ANTINOCICEPTIVE EFFECTS OF A NEW CENTRALLY-ACTING MUSCLE RELAXANT DS 103-282 ON CAT DORSAL HORN NEURONES

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Pharmacological data on a new centrally acting muscle relaxant 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiodazole (DS 103-282) indicates that it may have some analgesic activity (Sayers, Burki & Eichenberger, 1980). We have, therefore, examined the effects of iontophoretic and intravenous administration of DS 103-282 on excitation of lamina IV and V spinal neurones induced by noxious (radiant heat > 45°C) and non noxious (movement of interdigitol hairs by an electronically controlled air-jet) stimulation applied to the ipsilateral foot in the α-chloralose anaesthetized cat.

In 30/32 tests on 19 dorsal horn neurones DS 103-282 (19 $\pm$ 2.2 nA, mean $\pm$ S.E.M., from 100 mM solutions pH 5.5) reduced responses to noxious radiant heat by 66 $\pm$ 5%. Recovery of these responses occurred 9-30 min (27 $\pm$ 7 min) after terminating the DS 103-282 ejection. Spike configuration was not affected by DS 103-282 although spontaneous firing, when present, was frequently markedly reduced.

In contrast to the effects of DS 103-282 on excitation induced by noxious heat, excitation induced by non-noxious stimuli was either unaffected (8 neurones) or reduced less than responses to noxious heat (4 neurones) in tests on the same cells.

The muscle relaxant, (-) baclofen, when compared with DS 103-282 on 10 neurones also reduced responses to noxious heat (by 46 $\pm$ 7%) with ejection currents of 20 $\pm$ 3.5 nA (from 0.005M solutions in 0.165 mM NaCl) but this effect was more rapidly reversible (4 $\pm$ 1 min, recovery time) and was non selective since excitation induced by hair movement in 5 of these neurones was also reduced (by 63 $\pm$ 9%).

When given intravenously, DS 103-282 (0.05 - 0.1 mg/kg) produced a marked (>70%) and prolonged (> 25 min) reduction in the excitation induced by noxious heat (3 neurones) whereas responses to hair movements evoked in two of these neurones was not depressed. On two further neurones responses to noxious heat were enhanced by intravenous DS 103-282.

These results indicate that DS 103-282 has a preferential antinociceptive effect on spinal neurones. It is of particular interest that this effect of DS 103-282 is observed with similar doses to those previously reported to selectively depress the activity of spinal excitatory interneurones (iontophoretic and intravenous doses) and spinal polysynaptic reflexes (intravenous doses) (Davies, 1982). Thus, it is possible that analgesic effects of DS 103-282 may be evident at therapeutic doses.

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INHIBITION BY DS 103-282 OF D-(^{3}H)ASPARTATE RELEASE FROM SPINAL CORD SLICES

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A new muscle relaxant, 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiodazole (DS 103-282) has recently been shown to preferentially depress both, polysynaptic rather than monosynaptic and nociceptive compared to non-nociceptive excitation of dorsal horn spinal neurones (Davies, 1982; Davies and Johnston 1983). These differential effects suggest that DS 103-282 might possibly be acting presynaptically to inhibit the release of an excitatory transmitter. Excitatory interneurones may utilise aspartate as a transmitter (Davidoff, Graham, Shank, Werman and Aprison, 1967), and thus the present experiments have examined the effects of DS 103-282 on the release of D-[^{3}H]aspartate in vitro.

Transverse rat spinal cord slices (0.25 mm thickness) were rapidly prepared from freshly dissected tissue using a mechanical chopper and incubated for 30 min in an oxygenated Krebs-Ringer bicarbonate solution containing D-[^{3}H]aspartate at a concentration of 5×10^{-8} M. Slices were placed in small chambers and superfused for 20 min with either drug containing or drug free Krebs solution. The spontaneous and evoked release (45 mM K^+ or veratridine 67 µg/ml) from treated and non treated slices was thus compared in parallel experiments.

The K^+-evoked release of D-[^{3}H]aspartate was reduced by 68.5 $\pm$ 6% ($n = 8$, $p<0.05$) in the absence of calcium.

DS 103-282, (50-500 µM) did not effect the uptake of D-[^{3}H]aspartate into the slices or the small amount of calcium independent release.

However, the K^+- and veratridine-evoked release of radioactivity were consistentely reduced in the presence of DS 103-282. Low concentrations of the drug (50 µM) reduced only K^+-evoked release. Higher concentrations inhibited the release evoked by both agents, e.g. DS 103-282 (500 µM) reduced K^+-evoked release by 35.6 $\pm$ 7% ($n = 8$, $p<0.05$) and veratridine-evoked release by 60.2 $\pm$ 9% ($n = 4$, $p<0.001$).

Glycine, serotonin, morphine and baclofen at concentrations up to 500 µM were without effect on the release of D-[^{3}H]aspartate.

These results suggest that the inhibitory effects observed in vivo following DS 103-282 administration might be due, in part, to the depression of release of transmitter from excitatory nerve terminals.

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ELECTROPHYSIOLOGICAL ANALYSIS OF α_2 ADRENOCEPTOR ACTIVATION IN THE LOCUS COERULEUS

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In the nucleus locus coeruleus (LC) recurrent, inhibitory collaterals are thought to release noradrenaline (NA) on to α_2 adrenoceptors located on LC neurones themselves (Aghajanian et al, 1977; Cedarbaum & Aghajanian, 1977). Intracellular recordings in vivo have indicated that clonidine administered i.p. hyperpolarises the membrane of LC neurones. We have sought to characterise the response of LC neurones to adrenoceptor activation.

Intracellular recordings were made from rat LC neurones maintained in an in vitro slice preparation at 37°C superfused with a Krebs solution containing 2.5 mM potassium as described by Henderson et al (1982). Drugs were applied either by changing the superfusing medium to one which contained a known concentration or by pressure application from a micropipette located in the fluid just above the surface of the slice.

NA (1-30μM) and clonidine (3-100 nM) applied in the bathing medium produced dose related hyperpolarisations of LC neurones which were associated with an increase in membrane conductance. In neurones which fired action potentials spontaneously the hyperpolarisation decreased or abolished spontaneous firing. The hyperpolarisation was a direct effect on the impaled neurone since it persisted in a zero calcium, 20 mM magnesium solution which abolished evoked synaptic potentials.

When the membrane potential was made more negative by injection of current the response to pressure application of NA decreased and reversed at -104 ± 1 mV (mean ± s.e of mean, n=7) which approximates to the potassium equilibrium potential in these neurones. The reversal potentials calculated from the change in membrane conductance and change in membrane time constant were -121 ± 8 mV (n=4) and -123 ± 8 mV (n=4) respectively for NA and -140 ± 7 mV (n=7) and -125 ± 7 mV (n=4) respectively for clonidine. These more negative values for reversal potential indicate that in part the activation of α_2 adrenoceptors occurs at a site distant from the cell soma (Carlen & Durand, 1981).

