit extraneuronal uptake. The tissues were paced at 1Hz (threshold voltage + 50%, 5ms pulse-width) and isometric tension recorded. Cumulative dose-response curves to isoprenaline were constructed followed after washout by curves to phenylephrine, clonidine or methoxamine. Increases in developed tension were calculated as a percentage of the isoprenaline maximum. Phenylephrine produced a maximum increase in developed tension 28.5±3.9% of the isoprenaline maximum. This response was abolished by propranolol (1μM). Clonidine also produced a positive inotropic response, with a maximum 62.3±3.9% of that to isoprenaline. The clonidine EC50 (6.9(5.2-9.0)μM) in the presence of propranolol (1μM) was similar to control values (4.6(3.2-6.4)μM). Phentolamine (5μM) resulted in a leftward shift (potentiation) of the clonidine curve, the EC50 being reduced ($P<0.001$) from 17.3(12.1-25.0)μM to 8.6(6.9-10.9)μM. However, the clonidine responses were blocked by the H_2-receptor antagonist, cimetidine (5μM), the control EC50 value of 8.8(5.3-14.5)μM increasing to 96.7(89.0-107.3)μM in the presence of the antagonist ($P<0.001$). A similar shift was obtained for blockade of histamine responses by cimetidine (5μM) on this tissue, the histamine EC50 increasing ($P<0.001$) from 0.33(0.2-0.7)μM to 3.3(2.0-5.6)μM. Clonidine responses on this tissue were therefore H_2-receptor mediated. The selective α_1-adrenoceptor agonist, methoxamine, produced only a negative inotropic response that was neither blocked by phentolamine (5μM) nor propranolol (1μM).

Finally, guinea-pig ventricular muscle was assayed for [^{3}H]prazosin and [^{3}H]dihydroalprenolol binding at 32°C, using phentolamine (10μM) and isoprenaline (200μM) respectively to calculate non-specific binding. Scatchard analysis of the saturation curves revealed that the K_D for β-adrenoceptor binding assessed with [^{3}H]DHA was 0.63±0.11nM. The maximum number of binding sites (B_m=114.0±5.1 fmoles mg^{-1} protein) was 5.7 times that for α_1-adrenoceptor binding with [^{3}H]prazosin (B_m=20.0±4.9 fmoles mg^{-1} protein, K_D=0.60±0.27nM).

It is concluded that in normal guinea-pig papillary muscle, phenylephrine exerts its action directly or indirectly through β-adrenoceptors, clonidine via H_2-receptors, while methoxamine only has a non-specific depressant action. The few α_1-adrenoceptors identified by [^{3}H]prazosin binding are probably located on the coronary vessels.

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THE INFLUENCE OF BLOOD GASES ON α_1 AND α_2 ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES IN THE PITHED RAT

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In a preliminary study of oxygen-ventilated pithed rats (McGrath et al, 1982) it was found that altering the artificial ventilation volume and hence varying the blood gases had contrasting effects on the peak pressor responses to intravenous boluses of agonists selective for either vascular α_1 or α_2 adrenoceptors. This communication confirms these earlier findings and further examines the influence of blood gases on responses mediated by adrenoceptor sub-types.

Male Wistar rats (245-265g) were pithed by the method of Gillespie et al (1970) and ventilated at 60 strokes/min. with one of the following gas mixtures (i) air (ii) 100% O_2 (iii) 30% O_2 with varying CO_2 levels (0-4%) or (iv) varying O_2 levels (15-100%) with different background CO_2 levels (0 or 4%). To alter blood gases in (i) and (ii) the stroke volume was varied (1.8-4ml/stroke) but was fixed at 3.5ml/stroke for the other gas mixtures. In all cases the remainder of the gas was N_2. Carotid arterial blood samples (0.6ml) were taken for blood gas analysis. Pressor responses were assessed as changes in diastolic arterial blood pressure to a range of doses of adrenoceptor agonists; Phenylephrine (0.3-30ug/Kg) α_1 and Xylazine (0.05-5mg/Kg) α_2.

The results confirm that hyperventilation (with 100% O_2 or air) causing respiratory alkalosis increases α_1 responses while hypoventilation causing respiratory acidosis increases α_2 responses. However, when the PaO_2 was allowed to fall to hypoxic levels the α_1 responses were additionally depressed whereas the α_2 responses were still near maximum. Gas mixtures were then used to vary CO_2 or O_2 independently. "Normoxic" mixtures (PaO_2 approx. 100mmHg) with varying CO_2 levels showed that α_2 responses still increased with increasing $PaCO_2$ but α_1 responses were no longer clearly depressed. When PaO_2 was varied against different background levels of CO_2, the α_1 responses increased as PaO_2 increased, in both cases.

The results suggest that α_2 responses are affected by CO_2 alone but that α_1 responses are altered by changing CO_2 or O_2 levels. Physiologically, the alteration of the responses, mediated by the two receptors, are in opposite directions. α_2 responses are increased by rising CO_2 whereas α_1 are decreased by rising CO_2 or falling O_2.

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THE INFLUENCE OF VASOPRESSIN ON BP FOLLOWING α-ADRENOCEPTOR ANTAGONISM

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In a previous communication (Bennett et al, 1984), in which Long Evans (LE) and Brattleboro rats were compared, we presented results consistent with the proposal that vasopressin acts to limit the hypotensive action of α_1- and α_2-adrenoceptor antagonism. These results differ from those of Waeber et al (1983) in Wistar rats, but our experiments were carried out in the presence of atropine and propranolol whilst those of Waeber et al (1983) were not. We have now measured BP responses to the α_1-antagonist prazosin (1 $mgkg^{-1}$; 1 $mgkg^{-1}h^{-1}$ i.v.) followed by the specific antagonist of the pressor action of vasopressin ([1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-8-D-arginine] vasopressin; $d(CH_2)_5$DAVP; 10 μgkg^{-1}; 10 $\mu gkg^{-1}h^{-1}$ i.v.), and lastly, the α_2-adrenoceptor antagonist RX781094 (0.75 $mgkg^{-1}$ i.v.; Doxey et al, 1983) in LE rats in the absence of atropine and propranolol; half the rats were treated with the angiotensin converting enzyme inhibitor captopril (2 $mgkg^{-1}$; 1 $mgkg^{-1}h^{-1}$ i.v.). LE rats (n=6) were anaesthetised (sodium methohexitone i.p.), and the jugular vein and abdominal aorta were cannulated. Systolic and diastolic BP and HR recordings were begun after a 4½-5 h recovery period.

The results (Table) show that in the absence of captopril, prazosin caused an immediate fall in BP but it recovered almost completely within 30 min. Administration of $d(CH_2)_5$DAVP 45 min after prazosin produced a small and transient fall in BP. RX781094, 45 min after $d(CH_2)_5$DAVP, caused a marked hypotension, but recovery was complete within 15-30 min. In the presence of captopril, prazosin produced a fall in BP no greater nor more sustained than in its absence. However, addition of $d(CH_2)_5$DAVP 45 min later caused a substantial and sustained fall in BP. Under these conditions, RX781094 lowered BP further, and there was little evidence of recovery.

Table: The Effects of Prazosin, $d(CH_2)_5$DAVP and RX781094 on BP (systolic/diastolic; mean±S.E. mean) in LE Rats.