The response to pressure application of NA was increased in amplitude and duration by desmethylimipramine (0.1 - 10 μM). Piperoxane (0.1 - 3 μM), yohimbine (0.01 - 0.1 μM) or phentolamine (0.1 - 1 μM) attenuated or abolished the response to NA whereas prazosin (1 μM) and propranolol (1 μM) did not. Perfusion of the slice with phenylephrine or isoprenaline did not alter resting membrane potential or membrane conductance.

These results demonstrate that the soma and dendritic regions of LC neurones posses α_2 adrenoceptors; the activation of these receptors appears to result in an increased potassium conductance.

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NEUROTENSIN POTENTIATES THE POTASSIUM-STIMULATED RELEASE OF (^{3}H)-DOPAMINE FROM RAT STRIATAL SLICES IN VITRO

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There is considerable pharmacological evidence suggesting a neurotensin-dopamine interaction in the mammalian central nervous system. The presence of neurotensin (NT) binding sites on dopamine (DA) neurones (Palacios and Kuhar, 1981), excitatory effects of NT on DA-ergic systems demonstrated in electrophysiological experiments (Andrade and Aghajanian, 1981) and behavioural tests (Kalivas et al, 1981), and changes in NT content of brain regions following neuroleptic treatment (Govoni et al, 1980) prompted the release experiments described here.

An in vitro model system was used to study pharmacological effects of NT, NT fragments and analogues on striatal (^{3}H)-DA release. Briefly, rat striatal tissue was chopped into slices 0.35 x 0.35 x 2 mm, suspended in oxygenated Krebs' bicarbonate medium at 37^{o}C and then incubated in 0.1 µM (^{3}H)-DA for 15 min to allow uptake of the (^{3}H)neurotransmitter. The slices were placed on gauze in release chambers and superfused at a rate of 0.4 ml/min with medium containing 1 µM nomifensine, 10 µM pargyline, 0.1% bovine serum albumin and 0.28 µg/ml bacitracin. 2 ml fractions were collected when the rate of tritium overflow had stabilised. Depolarisation of the slices was achieved with 2 consecutive pulses of 23.8 mM KCl (isomolar replacement of K^+ for Na^+) each of 2 minutes. The first pulse served as an internal control on tissue activity when no peptide was applied, but the second was used to investigate drug effects where the peptide was applied for 2 min with the high K^+ medium. NT effects on spontaneous release were tested in place of the second K^+ pulse.

Spontaneous tritium overflow was reduced, and K^+-evoked release abolished in high magnesium, calcium-free media. NT evoked tritium overflow in a calcium-dependent manner in doses of 0.1-10 µM, and dramatically facilitated K^+-evoked overflow. The dose response curve for the latter effect had a threshold at 0.1 nM and saturated in the micromolar range of concentrations. 1 µM NT caused an increase in (^{3}H)-DA overflow of 180% compared to controls. 1 µM NT also potentiated K^+-evoked endogenous DA release from striatal slices in a similar in vitro system, with an increase in the release above controls of 152%. Endogenous DA release was measured by HPLC with electrochemical detection.

The structure-activity profile demonstrated the involvement of a NT receptor in the facilitation of (^{3}H)-DA release. In accord with receptor binding and bioassay data (Kitabgi et al, 1980) the amino-terminal hexapeptide NT_{1-6} and the carboxy-terminal tetrapeptide NT_{10-13}, had no activity. Other peptides, substance P, somatostatin and thyrotropin-releasing hormone, tested at 1 µM did not potentiate K^+-evoked (^{3}H)-DA release. These results demonstrate a unique receptor-mediated facilitation of DA release by NT.

M.E. de Quidt is an MRC Scholar

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DIFFERENTIAL LOCALISATION OF (^{3}H)-SPIPERONE AND (^{3}H)-N,n -PROPYLNOR APOMORPHINE BINDING SITES IN RAT STRIATUM AND SUBSTANTIA NIGRA

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Dopamine receptors labelled by the antagonist ligand ^{3}H-spiperone are present on striatal (ST) cell bodies and the terminals of cortico-striate glutamate fibres (Schwarcz et al,1978). In substantia nigra (SN), specific ^{3}H-spiperone binding sites are found on dopamine (DA) cell bodies (Reisine et al,1979). However, there is controversy as to whether DA agonist ligands such as ^{3}H-N,n-propylnorapomorphine (NPA), label identical binding sites. Whether agonist sites in ST are present on cell bodies or are located on the terminals of nigro-striatal dopamine fibres is unclear (Nagy et al,1978; Creese & Snyder,1979). We have examined the anatomical localisation of specific ^{3}H-spiperone and ^{3}H-NPA binding sites in both ST and SN following lesions of afferent and efferent pathways in the rat.

Unilateral kainic acid (KA) lesions of ST 21 days previously decreased the number (Bmax) of ^{3}H-spiperone binding sites in ST (Table 1). Unilateral destruction of the medial forebrain bundle (MFB) using 6-hydroxydopamine (6-OHDA) or KA lesions of SN 21 days previously increased Bmax for ^{3}H-spiperone ST binding. Unilateral removal of frontal and parietal cortex 5 days previously reduced Bmax for ^{3}H-spiperone. Lesions had no effect on the dissociation constant (K_D) for ^{3}H-spiperone binding in ST. These results confirm the localisation of ^{3}H-spiperone binding sites to ST cells and terminals of cortico-striate fibres. In contrast, unilateral KA lesions of ST or SN or 6-OHDA lesions of MFB all reduced Bmax for ^{3}H-NPA binding in ST (Table 1). Unilateral decortication did not alter Bmax for ^{3}H-NPA binding. K_D was decreased by 6-OHDA lesions and KA lesions of SN but not by KA lesions of ST. This suggests ^{3}H-NPA binding sites are located both on ST cell bodies and on presynaptic DA terminals in ST.

Table 1 Bmax (pmoles/g tissue) and K_D (nM) values following lesions

	^{3}H-spiperone (0.1-4.0 nM)[1,3]				^{3}H-NPA (0.05-2.0 nM)[2,3]			
	Control		Lesion		Control		Lesion	
Lesion	Bmax	K_D	Bmax	K_D	Bmax	K_D	Bmax	K_D
KA striatum	19±1	0.12±0.01	11±1*	0.13±0.03	16±2	0.9±0.1	12±1*	0.9±0.1
KA SN	19±2	0.15±0.02	25±2*	0.19±0.02	17±2	0.9±0.1	10±2*	0.6±0.1*
6-OHDA MFB	20±2	0.19±0.03	27±1*	0.20±0.03	19±3	1.1±0.1	11±1*	0.8±0.1*
Decortication	21±2	0.13±0.02	16±1*	0.16±0.03	18±2	1.1±0.1	15±2	1.1±0.2

* $p < 0.05$ compared to the intact forebrain
1 defined using 10^{-5}M (-)-sulpiride; 2 defined using 10^{-6}M (±)-ADTN; 3 carried out according to Hall et al (1981)

Unilateral KA lesions of ST or 6-OHDA lesions of MFB or decortication increased, while KA lesions of SN decreased, ^{3}H-spiperone (4.0 nM) binding to SN. In contrast ^{3}H-NPA (2.0 nM) binding to SN was decreased by KA lesions of ST or SN and by 6-OHDA lesions of the MFB. Unilateral decortication did not alter SN ^{3}H-NPA binding.