		Control (n=6)	Captopril (n=6)
Basal		146±1.2/95±3.6	147±3.3/101±2.2
+Prazosin	1 min	92±6.5/62±4.8	98±4.8/ 65±4.4
	45 min	132±4.3/93±3.3	132±5.9/ 97±4.7
+$d(CH_2)_5$DAVP	1 min	124±5.7/87±3.7	104±7.7/ 73±6.9
	45 min	133±4.5/94±2.9	104±7.7/ 74±5.8
+RX781094	1 min	82±4.0/52±4.0	83±3.3/ 53±3.6
	30 min	136±7.6/97±4.4	86±3.7/ 53±3.6

These results indicate that, in the presence of captopril, vasopressin may act to maintain BP following α_1-antagonism in LE rats. In these circumstances, there may also be a postjunctional α_2-adrenoceptor-mediated contribution to BP maintenance.

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EVIDENCE FOR A SYMPATHOEXCITATORY ACTION OF α_2-ADRENOCEPTOR ANTAGONISTS

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Activation of central α_2 adrenoceptors causes a decrease in sympathetic nerve activity (Haeusler, 1982). If these α_2 adrenoceptors are under tonic activation their blockade would be expected to result in an increase in sympathetic nerve activity. Hence, the following experiments were carried out to ascertain whether the α_2 adrenoceptor antagonists yohimbine (Starke et al., 1975) Wy 26392 (Lattimer et al., 1982) and Idazoxan (RX 781094) (Doxey et al., 1983) cause an increase in preganglionic sympathetic nerve activity in the anaesthetised cat.

Cats were anaesthetised with α chloralose (70 mg kg^{-1}, i.v.) and pentobarbitone sodium (12 mg). Simultaneous recordings were made of brachial arterial pressure, heart rate, femoral arterial flow (from which conductance was derived) and preganglionic sympathetic nerve activity as previously described (Ramage, 1982) except that skeletal muscle paralysis was produced by decamethonium (0.25 mg kg^{-1}) In all experiments these parameters were recorded for 20 min before the intravenous injections of the test solution. A cumulative dose (0.01-1 mg kg^{-1}) response curve was produced for each drug with injections given at 15 min intervals. Control injections of vehicle (0.04M lactic acid) were also carried out.

Yohimbine (n=3), Wy 26392 (n=3) and idazoxan (n=3) caused dose related increases in preganglionic sympathetic nerve activity reaching maximum values of 260%, 429% and 177% of the background respectively. The increase in preganglionic sympathetic nerve activity for yohimbine was associated with a maximum rise in mean blood pressure of 12 mm Hg and in heart rate of 47 beats min^{-1}, while for Wy 26392 and idazoxan the maximum rises in blood pressure were 16 and 8 mm Hg and in heart rate 47 and 68 beats min^{-1} respectively. Femoral arterial conductance was decreased by low doses and increased by high doses of all three drugs.

The increase in femoral arterial conductance at high doses was associated with a fall in mean blood pressure and a decline in heart rate. In the control experiments (n=3) all the parameters measured remained fairly stable.

These results indicate that the α_2 adrenoceptor antagonists have a sympathoexcitatory action and suggest that in the anaesthetised cat preganglionic sympathetic nerve activity is subject to a tonic inhibitory input mediated via central α_2 adrenoceptors.

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TISSUE BLOOD FLOW AND ARTERIOVENOUS SHUNTING IN PIGS MEASURED WITH MICROSPHERES OF FOUR DIFFERENT SIZES

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With simultaneous use of microspheres of four different sizes (10, 15, 25 and 35 μm), we compared blood flows to a large number of tissues, and the complete distribution of common carotid arterial blood flow in pentobarbital anaesthetized pigs (Saxena & Verdouw, 1982). In addition, we studied the effects of 5-hydroxytryptamine (5-HT) on haemodynamic values obtained with 15 and 35 μm spheres.

When the microspheres were injected into the left atrium (n=6), the blood flows ($ml \cdot min^{-1} \cdot 100g^{-1}$) measured with 10 μm spheres were: cerebral hemispheres (40 ± 2), eyes (51 ± 7), left ventricle (112 ± 11), kidneys (425 ± 34), skin (2.2 ± 0.4), skeletal muscles (4.0 ± 0.6), stomach (23 ± 3), small intestines (39 ± 4), large intestines (37 ± 2), spleen (210 ± 25), adrenals (283 ± 34) and liver (77 ± 7). Tissue blood flows estimated with the larger spheres did not differ in most tissues, but were much elevated in the skin and eyes (fig. 1). The skin regions exhibited heterogeneity; the magnitude of changes between 10 and 35 μm spheres increased in the following order: thigh (+67%), trunk (+130%), neck (+246%), abdomen (+280%) and face (+446%). The distribution of carotid blood flow measured with 10 or 15 μm spheres was similar to that described earlier (Saxena & Verdouw, 1982). Only in the skin and ears (>300%) and in the tongue and eyes (about 50%) were the flows higher when measured with the larger spheres. After infusion of 5-HT (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, *i.v.* or 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, intracarotid arterially), the differences in the flow values obtained with 15 and 35 μm spheres were completely eliminated in the skin and ears.

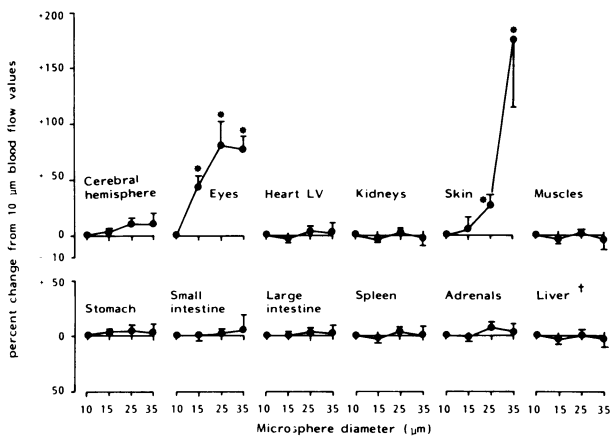

Fig. 1. Tissue blood flows measured with microspheres of 4 different sizes injected into the left atrium. *, P<0.05 (Duncan's new multiple range test). †, Hepatic artery.

Since it is known that the spheres of 9, 15 and 24 μm are trapped in vessels with a diameter of 12, 28 and 43 μm, respectively (Dickhoner *et al.*, 1978), we conclude that the dependence of blood flow values on the sphere size indicates that arteriovenous anastomoses (AVAs), which generally have diameters >20 μm, are located in the skin and ears and, to some extent, in the eyes and tongue. The reduction of the "size" gradient by 5-HT, which can constrict AVAs (Saxena & Verdouw, 1982), is in agreement with the above conclusion.

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α-ADRENOCEPTOR INVOLVEMENT IN CATECHOLAMINE-INDUCED HYPERGLYCAEMIA IN CONSCIOUS FASTED RABBITS

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Propranolol fails to block the hyperglycaemic effect of adrenaline (Ad) and noradrenaline in conscious fasted rabbits, and the hepatic glycogenolytic action of Ad is also unaffected (Moratinos et al., 1975). In keeping with this, more recent work suggests that α-adrenoceptors have a role in catecholamine (CA) induced hyperglycaemia though some conflicting results have been described (see Al-Jibouri et al, 1980; Nakadate et al, 1980). The aim of this study was to examine further the role of α-adrenoceptors in CA hyperglycaemia in conscious rabbits fasted for 24h.

I.v. infusion of the α-adrenoceptor agonist phenylephrine (PE) produced a dose-related increase in arterial blood glucose. In response to 20 μg PE/kg/min for 30 min, the increase (Δ) in glucose reached a peak of 3.3 ± 1.1 mmol/l (n=7; mean ± s.e. mean) 45 min after the start of the infusion, as compared with Δ=0.7 ± 0.13, n=7, with a saline control (arterial glucose 4.6 ± 0.45 mmol/l).

The hyperglycaemia was accompanied by a transient and not statistically significant rise in blood lactate (Δ at the end of the infusion -0.81 ± 0.49 mmol/l, versus 0.01 ± 0.11, n=7, after saline). Plasma immuno-reactive insulin (IRI) levels rose somewhat though to a very variable extent (Δ at 30 min, 71 ± 40%, n=7, compared with -8 ± 20%, n=7, with saline: $P<0.2$). At the end of the infusion, PE had reduced liver glycogen to 3.2 ± 0.8 mg/g wet wt (n=6) from 5.9 ± 1.0 mg/g (after saline). Muscle glycogen did not change.

The administration of the α_1-selective antagonist prazosin (PRZ) either as an i.v. infusion (0.5 - 10 μg/kg/min, for 30 min) or as a s.c. injection (1 mg/kg) did not completely block the effect of PE on blood glucose. Thus the increase in glucose with PE (at the same infusion rate, and after 45 min, as before) was 3.3 ± 1.1 mmol/l (n=7) in the controls as compared with 1.53 ± 0.23 mmol/l (n=7) in the presence of prazosin (s.c. at 1 mg/kg, 30 min before PE).

The α_1- and α_2-adrenoceptor blocker phenoxybenzamine (PBZ) was tested against equiactive doses of PE and Ad. When slowly infused over a 15 min period, PBZ (total dose 0.25 mg/kg) evoked small fluctuations in blood glucose and IRI plasma levels. When given 50 min before PE, the hyperglycaemia was completely suppressed and the response to Ad was attenuated (Ad, at 0.3 μg/kg/min for 30 min, raised blood glucose by 4.3 ± 1.0 mmol/l, n=5, at the end of the infusion: after PBZ the corresponding value was 2.4 ± 0.25 mmol/l, n=9, $P<0.05$). Interestingly, the pattern of IRI plasma levels found after PE and Ad infusion was not altered by the antagonist. PBZ also completely blocked the reduction in liver glycogen induced by PE (3.2 ± 0.8 mg/g wet tissue after PE, no PBZ; 6.6 ± 1.2, n=7 after PE and PBZ).

Our results add to the evidence for α-adrenoceptor involvement in the hyperglycaemic and hepatic glycogenolytic actions of CA in conscious rabbits.

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ACTIVATION-INDUCED POSTSYNAPTIC BLOCK OF INSECT NERVE-MUSCLE TRANSMISSION BY A LOW MOLECULAR WEIGHT FRACTION OF SPIDER VENOM

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Two species of Old World, Araneid spiders, Nephila clavata and Araneus ventricosus have been reported to possess a venom component which acts on glutamate receptors of either lobster muscle (Kawai et al., 1982a, Abe et al., 1983) and/or mammalian brain (Kawai et al., 1982b). Specific competitive antagonism by these venoms leads to irreversible inhibition of the glutamate induced response in these systems. We have studied the effects of crude and partly purified toxin from venom of two New World spiders, Araneus gemma and Argiope trifasciata on nerve-muscle preparations of the metathoracic (jumping) leg of the locust, Schistocerca gregaria.

Venom glands were dissected either from specimens frozen in liquid nitrogen or from anaesthetized spiders and homogenised in standard locust saline to produce a crude extract. A venom concentration of 1U (i.e. 1 venom gland homogenised in 1 ml of locust saline) produced a rapid abolition of the neurally evoked twitch contraction of the isolated retractor unguis nerve-muscle preparation and also blocked the muscle contraction evoked by perfusion of this preparation with saline containing 10^{-4}M L-glutamate. Recovery from the effects of either venom was slow and incomplete. A transient increase in the rate of recovery could be obtained by resting the preparation during the wash period after removal of the venom but a further decline in twitch amplitude occurred when stimulation was recommenced at the normal frequency (ca. 0.25Hz).

Intracellularly recorded postsynaptic potentials evoked by ionophoresis of pulses of L-glutamate onto an excitatory junctional site of locust extensor tibiae muscle were abolished by low (0.05U) concentrations of the venom. Inhibition of the ionophoretic potential was dependent upon the ionophoretic pulse repetition rate suggesting again that activation of the glutamate receptor-channel complex is a prerequisite for venom induced block. The fact that glutamate potentials could still be evoked at neighbouring nerve-muscle junctions, not previously exposed to agonist, following removal of the venom supports this contention. Dose-response curves constructed from data obtained during ionophoresis of L-glutamate to single junctional sites were shifted to the right in a non-parallel fashion when venom was added to the bathing medium.

Crude homogenates of venom glands were boiled (2 min) and centrifuged (15,000g, 10 min). SDS gel electrophoresis of the resulting supernatant revealed a dense stained band at < 20,000 Daltons with a loss of higher molecular weight fractions compared to untreated venom. Further purification of the venom by passage through a membrane filter with a cut-off at 10,000 Daltons yielded a component which was still as potent as the crude homogenate. The partly purified toxin would thus appear to be a low molecular weight compound which blocks nerve-muscle transmission in the locust in a slowly reversible manner and only after the post-junctional glutamate receptors have been activated by L-glutamate.

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A β_2-ADRENOCEPTOR INVOLVEMENT IN THE DEVELOPMENT OF SPONTANEOUS HYPERTENSION IN RATS

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Adrenaline has been shown to facilitate ^{3}H-noradrenaline release from sympathetic nerves (Majewski, 1983), by an action at pre-junctional β_2-adrenoceptors and we have demonstrated that salbutamol and procaterol, two selective β_2-adrenoceptor agonists, mimic the effect of adrenaline in selectively enhancing pressor responses induced by electrical stimulation of the entire sympathetic outflow in pithed adrenal demedullated rats (Borkowski & Quinn, 1984). Since a role for adrenaline in the development of a raised blood pressure has been suggested by Majewski (1981) and supported by observations that bilateral adrenal demedullation of young spontaneously hypertensive (SHR) rats attenuates their hypertension (Borkowski & Quinn, 1983) this study examines the involvement of β_2-adrenoceptors in the development of spontaneous hypertension.

Systolic blood pressure (SBP) was monitored in 4 groups of 6-9 male SHR rats, from 3 to 14 weeks of age, using the indirect tail cuff method. At 4 weeks of age the rats were anaesthetised with ether, one group was sham operated, the rest underwent bilateral adrenal demedullation. Two groups of demedullated rats were then implanted with slow release depot preparations of salbutamol (0.165 µmol/kg s.c.) or procaterol (5 nmol/kg s.c.) every 14 days. At 14 weeks the rats were pithed and pressor responses to electrical stimulation (0.125 - 4Hz, 30v, 1msec, 15sec) of the entire sympathetic outflow and bolus injections of phenylephrine (30-10000 ng, i.v.) were measured, while adrenal and plasma catecholamine concentrations were determined using HPLC.