These data suggest that binding sites labelled by ^{3}H-spiperone and ^{3}H-NPA may be distinct entities with different anatomical locations with the nigro-striatal complex.

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$GABA_B$ BINDING SITES ON RAT STRIATAL SYNAPTIC MEMBRANES

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Recently, a class of γ-aminobutyric acid receptors ($GABA_B$ sites) which is relatively insensitive to traditional GABA agonists such as muscimol, and the antagonist bicuculline, has been described (Hill & Bowery, 1981; Bowery et al., 1982). Interaction of GABA with the $GABA_B$ site is potently and stereospecifically inhibited by baclofen (β-p-chlorophenyl GABA), whereas the activity of this drug at the $GABA_A$ receptor is minimal. We have measured the binding of ^{3}H-GABA to rat striatal synaptic membranes, and have investigated the effects of (a) prior lesioning of a major afferent neuronal input by decortication and (b) the destruction of intrinsic striatal neurones with kainate.

Crude synaptic membranes were prepared from striata of 200 g female Wistar rats (Zukin et al., 1974). The membranes were frozen overnight at -20 °C prior to their use in binding assays. $GABA_B$ binding was determined by incubation of extensively washed membranes for 10 min at 20 °C with ^{3}H-GABA (usually 10 nM) in 50 mM Tris-HCl buffer (pH 7.4) containing 2.5 mM $CaCl_2$ and 50 μM isoguvacine. Membranes were recovered by centrifugation for 1 min in a Beckman microfuge. Specific binding was defined as that displaced by 100 μM baclofen (or GABA). No significant differences were seen between these two compounds, and IC_{50} values for (±)-baclofen and GABA were 0.50 and 0.18 respectively.

The characteristics of binding were essentially similar to those described for $GABA_B$ sites on whole brain membranes (Bowery et al., 1982). Binding was calcium-dependent; linear with tissue concentration (0.03-0.5 mg protein); it was optimal at 20 °C with total loss of binding at 50 °C, and in an experiment to investigate binding over the pH range 6.4-8.2, was maximal between 7.4 and 8.2. The time course of $GABA_B$ binding was extremely rapid, having attained equilibrium within 30 s and remaining constant over the next 15 min. Using 10 nM ^{3}H-GABA, membranes bound specifically 43.4 ± 5.4 fmol GABA/mg protein (n = 7). This was comparable to the binding observed following incubation with ^{3}H-baclofen (52.8 ± 10.3 fmol/mg protein) (n = 6). Binding was saturable, and over the ^{3}H-GABA concentration range investigated (25 nM-1 μM) Scatchard analysis yielded a linear plot, indicating a single population of $GABA_B$ sites of K_D 475 ± 43 nM, and Bmax 3.3 ± 0.2 pmol/mg protein (replicates of 6 independent determinations).

Preliminary experiments using rats with striatal lesions suggest a major localisation of $GABA_B$ sites on cortico-striatal terminals, since 9 days following decortication, there was a 33% reduction in striatal binding. In contrast, there was no apparent reduction in binding to membranes 5 days after intrastriatal injection of a high concentration of kainate (10 nmol). The demonstration of $GABA_B$ receptors on cortico-striatal terminals provides a rational basis for the possible value of baclofen therapy in Huntington's Disease (Shoulson, 1982).

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SOLUBILISATION OF HUMAN STRIATAL DOPAMINE (D_2) RECEPTORS

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Digitonin and lysolecithin are the two detergents which have been used to solubilise successfully mammalian striatal dopamine (D_2) receptors (Gorissen & Laduron, 1979; Lerner et al., 1981; Davis et al., 1981). We have now shown that sodium cholate at high ionic strength, previously shown to be effective in solubilising muscarinic receptors (Carson, 1982; Hulme et al., 1982), will also solubilise human brain dopamine receptors. This method is inexpensive and gives a high yield of a stable soluble preparation.

A 1:10 homogenate of human striatal mitochondrial (P_2) and microsomal (P_1) membrane in a Tris buffer (50 mM, pH 7.5) is prepared. EDTA (1 mM, sodium chloride (1 M) and sodium cholate (0.3%, final concentration) are then added sequentially with stirring at 0°C for 30 min. Soluble proteins were separated by centrifugation at 105,000 g for 30 min. Electron microscopy demonstrates the virtual absence of membrane fragments. The solubilised D_2 receptor was characterised by the inhibition of binding of ^{3}H-spiperone (25 Ci/mmole) by antagonists (16 hr, 4°C). The binding of 1 mM ^{3}H-spiperone was in equilibrium at 4 hrs. Specific binding was defined as that displaced by 10^{-5} M (+)-butaclamol. Bound ^{3}H-spiperone was separated from free ligand by gel filtration on a small (2 ml) column of Sephadex G50 (Hulme et al., 1982).

The soluble receptor is quite stable at 4°C in the presence of 3 nm ^{3}H-spiperone, less than 10% loss of binding being observed after 3 days. In the absence of dopaminergic ligands, the receptor is less stable ($t_{\frac{1}{2}} \sim 2$ days). The yield of receptor is reasonable (30-50%) and a high ratio of specific to non-specific binding is obtained (1.5:1 at 3 nM ^{3}H-spiperone).

When the apparent dissociation constants for ^{3}H-spiperone and other antagonists are compared with values obtained on membrane receptors (Table 1), a decrease in affinity of at least 5-fold for all ligands is observed. At least part of this discrepancy might be ascribed to the different assay conditions. Nevertheless, the rank order of potency was preserved. No evidence of a 5HT component of binding of the ^{3}H-spiperone to the soluble preparation was observed.

Table 1. K_i values (nM) for inhibition of ^{3}H-spiperone binding*

	Soluble	Membrane
Spiperone	1.5	0.3
(+)Butaclamol	80	4
(-)Butaclamol	$>10^5$	$>10^5$
Chlorpromazine	1800	51
Haloperidol	200	11
Pimozide	350	36
Ketanserin	$>10^5$	$>10^5$

* Values for unlabelled compounds were determined by the inhibition of binding of 1 nM ^{3}H-spiperone in membranes (Owen et al., 1978) and soluble preparations and corrected for occupancy by labelled ligand.