At 14 weeks, sham operated rats had an SBP of 208 ± 4 mmHg, while rats demedullated 10 weeks previously had an SBP of 177 ± 3 mmHg and those implanted with slow release depots of procaterol or salbutamol developed SBP's of 205 ± 7 mmHg and 211 ± 5 mmHg respectively. In pithed animals, pressor responses to electrical stimulation were significantly ($p<0.001$) reduced in demedullated rats (maximal increase in DBP being 109 ± 2 compared to 134 ± 2 mmHg in sham operated animals), while responses to phenylephrine were only slightly reduced. In demedullated rats implanted with procaterol or salbutamol pressor responses to electrical stimulation and phenylephrine were not significantly different from those seen in sham operated rats.

Paired adrenals from sham operated rats contained 27.8 ± 1 µg of adrenaline and 9.8 ± 0.5 µg of noradrenaline. Adrenal demedullation reduced the adrenaline content by 99.7% and noradrenaline by 99.1% and these reductions were unaffected by procaterol or salbutamol. Plasma noradrenaline and adrenaline levels were 182 ± 31 pg/ml and 19.4 ± 7 pg/ml respectively in pithed sham operated rats and 67 ± 11 pg/ml and 0 in demedullated animals. However, in demedullated rats implanted with salbutamol and procaterol, plasma noradrenaline levels were 140 ± 19 pg/ml and 349 ± 56 pg/ml respectively, while plasma adrenaline was again undetectable.

These results confirm that bilateral adrenal demedullation attenuates the development of hypertension in SHR rats. Implants of salbutamol and procaterol restored the development of hypertension, possibly by facilitating sympathetic neurotransmission.

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THE EFFECTS OF PROPRANOLOL AND TIMOLOL ON RAT HEART MITOCHONDRIAL FUNCTION

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Beta-receptor-blocker drugs are commonly used as antihypertensive agents and although they have other clinical effects it is generally assumed that the therapeutic action of drugs like propranolol and timolol is mediated exclusively through beta-adrenoreceptor blockade. Propranolol differs in several important respects from timolol. Propranolol, for example, has a hydrophobic character and tends to partition into the hydrophobic domain in membranes and clefts of proteins. In consequence, it is largely in a bound form in blood plasma compared with timolol which is mostly in a free form. (Taylor & Turner 1981) Clinically, propranolol is regarded as having a more depressant effect on the myocardium than does timolol. Evidence for the effect of beta-receptor blockers on cardiac mitochondrial function and energy metabolism is somewhat conflicting. (Naylor et al 1975; Sobel et al 1966; Sakurado et al 1972). Experiments have been undertaken to investigate the direct effects of these drugs on rat heart mitochondria in vitro.

The initial experiments aimed to compare the effect of drug concentration on uncoupled rat heart mitochondria respiring on substrates of α-oxoglutarate, pyruvate-malate or succinate. No significant effects were observed with either of these drugs when present in concentrations of less than 2mM. A marked inhibition of respiration supported by succinate (>50%) was observed when the mitochondria were broken to allow access of the substrate and drugs into the organelle. Oxidation of succinate by broken mitochondria was inhibited to about the same extent by both drugs. No respiration of α-oxoglutarate or pyruvate-malate was observed in broken mitochondria consistent with the fact that soluble enzymes required for the metabolism of these substrates had been leached from the mitochondria. Propanolol, but not timolol, caused a marked inhibition of NADH oxidase activity and NADH-supported oxygen up-take in sub-mitochondrial particles. With 2mM propranolol respiration rate decreased from about 55 to less than 10 nmoles O_2 consumed per mg protein per minute. The site of inhibition by propranolol was found to be on the reductase of Complex I and the associated flavoprotein. The rate of ferricyanide reductase of Complex I in preparations inhibited by rotenone was unaffected by propanolol.

These data show that the inhibitory effects of propranolol and timolol on mitochondrial function are different and that propanolol has a considerably greater effect than timolol. Further studies will be required to establish the role of beta-receptor blocker drugs on cardiac energy metabolism..

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RESPONSES MEDIATED VIA β_1 BUT NOT β_2-ADRENOCEPTORS EXHIBIT SUPERSENSITIVITY AFTER 6-HYDROXYDOPAMINE PRETREATMENT

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Chronic pretreatment of animals with 6-hydroxydopamine (6-OHDA) results in chemical sympathectomy and an increased sensitivity of guinea-pig atria to sympathomimetic amines which is post-junctional in origin (Broadley et al. 1983). It has been suggested that the β_1-adrenoceptor subtype responds primarily to neurotransmitter and is therefore innervated, whereas the β_2-adrenoceptor is non-innervated and responsive to circulating catecholamines (Ariens 1981). The present study examines this concept by determining the effect of 6-OHDA on the sensitivity of guinea-pig isolated tissues which display β_1- or β_2-mediated responses.

Male guinea-pigs were pretreated with 6-OHDA (460mgkg^{-1}, 6 intracardiac doses over 20 days). Controls were sham injected with vehicle. Isolated tissues were set up in Krebs-bicarbonate solution at 38°C, gassed with 5% CO_2 in O_2 and containing metanephrine (10μM) and phentolamine (5μM). Increase tension and rate responses of paced left (2Hz) and spontaneous right atria were recorded. Lung strips and tracheal spirals under 2g tension were washed every 20 min for 1h before obtaining relaxation responses. Ileal segments were set up under 0.5g tension and relaxation responses recorded as inhibition of carbachol-induced contractions. Vas deferens under 1g tension were field stimulated every 30 sec for 3 sec (100v, 10Hz, 1ms) and responses measured as inhibition of twitch height. Cumulative dose-response curves were obtained to isoprenaline, followed after washout by a partial agonist.

Tissue	Isoprenaline EC50 (nM)[1]		Partial agonist (% iso max)[2]	
	SHAM	6-OHDA	SHAM	6-OHDA
L.Atria	12.5(2.9-53.0)	1.33(0.2-8.2)*	23.0(21-25.0)	38.2(34.0-42.0)*
R.Atria	4.7(2.5-8.8)	2.2(1.21-3.9)*	63.5(61.0-66.0)	74.4(71.9-76.8)*
Lung	10.8(6.6-17.6)	9.25(5.8-14.7)^{NS}	56.3(52.3-60.3)	63.8(56.6-68.1)^{NS}
Trachea	1.24(0.52-2.98)	1.29(0.84-4.37)^{NS}	47.9(41.5-54.3)	68.9(64.5-73.3)*
Ileum	75.0(24.7-142)	11.5(30.4-43.9)*	-	-
Vas deferens	13.7(4.7-39.6)	8.47(4.4-21.2)^{NS}	90.9(85.6-96.2)	76.9(71.4-82.0)^{NS}

[1]Geomtric mean and 95% confidence limits. [2]Mean ± s.e.m. *Significant difference from controls (Student's t-test), $P<0.05$. Partial agonists - salbutamol (atria, vas deferens), ritodrine (lung) and prenalterol (trachea). $n\geq4$.

Increases in sensitivity after 6-OHDA were demonstrated as a significantly lower EC50 for isoprenaline or increased maxima to partial agonists in atria and ileum, the responses of which are via β_1-adrenoceptors only (Zaagsma et al. 1983 and unpublished data). No increase in sensitivity occurred for lung and vas deferens responses which are β_2-adrenoceptor-mediated (Zaagsma et al. 1983; Gerthoffer and Westfall 1976). In the trachea there was no supersensitivity to isoprenaline, but an increased partial agonist maximum. This tissue contains both β_1 and β_2-adrenoceptors (Furchgott et al. 1975).