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EVALUATION OF SYNAPTOSOMAL DOPAMINE UPTAKE IN RAT BRAIN

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Catecholamine neurotransmitter action in the synaptic cleft is terminated mainly by re-uptake across the presynaptic membrane. This re-uptake system is energy-dependent and monoamine specific (Iversen, 1975). Using corpus striatum as starting material a synaptosomal preparation containing a large proportion of dopaminergic synaptosomes can be prepared. Although crude synaptosomal preparations (P_2) reduce preparation time, subcellular contamination from myelin and mitochondria militates against the use of this type of preparation for studies on noradrenaline (NA) and 5-hydroxytryptamine (5-HT) uptake (Wood and Wyllie, 1982). In the present study therefore, we compare the uptake of DA into crude (P_2) and purified (P_{2B}) synaptosomal preparations.

Synaptosomes and subcellular fractions were prepared by the method of Gray and Whittaker (1962), modified by the use of a vertical rotor as described in detail by Wood and Wyllie (1982). Synaptosomes and other fractions were resuspended in 0.32M sucrose ($3ml g^{-1}$ original tissue). Uptake of DA was determined in Tris-Krebs medium of the following composition (mM):- NaCl(136); KCl(5); $MgCl_2$(1.2); $CaCl_2$(2.5); glucose (10); ascorbate (1) and Tris-base (20), buffered to pH 7.4 with HCl. The medium was gassed with oxygen prior to use; the reaction was initiated by addition of (2,5,6-3H) dopamine 5-15Ci $mmol^{-1}$, Amersham (^{3}H-DA) and terminated by filtration through glass fibre (GF/B) filters.

It has previously been shown, at least for NA and 5-HT, that the most reliable estimate of the energy-dependent component of uptake is achieved by sodium-ion removal (Wood and Wyllie, 1982). On this basis, the accumulation of DA into purified synaptosomes was found to be completely energy dependent. Kinetic analysis of the uptake processes revealed that the apparent high affinity, Ku, of the energy-dependent uptake process in both P_2 and P_{2B} fractions were similar ($\sim$400nM). However, the Vu values dissimilar, being six fold higher in the P2 than the P_{2B} fraction. This is probably related to the different preparation times for the two fractions and the lability of the synaptosomes.

From kinetic analysis, a concentration of 10^{-7}M ^{3}H-DA was considered to be predominantly accumulated by the high affinity (and therefore presumably physiological) uptake process. The validity of using crude (P_2) fractions was further substantiated from analysis of the relative effects of inhibitors on DA uptake at this concentration. In general, NA, 5-HT, DA, ciclazindol, mazindol and nomifensine were found to have similar potencies in both fractions. Noradrenaline and 5-HT were less potent inhibitors of the uptake system than DA itself; a finding which reflects the structural requirements of the DA uptake system.

IC_{50} nM P2:- DA 370 ± 73; NA 2600 ± 420; 17000 ± 1300; mazindol 110 ± 16; ciclazindol 2200 ± 420, nomifensin 420 ± 120.

IC_{50} nM P_{2B} DA 290 ± 85; NA 3600 ± 140; 5-HT 6900 ± 450; mazindol 147 ± 23; ciclazindol 1000 ± 350; nomifensin 490 ± 140.

The use of crude synaptosomal (P_2) fractions to measure DA uptake can be justified in terms of kinetic analysis and drug sensitivity. In order to analyse the energy-dependent uptake, the final DA concentration should be in the range $5x10^{-8}$M - $3x10^{-7}$M.

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INVESTIGATION OF THE EFFECTS OF GANGLION-BLOCKING AGENTS AND METHERGOLINE ON THE NICOTINE CUE IN RATS

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Nicotine produces a highly specific cue (discriminative stimulus) which is blocked by ganglion-blocking drugs (eg mecamylamine) thought to penetrate to the CNS (Rosecrans and Chance, 1977). However, mecamylamine does not displace nicotine from a binding site in rat brain (Romano and Goldstein, 1980). One explanation for this dissociation between the behavioural and biochemical findings would be that mecamylamine acts through non-competitive mechanisms. The present investigation has determined some characteristics of the nicotine-mecamylamine interaction, and we also report further studies of the specificity of the cue.

Rats were trained to discriminate the effects of nicotine tartrate (0.4 mg/kg sc) from saline in a standard two-bar, operant conditioning procedure (Stolerman and D'Mello, 1981; Garcha et al, 1982). All doses given are those of the bases. Pretreatment with mecamylamine hydrochloride shifted the dose-response curve for the nicotine cue downwards in a dose-related manner. In control animals with saline pretreatment, nicotine produced a dose-related increase in drug appropriate responding. The mean percentage ($\pm$ SEM) of drug-appropriate responding following nicotine (0.4 mg/kg) was 93.2 $\pm$ 2.4%, as compared with 4.2 $\pm$ 2.2% after saline. After mecamylamine (0.25 mg/kg sc 15 min prior to nicotine), the response to nicotine was reduced to 49.5 $\pm$ 13.6% ($p < 0.01$). Increasing the dose of nicotine to 0.8 mg/kg and 1.6 mg/kg yielded scores of 73.7 $\pm$ 7.4% and 41.6 $\pm$ 12.8% respectively. After mecamylamine (0.75 mg/kg) the response to nicotine (0.4 mg/kg) was completely blocked (mean score = 4.6 $\pm$ 2.3%). Doses of nicotine as large as 3.2 mg/kg increased drug-appropriate responding to not more than 41.9%. Pretreatment with hexamethonium bromide (2.5 or 7.5 mg/kg) did not alter the dose-response curve for the nicotine cue.

Although the nicotine cue has generally been found to be pharmacologically highly specific, we recently found that the directly-acting 5HT agonist quipazine maleate (0.125-2.0 mg/kg sc) increased nicotine-appropriate responding to 50.5 $\pm$ 10.6%. We therefore investigated the effects of the 5HT receptor antagonist, methergoline, on the nicotine cue. Pretreatment with methergoline (0.25-4.0 mg/kg sc 3 h prior to testing) did not influence nicotine-appropriate responding; after methergoline (4.0 mg/kg) responses to nicotine (0.4 mg/kg) were 90.5 $\pm$ 1.9%, as compared with 93.1 $\pm$ 2.5% in control animals receiving nicotine (0.4 mg/kg) but pretreated with saline.

Thus we have confirmed that the nicotine cue is blocked by mecamylamine but not hexamethonium. Our findings suggest that one characteristic of a competitive relationship, ability to overcome the block by increasing the dose of agonist, is absent from the nicotine-mecamylamine interaction. This fits in with neurochemical studies (Romano and Goldstein, 1980), and is compatible with the view that a cholinergic site may be the receptor mediating the nicotine cue. The 5HT receptors upon which methergoline acts do not seem to be involved in the nicotine cue.

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THE EFFECTS OF α_1- AND α_2-ADRENOCEPTOR BLOCKING AGENTS ON APOMORPHINE-INDUCED CLIMBING BEHAVIOUR IN MICE

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Yohimbine (YOH) has been considered as a prototype drug for α_2-adrenoceptor blockade, and thus is widely used to assess neurochemical and pharmacological indices of α_2-adrenoceptor dependent functions (Cf. Langer, 1980), including the involvement of these receptors in the regulation of brain dopaminergic neurotransmission (Al Shabibi and Doggett, 1978). However, it was recently shown that YOH also blocks dopamine receptors, independent of its effects of noradrenergic transmission (Scatton et al, 1980).

In the present study, we have thus investigated the effect of YOH and other α_2 and α_1 adrenoceptor blocking agents, in a classical model for testing neuroleptics, the antagonism of apomorphine-induced climbing behaviour in mice (Worms and Lloyd, 1979).

Male Swiss mice (CD_1 strain, Charles River France, 18-22 g) were used. All compounds were injected ip. The antiapomorphine test was performed as described previously (Worms and Lloyd, 1979). In a parallel series of experiments, YOH and RX 781094 (Dettmar et al, 1981) were tested for their ability to antagonize clonidine-induced sedation in mice after administration 1 hour before clonidine (0.2 mg/kg, ip). The mice were then placed in a photocell activity cage and activity counts were noted 20 min. later.

The α_2 antagonists, YOH (ED 50 = 2 mg/kg), rauwolscine (ED 50 = 1.5 mg/kg) and piperoxane (ED 50 = 20 mg/kg) dose-dependently antagonized apomorphine-induced climbing. In contrast the other α_2 blockers tolazoline (3-30 mg/kg) and RX 781094 (0.3-10 mg/kg), as well as the α_1 or mixed antagonists phenoxybenzamine (3-30 mg/kg), prazosin (1-3 mg/kg), corynanthine (1-10 mg/kg) and phentolamine (1-10 mg/kg), did not affect the climbing behaviour.

In this model, the ED 50's for classical neuroleptics were 0.04, 0.85 and 19 mg/kg ip, for haloperidol, chlorpromazine and sulpiride, respectively.

The anti-apomorphine effect of YOH (3 and 10 mg/kg) was not antagonized by pretreatment (30 min.) with clonidine (0.1 or 0.3 mg/kg, ip).

Both YOH (3 mg/kg) and RX 781094 (1 mg/kg) antagonized clonidine sedation in mice (percent of saline controls : clonidine = 34 ± 4 ; clonidine + YOH = 69 ± 10, $p < 0.01$; clonidine = 26 ± 3 ; clonidine + RX = 74 ± 12, $p < 0.01$).

These data, together with previously reported neurochemical data (Scatton et al, 1980 ; Dedek et al, 1982) suggest that YOH, rauwolscine and piperoxane antagonize the effect of apomorphine through a direct blockade of DA receptors, independent of their α_2 blocking properties, as RX 781094 (which is more potent than YOH in blocking clonidine-induced sedation), tolazoline and the α_1 blocking drugs fail to modify apomorphine-induced climbing. This indicates that α-mediated neurotransmission is probably not directly involved in the regulation of this dopamine receptor dependent behaviour.

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EFFECT OF OXYPERTINE VERSUS HALOPERIDOL ON AMPHETAMINE-INDUCED BEHAVIOUR

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We have postulated (Ashcroft et al. 1981) that the biochemical changes underlying schizophrenia are not primarily in aminergic activity but in the limits within which aminergic activity is consistent with organised behaviour. In schizophrenia these limits would be constricted and therefore we must look for treatments that expand them rather than drugs which block the activity. For this we need an animal model in which we can test firstly the existence of tolerated limits for aminergic activity and, secondly, the possibility of modifying them pharmacologically. The animal model for these experiments was amphetamine induced stereotyped behaviour (ST) (as an example of disorganised activity) versus amphetamine induced exploratory behaviour (EX) (as an index of organised activity) (Makanjuola et al. 1977 and Palomo et al. unpublished). We have, therefore, exploratory activity as an index of aminergic activity within the tolerated limits, an upper stereotyped threshold and a lower exploratory threshold. This should allow us to look for drugs that increase the ST threshold (so that higher doses of amphetamine are tolerated without ST) and decrease the EX threshold (so that smaller doses of amphetamine produce stimulation).

90 Hooded Lister rats (Animal Suppliers, London) were used for these experiments. They received a dose of d-amphetamine (8 mg/Kg.) plus one dose of oxypertine or haloperidol. Immediately after the i.p. injection, one animal at a time was placed into a hole-board apparatus designed for this study and the rat behaviour was recorded for four minutes every 20 minutes during two hours. We used for our comparisons in the present paper the pooled activity of periods 3 and 4 (40 and 60 minutes after drug administration). Oxypertine (0, 0.25, 1, 4 or 16 mg/Kg.) given alone had no significant effect on EX (6.8 ± 2.2; 11.6 ± 7.3; 8.2 ± 2.6; 2.3 ± 2.1; and 2.2 ± 2.1 respectively) or ST (0.7 ± 0.3; 1.0 ± 0.5; 0.2 ± 0.1; 0.3 ± 0.2 and 0.1 ± 0.1 respectively). When given in combination with amphetamine, oxypertine has a differential effect on EX and ST in that both 1 and 4 mg. oxypertine blocksthe 8 mg. amphetamine induced ST ($p<0.05$) while EX increases ($p<0.02$)(Table 1). Haloperidol blocks ST at doses of 0.025 mg/Kg. or more ($p<0.05$). EX was reduced ($p<0.05$) and, although it seemed somewhat boosted at 0.025 mg. dose, the deviation from linearity was not significant. (Table 1).

Table 1: $\bar{x} \pm$ S.E. of mean. All animals received 8 mg/Kg. Amphetamine

Oxypertine(mg/Kg.)	0	0.25	1	4	16
Stereotypy	129.4 ± 27.3	122.6 ± 30.3	44.2 ± 22.8	8.7 ± 3.9	0.2 ± 0.2
Exploration	50.0 ± 18.4	27.6 ± 9.5	150.6 ± 38.1	201.8 ± 25.4	3.5 ± 1.4
Haloperidol(mg/Kg.)	**0**	**0.025**	**0.1**	**0.4**	**1.6**
Stereotypy	129.4 ± 27.3	39.1 ± 35.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Exploration	50.0 ± 18.4	98.7 ± 29.3	34.4 ± 12.4	5.3 ± 1.9	0.9 ± 0.7

In summary we can conclude first that the amphetamine model is a useful experimental tool to test our proposed model for schizophrenia and its treatment and second, that oxypertine looks promising as a possible drug which may expand the tolerated limits for aminergic activity.

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12P

A SPECIFIC UPTAKE SYSTEM FOR TRYPTAMINE IN THE RAT CEREBRAL CORTEX?

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Electrophysiological studies (Jones, I982 ; Jones and Broadbent, I982) have indicated that tryptamine(T) as well as 5-hydroxytryptamine(5HT) may play an active role in neurotransmission in the rat cerebral cortex. The major means of inctivation of 5HT in the cortex is by reuptake but it is not known whether such a system exists for T. The present study has investigated this possibility.