These results suggest that supersensitivity occurs after chemical sympathectomy only at β_1-adrenoceptors and that these receptors are innervated.

P.F.G. is a CASE student in collaboration with Beechams Pharmaceuticals.

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ANTIARRHYTHMIC EFFECTS OF A METOPROLOL THROMBOXANE SYNTHESIS INHIBITOR COMBINATION IN CONSCIOUS RATS

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Recent studies have demonstrated that metoprolol, alone and in combination with the thromboxane synthetase inhibitor UK38,485, reduces the severity and incidence of the serious ventricular arrhythmias resulting from coronary artery ligation in anaesthetised rats (Parratt & Wainwright, 1983). The present study was carried out to determine the effects of metoprolol, UK38,485 and a combination of the two on post-ligation arrhythmias and survival in conscious rats.

Male Sprague-Dawley rats were prepared for coronary artery ligation as described by Leprân et al. (1979). Seven days after the preliminary operation the rats were administered metoprolol (2 mg/kg i.v.), UK38,485 (5 mg/kg i.v.) or a combination of the two, fifteen minutes prior to occlusion of the left coronary artery. The effects on the arrhythmias and survival are shown in Table 1.

Table 1 Survival rate and occurrence of arrhythmias after acute ligation of the main coronary artery.

Treatment	n	Survival rate (%)		Incidence of arrhythmias (%)		
		20 min	16h	VF	VT	VEB
None	11	36	27	55	55	64
Metoprolol (2mg/kg)	10	20	20	70	70	80
UK38,485 (5mg/kg)	10	20	20	80	60	70
Metoprolol + UK38,485	12	69	69*	46	54	85

*P<0.05 (Fishers exact probability test)

Pretreatment with either metoprolol or UK38,485 alone did not protect against either mortality or the occurrence of ventricular fibrillation (VF), ventricular tachycardia (VT) or ventricular ectopic beats. However, administration of a combination of the drugs significantly improved survival at 16 hours.

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THE EFFECTS OF BLOOD GASES AND NIFEDIPINE ON PRESSOR RESPONSES TO ANGIOTENSIN II IN THE PITHED RAT

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Prolonged intravenous infusion of "sub-pressor" doses of angiotensin II (AII) in the conscious rat produces a "slow pressor response" (Brown et al,1981). As a first step towards developing an acute model of this effect we have examined some properties of pressor responses to AII employing manouvres to which we have previously subjected α-adrenoceptor-mediated responses. Bolus injections of most α_1-agonists, e.g. phenylephrine, produce an immediate pressor response which is short-lived, nifedipine-insensitive and increases with increasing O_2. This component contrasts with other, more prolonged, responses which are nifedipine-sensitive, relatively unaffected by O_2 but increase in acidosis (McGrath et al,1982; Flavahan & McGrath,1982; Grant et al, this meeting). Since bolus injections of AII produce rapid, transient pressor responses, we have examined the properties of responses to boluses and infusions of AII with respect to blood gases and nifedipine-sensitivity.

Male wistar rats (245-265g) were pithed by the method of Gillespie et al (1970). All rats were ventilated at 60 st/min and responses measured as Δ DBP (carotid artery). For studies on blood gases, rats were ventilated either with 100% O_2, varying the respiratory stroke volume, or varying the inspired gas mixture at 3.5ml/st. For subsequent studies with nifedipine, rats were ventilated at 2.5 ml/st with 40% O_2 to produce approx. physiological blood gases (pH=7.37 $\pm$ 0.004, $PaCO_2$ =37.9 $\pm$ 0.8, PaO_2 =125.0 $\pm$ 3.7, n=63).

Pressor responses to AII (10-300ng/kg i.v., jugular vein) in rats ventilated with 100% O_2 were increased in respiratory alkalosis and depressed in respiratory acidosis. In rats ventilated at 3.5 ml/st with 30% O_2, varying the $PaCO_2$ had no effect on responses to AII. However, when the $PaCO_2$ was kept at approx. physiological levels (35-40 mmHg) and the PaO_2 was varied, responses to AII increased with increasing PaO_2 ($R \geq 0.82$, $p < 0.001$, n=13). Furthermore, this trend remained, although the responses were depressed, when PaO_2 was varied under alkalotic or acidotic conditions. In rats respired to produce physiological blood gases, nifedipine (0.3mg/kg) reduced responses to bolus AII (100 & 300ng/kg) by 24 and 22% respectively ($p < 0.05$, n=4). In contrast, nifedipine (0.3mg/kg) reduced responses to an AII infusion (200 & 400ng/kg/min) by 71 and 68% respectively ($p < 0.001$, n=5-6).

Thus, responses to bolus injections of AII, like those to α_1-agonists, increase with O_2 and are nifedipine-resistant. In contrast to either α_1 or α_2 they are optimal at physiological $PaCO_2$ and are depressed in both acidosis and alkalosis. However, the responses to infusion of AII are nifedipine-sensitive. It is concluded that the analysis of responses to injected boluses may shed some light on basic mechanisms but that the physiological actions of AII, like those of catecholamines, and the basis of the "slow pressor" response may be more fruitfully explored through the response to infusions.

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CHARACTERIZATION OF THE HYPOTENSIVE MECHANISM OF KETANSERIN IN CONSCIOUS RENAL HYPERTENSIVE RABBITS

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Ketanserin is a 5-HT_2 receptor antagonist with α_1 adrenolytic activity (Leysen *et al.*, 1982). Although in man the anti-hypertensive properties of ketanserin have been attributed to blockade of 5-HT_2 receptors (Wenting *et al.*, 1982), in rats the blood pressure lowering effects appeared to be mainly due to α_1 adrenoceptor blockade (Fozard, 1982; Kalkman *et al.*, 1982). In order to characterize the hypotensive mechanism in conscious rabbits with cellophane perinephritic hypertension (Bolt & Saxena, 1984), we compared the cardiovascular profile of ketanserin with that of the α_1 adrenoceptor antagonist prazosin.

Heart rate (HR) and mean blood pressure (MBP) were recorded continuously after the *i.v.* administration of ketanserin (0.1, 0.3 and 1.0 mg/kg, n=8) and prazosin (0.01, 0.03 and 0.10 mg/kg, n=8). Using the radioactive microsphere technique (Bolt & Saxena, 1984), changes in cardiac output (CO) and regional haemodynamic variables were determined 10 minutes after each ketanserin dose and 15 minutes after each prazosin dose. The inhibitory effects of both compounds on the pressor response induced by phenylephrine (PE) (10 µg/kg, *i.v.*) were determined in two groups of 6 conscious animals.

At the doses used both compounds induced a comparable degree of α_1 adrenoceptor blockade. Ketanserin reduced the PE-pressor response by -29 ± 9, -49 ± 9 and -58 ± 8%, respectively, and prazosin by -24 ± 8, -36 ± 6 and -48 ± 6%, respectively. Fifteen minutes after the successive prazosin administrations a decrease in MBP was measured of -5 ± 2, -13 ± 2 and -25 ± 2%, respectively. Although pronounced tachycardia accompanied the blood pressure lowering effects of prazosin during the first few minutes, by 15 minutes only moderate changes in HR were measured (+10 ± 3, +12 ± 3 and +9 ± 4%, respectively). Due to a reduction in stroke volume, CO was lowered by -12 ± 3% at the highest dose used. After each ketanserin dose a marked transient decrease in MBP was observed, which stabilized at 1 ± 2, 4 ± 2 and 12 ± 2% below baseline levels, respectively, by the end of 10 minutes. Reflex tachycardia was noticed only during the first two minutes after each ketanserin administration. In contrast to prazosin, ketanserin increased the cardiac output by 21 ± 6, 22 ± 9 and 9 ± 3%, respectively. After prazosin administration a dose-dependent decrease in vascular resistance was observed in the kidneys, gastro-intestinal tract and bones. Apart from these vascular beds, ketanserin also caused significant vasodilatation in the heart and brain.

Considering the α_1 adrenolytic action of ketanserin at hypotensive doses and the similarities in the cardiovascular profile of ketanserin and prazosin, we conclude that blockade of α_1 adrenoceptors plays a predominant role in the hypotensive mechanism of ketanserin in conscious hypertensive rabbits. A contribution of 5-HT_2 receptor blockade can neither be excluded nor confirmed.

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CAPACITY LIMITED METABOLISM OF ACETYLSALICYCLIC ACID IN THE RAT

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Over the years, the metabolism and pharmacokinetics of aspirin has received a great deal of attention both in man (Hucker et al, 1980) and in various animal species (Davis & Westfall, 1972; Haberland et al, 1957). Capacity limited metabolism of aspirin in man is well established specifically in relation to the formation of the glycine conjugate salicyluric acid (SUA) (Levy, 1979). Salicylic acid (SA) however forms a number of other products the metabolism of which has not been fully elucidated. Consequently we have investigated the metabolism of aspirin in the rat over a ten fold dose range.

Wistar albino rats weighing between 240 and 260 grams received ^{14}C-labelled aspirin (carboxyl-14 C) by mouth in doses of 10, 20, 50, and 100mg/kg (2 males and 2 females per dose level). The animals were housed individually in metabolism cages and all urine passed in the subsequent 72 hours was collected in 24 hour aliquots. Metabolites of aspirin were assayed by radio-TLC. Aliquots of the 0 - 24h urine were treated with β-glucuronidase (pH 5, 37°C for 24h) - for phenolic glucuronides; 2M NaOH (37°C for 24h) - for acyl glucuronides; or with 6M HCl (100°C for 24h) - for total conjugates and reassayed with the following results:-

		10mg/kg	20mg/kg	50mg/kg	100mg/kg aspirin
Salicylic acid (SA)	mean	51.47	49.06	48.88	43.30
	s.d.	1.61	17.01	4.63	5.72
Phenolic SA glucuronide	mean	6.21	13.29	13.65	19.30
	s.d.	3.28	12.05	2.24	4.03
Acyl SA glucuronide	mean	11.20	16.75	18.65	24.47
	s.d.	5.04	1.48	1.04	5.28
Salicyluric acid (SUA)	mean	18.56	13.38	7.75	4.58
	s.d.	0.39	3.38	1.34	0.62
SUA glucuronide	mean	3.70	2.43	4.02	1.25
	s.d.	3.21	3.04	1.88	2.50
Gentisic acid (GA)	mean	0.56	0.95	2.27	6.43
	s.d.	0.18	0.83	0.94	1.84
Glycine GA conjugate	mean	0.70	0.46	3.77	4.45
	s.d.	0.63	0.69	5.65	3.03

Figures represent % total dose excreted in 0 - 24h urine

Both SUA and its conjugate decreased with increasing dose compatible with capacity limitation. There was a compensatory increase in acyl and phenolic glucuronides of SA and of the Phase I metabolite gentisic acid. Furthermore, excretion of another glycine conjugate, that of gentisic acid (gentisuric acid) also increased with dose. Saturation of aspirin metabolism in the rat appears to be confined to formation of SUA.

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EFFECT OF PREGNANT MARE SERUM GONADOTROPHIN ON MAMMARY TUMOURS INDUCED BY 7,12-DIMETHYLBENZ (a)ANTHRACENE (DMBA) IN THE RAT

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The regression of DMBA induced rat mammary tumours produced by the superactive gonadoliberin analogue ICI 118630 may be the result of reduced ovarian response to the high circulating levels of LH (Furr & Nicholson 1982). Pregnant mare serum gonadotrophin (PMSG) elicits both LH and FSH type responses in the rat (Moore et al 1980). This duality of activity and a long circulating half life in rodents (Sasamoto et al 1972) provides an agent which allowed us to mimic the continuously high gonadotrophin levels produced by ICI 118630 and examine its effect on the growth of DMBA-induced, oestrogen dependent, rat mammary tumours and of the host animal's hormonal milieu.

Nine Sprague Dawley rats bearing DMBA induced tumours (mean diameter >0.9cm) were injected s.c. twice daily for 14 days, four receiving 200IU of PMSG in distilled water and five distilled water alone. Tumour volumes were determined at 3 or 4 day intervals. Ten tumours on 8 rats were available for comparison after any cystic tumour had been eliminated. Excluding one tumour which continued to grow very rapidly the mean percentage increase in the volumes of tumours on PMSG treated rats following 2 weeks treatment was approximately half that of control animals (see table)

Treatment	% increase in tumour vol in 14 days	ovarian weight (mg)	uterine wet weight (mg)	plasma oestradiol (pg/ml)	plasma prolactin (ng/ml)
Control	223±40.6	135±4	364±35	55±6.6	160±85.6
PMSG	108±26.3	708±116*	471±18*	281±60.9*	660±160*

*P <0.05 mean ± sem.

Vaginal smears taken after one week's treatment with PMSG indicated continuous oestrogen stimulation. At sacrifice, uterine wet weights, ovarian weights and plasma oestradiol and prolactin levels were significantly higher than those of control, the oestradiol level being even higher than that reported (Butcher et al 1974) to occur at the oestradiol peak of proestrus. (see table) These findings contrast markedly with those of Furr and Nicholson (1982) who reported ovarian suppression with significantly reduced circulating levels of oestradiol and prolactin following the administration of ICI 118630. The down-regulation of LH receptors suggested by the above authors to account for the low oestradiol levels has clearly not occurred with PMSG treatment. Tumour regression is known to occur following the administration of pharmacological doses of oestrogen (Meites et al 1971) which also results in high prolactin levels. The reduced tumour growth following PMSG administration may thus be the consequence of the high levels of circulating oestradiol and prolactin stimulated but the possibility that PMSG modifies the response of oestrogen target tissues cannot be excluded.