Uptake studies were performed on slices of rat fronto-parietal cortex using the method described by Dyck (I978). Slices were pre-incubated at $37^{o}C$ in Krebs-Henseleit medium for I5 min and then transferred to fresh buffer containing either ^{3}H-T or ^{I4}C-5HT for IO min. Labelled amine accumulated in the slices was determined by scintillation counting.

T was significantly accumulated by cortical slices, the uptake being linearly related to substrate concentration over the range tested ($5xIO^{-8}$-$IO^{-6}M$). Incubation at $O^{o}C$ or in a low Na^{+} medium reduced the amount of amine taken up considerably.Kinetic analysis of the active uptake (i.e.37- $O^{o}C$) gave an apparent Km of $0.6xIO^{-6}M$ and a Vmax of 6.0 pmol/mg tissue/IOmin. This compares with a Km of $0.7xIO^{-6}$ and a Vmax of II.2 for the uptake of 5HT in the same preparation.

Inclusion of unlabelled T in the incubation medium considerably reduced the uptake of ^{I4}C-5HT. Kinetic analysis of this inhibition revealed that it was of a non-competitive nature however. Also, unlabelled 5HT had very little effect on the uptake of ^{3}H-T. It was possible to discriminate between T and 5HT uptake by use of the 5HT uptake blocker fluoxetine. At a concentration of $5xIO^{-6}M$ fluoxetine caused a 50-60% reduction in 5HT uptake but did not alter T uptake. However,T uptake was reduced by 40-50% in the presence of $5xIO^{-5}M$ fluoxetine.

Investigation of the release of newly taken up T and 5HT also suggested that the two uptake systems may be distinct. Thus T was able to release ^{I4}C-5HT at relatively low concentrations(IO^{-6}-$IO^{-5}M$) but 5HT did not evoke any release of ^{3}H-T (up to $5xIO^{-5}M$).In addition fluoxetine at a concentration which caused a marked inhibition of 5HT uptake did not prevent the ability of unlabelled T to release ^{I4}C-5HT but rather,potentiated it.

The data indicate therefore that T can be actively accumulated in rat cortex by a sodium and temperature dependant mechanism.The system exhibits a similar substrate affinity to the 5HT uptake but has a reduced maximal velocity. Several experiments suggest that the uptake may be distinct from that of 5HT however. The prescence of an active uptake for T may be consistent with the proposal that T may be involved in synaptic transmission in the cerebral cortex.

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INCREASED RESPONSIVENESS TO TRYPTAMINE IN MICE FED A TRYPTOPHAN DEFICIENT DIET

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Tryptamine is formed in brain by decarboxylation of tryptophan (Saavedra and Axelrod 1973) and can also enter the brain from the circulation. Tryptamine injected peripherally produces behavioural effects. These effects may either involve release of pre-synaptic 5-hydroxytryptamine (5HT) (Marsden and Curzon 1979) or post-synaptic effects independent of 5HT (Jones 1981). Brain 5HT synthesis (Zambotti et al 1975) and release (Irons et al 1982) is reduced in mice feed a diet low in tryptophan (TP). The present study compares the head twitch response produced by tryptamine administered to mice fed diets containing different amounts of TP and investigates the effects of tryptamine on release of 5HT from mouse brain slices in vitro.

Mice (CS-1 males) were maintained on a normal powdered diet (41B modified, TP 0.21%), TP deficient (BP Nutrition-based on cooked maize meal and containing TP 0.07%) or the TP deficient diet with added TP (0.2%) for 14 days. Tryptamine (5 mg/Kg) was then given 30 mins after tranylcypromine (TCP 10 mg/Kg) while controls were given TCP plus saline. The number of head twitches were counted every 2 min for 45 min after the second injection. Brain 5HT and its metabolite 5-hydroxyindole acetic acid (5HIAA) were determined by HPLC with electrochemical detection (carbon paste working electrode set at +0.65 V). The indoleamines were separated on a spherisorb reverse phase column (5 μm) using 0.1 M acetate-citrate buffer pH 4.1 containing 10% methanol. 5HT release was measured in vitro using whole brain slices and HPLC with electrochemical detection.

Tryptamine caused a marked head twitch response (22.5 head twitches/45 min ± 1.3 (s.e.) n=6) compared with mice given a normal diet then TCP + saline (1.25 ± 0.5 (6)) while 5HT values were unchanged. The tryptamine response was significantly increased in the TP deficient mice (44.7 ± 1.8 (6)) and 5HT levels decreased (-41%) but was not in mice given the TP deficient diet plus TP (20.5 ± 3.4 (6)) when the 5HT values were not significantly different from normal diet mice. There were no significant differences in the number of head twitches observed after TCP plus saline between mice given the three different diets. In a second experiment, using the same three diets, half the mice were given p-chlorophenylalanine (PCPA 400 mg/Kg) 48 and 24 h before TCP plus tryptamine. Again the tryptamine response was markedly enhanced in the TP deficient group ($P<0.001$) compared with the normal and TP deficient plus TP diets. PCPA decreased 5HT in all diet groups (-40 to -65%) and also significantly reduced the tryptamine response in mice on the normal diet (-56%, $P<0.01$) and prevented the increased head twitch response in the TP deficient group. Tryptamine (10^{-7}M) significantly increased 5HT release (+30%) from brain slices from mice feed the normal diet and those feed the TP deficient diet (+58%) or the low TP diet plus TP (+62%). However, basal (no added tryptamine) 5HT release was signficantly decreased between the normal and the TP deficient diets (-47%) but returned to near normal values in the mice fed the TP deficient diet plus TP.

The results suggest that the head twitches induced by tryptamine are dependent upon the presence of 5HT and that the increased response in the TP deficient mice may reflect release of 5HT onto supersensitive postsynaptic receptors.

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EFFECT OF WITHDRAWING CHRONIC HIGH DOSE PROGESTERONE ON BRAIN TRYPTOPHAN, 5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLEACETIC ACID

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In human pregnancy, plasma progesterone rises approximately 1000 times, these high concentrations then rapidly fall to normal values within 72 hours of delivery (Yonnone et al., 1968). It has also been shown that brain concentrations of 5-Hydroxytryptamine (5HT) and 5-Hydroxyindoleacetic Acid (5HIAA) in mice are significantly decreased on the fifth day post partum, whilst tryptophan (TP) is slightly elevated (Greengrass & Tonge, 1972). This study therefore investigates whether the sudden fall in plasma progesterone plays a role in these changes of brain indoleamine concentrations.

Groups of 5 female mice received either natural progesterone, 10 mg/kg in gum acacia suspension p.o., or gum acacia control 5 ml/kg p.o. daily for 15 days. The treatment was then withdrawn. On the last day of drug administration and on each of the 3 days following withdrawal, brains were removed and assayed for TP, 5HT and 5HIAA using the fluorimetric method of Curzon and Green (1970).

On the final day of drug administration whole brain TP and 5HT concentrations were significantly increased above control values (TP: control = 2.4 ± 0.4; prog = 6.2 ± 1.2 μg/g brain tissue. 5HT: control = 360 ± 60; prog = 710 ± 10 ng/g brain tissue. P = 0.002 & 0.003 respectively). However there were no significant differences in whole brain 5HIAA concentrations

On the first day following drug withdrawal the whole brain TP and 5HT concentrations remained significantly increased above control (TP: control = 2.2 ± 0.2; prog = 5.3 ± 1.9 μg/g brain tissue. 5HT: control = 320 ± 30; prog = 790 ± 130 ng/g brain tissue. P = 0.035 & 0.01 respectively), again there were no significant differences in brain 5HIAA concentrations. On the second day of withdrawal whole brain TP and 5HIAA were significantly decreased below control values (TP: control = 5.1 ± 0.5; prog = 2.6 ± 0.9 μg/g brain tissue. 5HIAA: control = 1270 ± 170; prog = 440 ± 20 ng/g brain tissue. P = 0.03 & 0.004 respectively). There was no significant differences in brain 5HT concentrations between drug group and control. Whole brain 5HT was significantly decreased on day 3 following treatment withdrawal (control = 960 ± 150; prog = 380 ± 80 ng/g brain tissue. P = 0.032).

In a separate group of mice, with exactly the same experimental procedure, progesterone 10 mg/kg did not significantly alter plasma total tryptophan concentrations when compared with vehicle controls.

The results therefore suggest that 48 h after progesterone treatment withdrawal, there is a decrease in the entry of TP into the CNS, this seems to be due to some other mechanism than alterations of plasma TP. The decreased 5HT and 5HIAA at this time suggest decreased 5HT synthesis which may be due to the fall in brain TP. This effect however seems to last only for about one day, possibly reflecting the rapid turnover of progesterone.

This result may be of importance in understanding the processes involved in the aetiology of the transient mood changes often seen immediately post partum.

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ALTERATIONS OF STRIATAL ACETYLCHOLINE AND GLUTAMATE RELEASE FOLLOWING ACUTE AND CHRONIC NEUROLEPTIC ADMINISTRATION TO RATS

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Striatal dopamine receptor supersensitivity occurring during chronic neuroleptic administration to rats may be of functional significance for it is accompanied by increased striatal acetylcholine concentrations (Murugaiah et al,1982). Dopamine acts to inhibit striatal acetylcholine and glutamate release while acute administration of neuroleptic drugs to rats enhances in vitro release (De Belleroche & Neal,1982). We have compared the effect of neuroleptic drugs following acute and chronic administration to rats on the release of ^{3}H-acetylcholine and ^{3}H-glutamate from rat striatal slices preloaded with ^{3}H-choline and ^{3}H-glutamate respectively (see Kerwin & Pycock,1979).

Potassium chloride (K^+;5-50 mM) stimulated the efflux of both ^{3}H-acetylcholine and ^{3}H-glutamate from striatal slices in a Ca^{++} dependent manner. The effect of K^+ in both cases was abolished when dopamine (100 uM) was present throughout the superfusion. Acute administration of haloperidol (1-5 mg/kgip; HPL), trifluoperazine dihydrochloride (2-10 mg/kg; TFP) and clozapine (10-40 mg/kg ip; CLOZ) 90 min previously increased K^+ (25 mM) evoked ^{3}H-acetylcholine release compared to control animals; sulpiride (10-100 mg/kg ip; SULP) 3 h previously was without effect (Table 1). None of the drugs administered acutely in these doses had any effect on K^+ evoked ^{3}H-glutamate release. Striatal K^+ evoked ^{3}H-acetylcholine release was not different in animals receiving HPL (1.4-1.6 mg/kg/day), TFP (4.5-5.1 mg/kg/day) or SULP (102-110 mg/kg/day) for 12 months compared to age-matched control rats (Table 1). Administration of CLOZ (23-26 mg/kg/day) for 12 months decreased K^+ evoked release of ^{3}H-acetylcholine compared to controls. All drugs caused a decrease in striatal K^+ evoked release of ^{3}H-glutamate from striatal slices compared to age-matched control animals. Acute and chronic drug treatment had no apparent effect on spontaneous efflux of ^{3}H-acetylcholine or ^{3}H-glutamate.

Table 1 Release of ^{3}H-acetylcholine and ^{3}H-glutamate evoked by 25 mM K^+ in striatal slices from rats following acute or chronic neuroleptic intake

			Amount released			
			^{3}H-Glutamate		^{3}H-Acetylcholine	
		Dose(mg/kg)	Control	Drug	Control	Drug
ACUTE	HPL	5	9.1±1.7	10.3±2.1	10.5±2.0	20.8±4.5*
	TFP	10	11.0±1.9	9.8±0.9	7.0±0.8	12.0±1.5*
	CLOZ	40	10.4±1.2	12.5±2.1	7.2±0.5	30.0±2.8*
	SULP	100	8.5±1.3	7.5±0.8	6.1±0.8	6.4±0.5
CHRONIC	HPL	1.4-1.6	8.2±1.0	2.4±0.5*	5.0±0.4	3.0±2.1
	TFP	4.5-5.1	7.0±1.0	2.6±0.4*	6.0±1.2	7.2±1.5
	CLOZ	23-26	6.3±1.3	1.6±0.5*	6.2±0.2	1.8±0.3*
	SULP	102-110	7.2±1.2	2.4±0.5*	7.0±2.0	10.0±1.8

The results are shown as the amount released over the spontaneous levels during a 6 min period following K^+ stimulation and represent the amount of radioactivity collected as a percentage of the total radioactivity in the tissue at that time. Each result is the mean ± SEM of 4-12 individual animals. * $p < 0.05$

The reversal of the acute action of neuroleptic drugs on striatal ^{3}H-acetylcholine and ^{3}H-glutamate release is consistent with a functional increase in striatal dopamine sensitivity occurring following long-term neuroleptic treatment.

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CHRONIC HALOPERIDOL PRODUCES SUPERSENSITIVITY OF DOPAMINE RECEPTORS MODULATING (^{3}H)-DA BUT NOT (^{3}H)-ACH RELEASE IN THE RABBIT CAUDATE

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Dopamine autoreceptors modulating dopamine release in the rabbit caudate develop supersensitivity to apomorphine after chronic haloperidol administration (Arbilla et al., 1981). We have explored if this supersensitivity of dopamine autoreceptors also occurs when dopamine is used as an agonist, and whether in the same structure, similar changes in sensitivity develop for the dopamine receptors modulating 3H-Ach release (Hertting et al., 1980).