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THE EFFECT OF INDOMETHACIN ON URETERAL MOTILITY

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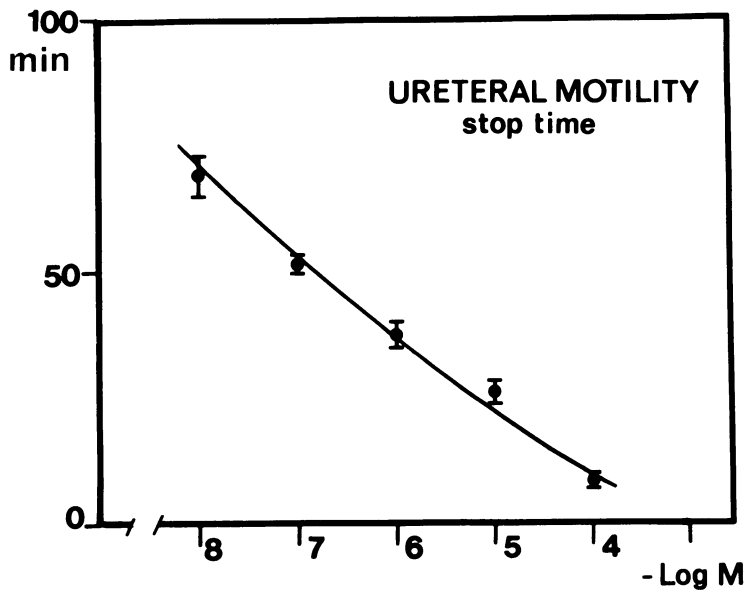
The effectiveness of parenteral indomethacin in renal colic has been documented (Holmlund and Sjodin, 1978). The mechanism is thought to be by inhibition of renal prostaglandin synthesis, reducing diuresis and consequently intra-ureteral pressure (Allen, et al, 1978). This study was set out to investigate a possible spasmolytic action of indomethacin on the ureter.

Experimental: Ureteral ring specimens (length = 4 mm) were obtained from 90 sheep and 11 patients (taken during surgery) and isometric tension was recorded in an organ bath. Spontaneous rhythmic contractions appeared within 10-30 min and reached a maximum after 100 min. The sodium salt of indomethacin in phosphate buffer (Dumex Ltd.) was then added to the bath.

Control activity in both human and sheep preparations was either continuous or intermittent; the tension ranged between 0.3 - 3.0 g and the average frequency of contraction was 13/min in sheep and 2/min in human subjects. Indomethacin dose dependently reduced both the frequency and amplitude of contraction. The threshold concentration for a reduction in frequency was 10^{-8}M and the EC_{50} was 10^{-6}M. Indomethacin ultimately caused a cessation of ureteral activity and the time taken for the contractions to stop was inversely related to indomethacin concentration as shown in the figure:



Hence the clinical efficacy of indomethacin in renal colic may be partly related to a direct spasmolytic action on the ureter.

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THE EFFECT OF LEUKOTRIENE D_4 ON TENSION RESPONSES AND CYCLIC NUCLEOTIDE LEVELS IN GUINEA-PIG LUNG STRIPS

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Contraction of guinea-pig tracheal smooth muscle in response to leukotriene D_4 (LTD_4) occurs subsequent to a rapid decrease in the intracellular concentration of cyclic AMP (Andersson et al, 1984). Both these effects appear to be a direct consequence of the LT since inhibition of cyclooxygenase with indomethacin did not significantly modify these responses (Andersson et al, 1984). We were interested to examine the effects of LTD_4 on tension responses and cyclic nucleotide levels in guinea-pig parenchymal lung strips (GPLS) where LTD_4-induced contractions are thought, at least in part, to be mediated indirectly via the synthesis and subsequent release of thromboxane A_2 (TXA_2; Zijlstra et al, 1983).

Sub-pleural GPLS were suspended in Krebs-Henseleit solution at 32°C which was bubbled with 5% CO_2 in O_2. Responses to LTD_4 and the TXA_2 mimetic U-46619 (Bundy, 1975) were recorded by conventional methods. After a fixed contact time each lung strip was frozen in liquid N_2 and assayed for cyclic nucleotides using standard kits (Amersham International, Bucks). The results are expressed as mean ± s.e.m. of 5-9 determinations, the level of significance with respect to control values being set at $P<0.05$. Cyclic nucleotide levels are expressed as pmol/mg tissue protein.

LTD_4 (200nM) produced a biphasic contraction of GPLS; an initial fast (phasic) response (1.57 ± 0.19 mN) followed by a slow (tonic) sustained contracture (1.33 ± 0.08 mN). These responses were accompanied by time-dependent increases in the levels of both cyclic AMP and cyclic GMP which peaked after about 5 min thereafter decaying (cyclic AMP - from 3.51 ± 0.41 to 28 ± 3.63: cyclic GMP - from 1.84 ± 0.65 to 13.41 ± 1.04).

Flurbiprofen (8 μM for 60 min) relaxed GPLS (1.33 ± 0.17 mN) and abolished the phasic response of the LTD_4-induced contraction. The tonic response was unaffected by this concentration of flurbiprofen. Flurbiprofen abolished the increase in both cyclic nucleotides induced by LTD_4.

The potent TXA_2 synthetase inhibitor dazmegrel (10 μM for 60 min : Parry et al, 1982), like flurbiprofen, relaxed GPLS (1.38 ± 0.21 mN) but did not abolish the increase in either cyclic nucleotide. In fact the accumulation of cyclic AMP induced by LTD_4 was significantly augmented by pretreatment with dazmegrel (from 4.01 ± 0.35 to 83.7 ± 6.63). Dazmegrel, however, did not potentiate the increase in cyclic GMP induced by LTD_4.

U-46619 (10 μM for 60 min) produced a slow sustained contracture of GPLS (3.91 ± 0.61 mN) which was accompanied by a time-dependent decrease in the levels of both cyclic nucleotides (cyclic AMP - from 3.61 ± 1.13 to 1.75 ± 0.07; cyclic GMP - from 1.62 ± 0.42 to 0.73 ± 0.05).

These data support the hypothesis that LTD_4 contracts GPLS by both direct and indirect mechanisms. The indirect (phasic) component, however, appears not to be mediated by TXA_2. Additionally, the increase in cyclic nucleotide content appears to be unrelated to a direct action of LTD_4 or to indirect effect of released TXA_2.

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SK&F 93944, A POTENT H_1-RECEPTOR HISTAMINE ANTAGONIST WITH NEGLIGIBLE ABILITY TO PENETRATE INTO THE CENTRAL NERVOUS SYSTEM

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SK&F 93944, 2-[4-(5-bromo-3-methylpyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl) methyl]-4-pyrimidone, is a potent, selective and competitive H_1-receptor histamine antagonist with negligible ability to penetrate into the central nervous system and which has insignificant anti-muscarinic activity.

SK&F 93944 is chemically related to the combined H_1- and H_2-receptor histamine antagonist, SK&F 93319 (Blakemore et al., 1983), differing only in the substituents of one of the pyridine rings (SK&F 93944 has 5-bromo-3-methylpyridyl instead of 3-methoxypyridyl present in SK&F 93319). SK&F 93944 differs chemically from most described H_1-receptor antagonists as it is not a tertiary amine and is mainly non-cationic at physiological pH.

SK&F 93944 caused concentration dependent antagonism of histamine-induced contractions of guinea-pig ileum over the concentration range, $2.2 \times 10^{-10} - 2 \times 10^{-9}$M, $pA_2 = 9.55$ (9.38 - 9.82, 95% confidence limits) and the regression of the Schild plot, 0.89 (0.63 - 1.15) was not significantly different to unity. A dose-ratio of 2 was obtained at a concentration of 2.8×10^{-10}M. SK&F 93944 is a weak, non-competitive antagonist at H_2-receptors on the guinea-pig atrium (dose ratio of 2 at 1.26×10^{-6}M) and a very weak, non-competitive antagonist of carbachol on guinea-pig ileum (dose-ratio of 2 requiring a concentration of 1.07×10^{-4}M).

SK&F 93944 caused dose-dependent antagonism of histamine-induced bronchoconstriction in guinea-pigs over the dose-range $1.25 \times 10^{-9} - 7.5 \times 10^{-8}$ mol/kg i.v., having a similar potency to that of mepyramine. The highest dose, 7.5×10^{-8} mol/kg, which shifted the histamine dose-response curve to the right with a dose-ratio in excess of 50 had no effect on carbachol-induced bronchoconstriction. SK&F 93944 is also an effective H_1-receptor antagonist in anaesthetised cats (inhibition of depressor responses to 2-(2-aminoethyl)pyridine) and conscious rats (inhibition of histamine-induced increases in cutaneous microvascular permeability).

In anaesthetised male rats, penetration into the central nervous system was measured during intravenous administration of ^{14}C-SK&F 93944 or ^{3}H-mepyramine. Counter-labelled inulin was used as a blood marker. At steady-state blood concentrations of about 3×10^{-9} moles/ml, the whole brain concentration of mepyramine was approximately 7×10^{-9} moles/g wet weight whereas that for SK&F 93944 was less that 1.5×10^{-11} moles/g wet weight, which is the limit of detection.

SK&F 93944 is thus a potent H_1-receptor histamine antagonist with insignificant antimuscarinic activity and which has negligible ability to penetrate into the central nervous system. Clinical pharmacology with SK&F 93944 has started in healthy human volunteers and preliminary results are presented at this meeting (Boyce, 1984).

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EFFECT OF PEPTIDOLEUKOTRIENES ON THE CUTANEOUS MICROVASCULATURE OF CONSCIOUS GUINEA-PIGS

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Intradermal injection of peptidoleukotrienes into the guinea pig flank causes a modest increase in microvascular permeability and a decrease in cutaneous blood flow (Ueno et al., 1981; Peck et al., 1981). However, in human volunteers, leukotrienes C_4, D_4 and E_4 (LTC_4, LTD_4, LTE_4) produce persistent cutaneous erythema and a distinct wheal with central pallor (Soter et al., 1983). In order to further investigate the difference in cutaneous responsiveness between guinea pigs and man, the microvascular effects of peptidoleukotrienes on the ear of the conscious guinea pig were examined. The guinea pig ear was chosen since it provides a highly vascular skin area which, like human skin, displays the "Lewis triple response" to intradermal histamine injection (Woodward and Owen, 1983). ^{125}I-bovine serum albumin (10 µCi/ml x 0.2 ml), ^{51}Cr-erythrocytes (50 µCi/ml x 0.5 ml) and Evans blue (2.5% soln. x 0.2 ml) were injected i.v. to permit both quantitative measurement of microvascular permeability and visualisation of albumin leakage. Drying the skin samples to constant weight and a 1 ml reference blood sample allowed extravascular albumin content to be calculated as ml/g dry weight of tissue. Ear surface temperature provided an indirect indication of cutaneous blood flow changes.

The microvascular effects of peptidoleukotrienes were evaluated over the dose range 0.1 ng - 1000 ng. Comparison of the natural 5S, 6R-LTD_4 and 5R, 6S-LTD_4 stereoisomers demonstrated the stereoselective nature of the vasopermeability response: ED_{50} values of 6.1 (2.3 - 9.9) and 700 (410 - 990) respectively were obtained. The increase in cutaneous microvascular permeability produced by 5S, 6R-LTD_4 was significantly ($P<0.05$) inhibited by FPL 55712 (5 mg/kg) but was unaltered by indomethacin (16 mg/kg), thus indicating the absence of an indirect component mediated by cyclo-oxygenase metabolites of arachidonic acid. LTD_4 appeared to be approximately 10 times more potent than LTC_4, ED_{50} 64 (36 - 92), as a permeability factor but LTE_4, ED_{50} 790 (210 - 1370), seemed no more active than 5R, 6S-LTD_4. A central blanching of the blue wheal area rarely occurred and was always transient.

In the conscious guinea pig, all peptidoleukotrienes caused a very modest increase in ear temperature, suggesting a small increase in cutaneous blood flow. It appears that the effect of peptidoleukotrienes on skin blood flow differ in guinea pigs and man, although the vasopermeability response is similar.

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PHARMACOLOGICAL MODULATION OF ALLERGEN INDUCED LUNG DYSFUNCTION USING A MODIFIED 'PRESSURE BOX' PLETHYSMOGRAPHIC TECHNIQUE

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We have previously reported on histamine independent lung dysfunction reaction following aerosolised ovalbumen in conscious, sensitised guinea-pigs (Clay and Thompson 1983). Such lung function abnormality was characterised by an elevation of thoracic gas volume (TGV) and reduction of specific airway conductance (S.Gaw), both of which could be exacerbated by indomethacin. We now report on a general pharmacological evaluation of antigen induced lung dysfunction using this technique.

Dose-response curves were determined for lung dysfunction following aerosolised ovalbumen provocation in groups of sensitised guinea pigs. Thirty animals were pre-treated, 30 minutes prior to provocation, with 1ml/kg i.p. saline and provoked for 5 minutes with serial antigen concentrations (0.01 - 0.32% w/v, n=5 for each concentration). Lung function was monitored for a further 10 minutes after completion of challenge. Elevation of TGV or depression of S.Gaw was assessed by area under curve analysis for both variables. A further twenty-four animals were pre-treated with 10mg/kg i.p. mepyramine and provoked with serial antigen concentrations (0.25 - 8% w/v; n=4 for each concentration). No clear dose-response relationship to antigen was observed in the former group, in terms of TGV elevation or S.Gaw reduction, although death resulting from antigen provocation was dose-related. In animals pre-treated with mepyramine, however, a clear dose-response relationship emerged without concomitant mortality. Mepyramine per se, failed to alter basal S.Gaw values (0.051 ± 0.001 (cm H_2O sec)$^{-1}$ after saline, 0.054 ± 0.002 (cmH_2O sec)$^{-1}$ after mepyramine). Reproducibility of a supra-maximal antigen exposure (4% w/v) in batches of mepyraminised guinea pigs was investigated over the course of eight months at frequent intervals. Eighteen groups of animals (n=7-12 per group) were investigated for lung function alterations after provocation. Overall, TGV increased by $60 \pm 7.5\%$ ($p<0.001$) from a basal value of 18.6 ± 0.8 ml and S.Gaw decreased by $30 \pm 2.5\%$ ($p<0.001$) from a basal value of 0.06 ± 0.0031 (cm H_2O sec)$^{-1}$. In each study, significant lung dysfunction was recorded with a range between studies of 16 - 46.5% decreases in S.Gaw.

Oral activity studies were performed with theophylline and salbutamol. ED_{50} evaluation of salbutamol yeilded a value of 3mg/kg after a 30 minutes pre-treatment, and 0.6mg/kg for a 120 minute pre-treatment. 100mg/kg theophylline produced 37% inhibition of allergic lung dysfunction. Control groups of animals were pre-treated with 1ml/kg acacia.

In conclusion, 'pressure box' plethysmography allows observation of histamine independent lung dysfunction in response to allergen challenge, which is dose-related, and reproducible in terms of gross reaction. Furthermore, drug induced modulation of antigenic changes confirms the dependence of dysfunction on an underlying bronchospastic reaction. One important observation is the reduced ED_{50} value found for salbutamol after two hours pre-treatment as compared to 30 minutes, which is consistent with pharmacodynamic/kinetic clinical observations (Walker et al 1972).

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