Rabbits which received haloperidol 1 mg/kg s.c. daily for 28 days were killed after 48, 72 or 96 h of withdrawal. Caudate nucleus slices were labelled with 3H-DA or 3H-Choline and superfused with Krebs' solution. Two periods of electrical stimulation (S1 and S2), at 0.3 or 3 Hz for 3H-DA or 0.3 Hz for 3H-Ach, were applied for a duration of 2 min, with an interval of 44 min. Dopamine, in the presence of nomifensine 10 μM, was added 6 min and apomorphine 20 min before S2.

Table 1 : Supersensitivity of release modulating dopamine autoreceptors in the rabbit caudate.

		S2 / S1			
		Untreated	Chronic Haloperidol (Time of Withdrawal)		
	μM		(48 hrs)	(72 hrs)	(96 hrs)
3H-Ach	CONT -	0.83±0.03	0.85±0.01	0.87±0.03	0.82±0.02
	APO 0.03	0.41±0.02*	0.40±0.03*	0.38±0.02*	0.40±0.01*
3H-DA	CONT -	0.98±0.04	0.95±0.02	N.D.	0.96±0.01
	APO 0.01	0.90±0.04	0.66±0.04*	N.D.	0.83±0.09
	CONT -	0.82±0.04	N.D.	0.82±0.04	N.D.
	DA 0.1	0.72±0.02	N.D.	0.56±0.02*	N.D.

* $p<0.05$; values are mean ± S.E.M. from 3 to 13 rabbits. N.D. : not determined.

The ratios S2/S1 are shown in Table 1. In untreated rabbits, apomorphine (0.001 to 1 μM) inhibited the evoked release of 3H-Ach. Identical concentration-response curves for the inhibition by apomorphine of 3H-Ach release were obtained after 48, 72 or 96 h of withdrawal from chronic haloperidol. The inhibitory effect of apomorphine at 0.03 μM on 3H-Ach release is shown in Table 1. In contrast with the results on 3H-Ach release, after 48 or 72 h of withdrawal, the dopamine receptors modulating 3H-DA release were more sensitive to apomorphine 0.01 μM or dopamine 0.1 μM (Table 1), and this supersensitivity faded after 96 h of withdrawal (Table 1). In the rabbits under chronic haloperidol treatment, the striatal haloperidol levels (ng/g) were : 229 ± 66 (n=12) after 48 h ; 51 ± 3 (n=3) after 72 h and undetectable after 96 h following the last injection.

The present data indicate that in spite of the residual levels of haloperidol in the striatum, there was supersensitivity for the dopamine autoreceptors modulating 3H-DA release but not for the dopamine receptors modulating 3H-ACh release. It is concluded that following chronic haloperidol treatment, the dopamine receptors modulating 3H-Ach release do not develop changes in sensitivity, while supersensitivity develops for the dopamine autoreceptors that modulate 3H-DA release.

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DURATION OF DOPAMINE RECEPTOR BLOCKADE AFTER A SINGLE I.M. DEPOT INJECTION OF FLUPHENAZINE DECANOATE

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We have noted extremely long-lasting dopamine (DA) receptor blockade following cessation of 6 months repeated treatment with the depot phenothiazine neuroleptic fluphenazine decanoate (FPZ-d)(Waddington & Gamble, 1981). This persistence of effect is consistent with elevated plasma levels of drug and prolactin (Wistedt et al, 1981) and prolonged protection against psychotic relapse (Levine et al, 1980) when chronic FPZ-d is terminated in the clinic. To clarify these unexpected effects we have studied the duration of action of a single i.m. depot of FPZ-d in a similar manner.

Male Sprague-Dawley rats (550-650 g) were given a single i.m. injection (0.2 ml of 25 mg/ml FPZ-d or oil vehicle alone) into rear leg muscle. At various times thereafter they were challenged with the DA agonist apomorphine (APOM) and resultant stereotyped behaviour assessed (Waddington & Gamble, 1980). With 0.15 mg/kg s.c. APOM stereotypy remained completely abolished ($P < 0.001$) for over 1 month. Between the 2nd and 3rd month there was some restoration of responsivity in the first 15 min of APOM action, though attenuation was evident ($P < 0.05$); in the 25-40 min phase of APOM action, responses remained markedly depressed ($P < 0.02$). At the 4 month point responses at 15 min were normal in FPZ-d animals with weak antagonism ($P < 0.05$) demonstrable in the 25-40 min period. Between the 5th and 6th month, responsivity was indistinguishable from that of vehicle controls. With 1.0 mg/kg APOM a characteristic syndrome of perimeter hyperlocomotion, punctuated by corner rearing/sniffing, was noted in 50% of FPZ-d animals (0% in controls, $N = 8$, $P = 0.1$) at 28 but not 14 days after depot injection; it was not observed 3 months later. This syndrome was identical to that induced similarly in 100% of animals given FPZ-d or oral FPZ for 6 months (Waddington et al, 1982).

Responsivity to low APOM doses was antagonised for at least 2-3 months after a single i.m. depot of FPZ-d. This long duration of action was less than that seen after 6 months of repeated FPZ-d, when responsivity was still attenuated 4-6 months after cessation (Waddington & Gamble, 1981). Svendsen & Aaes-Jorgensen (1979) reported the extremely slow disappearance of ^{14}C-labelled sesame oil, the vehicle for FPZ-d, after a single i.m. injection in the rat. Thus, the basis of these extremely prolonged effects may involve the persistence of the oil vehicle and its slow build-up during repeated treatment.

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THE EXCITATORY AMINO-ACID ANTAGONIST γ-D-GLUTAMYLGLYCINE MASKS RATHER THAN PREVENTS LONG-TERM POTENTIATION IN THE PERFORANT PATH

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Brief trains of high frequency stimulation delivered to monosynaptic hippocampal pathways including the perforant path (PP) can increase the amplitude of their evoked potentials for many hours (Bliss and Lømo, 1973). The basis of this long term potentiation (LTP) remains unclear, although it appears to have both pre- and post-synaptic components (see Dolphin et al 1982). It has been reported that LTP in the CA1 region may be prevented in hippocampal slices if the high-frequency train is given in the presence of the glutamate antagonist D,L-aminophosphonobutyric acid (APB; Dunwiddie et al 1978), indicating that glutamate receptor activation may be a prerequisite for the establishment of LTP. We have attempted to repeat these experiments in vivo, using another glutamate antagonist γ-D-glutamylglycine (DGG), which inhibits the field potentials evoked by stimulation of the PP in hippocampal slices (Collingridge et al 1982). This antagonist was used in place of D,L-APB in view of the differing properties of the D- and L-enantiomers of APB with respect to interference in glutamatergic transmission (Koerner and Cotman, 1981; Davies and Watkins, 1982).

Experiments were performed using the technique of local perfusion through a push-pull cannula introduced into the upper blade of the dentate gyrus in urethane (1.5g/kg)-anaesthetised rats (Dolphin et al., 1982). The potentials evoked by stimulation of the PP at 0.1 Hz were recorded in the granule cell layer using an insulated stainless steel wire recording electrode attached to and extending 0.9mm beyond the outer cannula. The stimulus strength was initially adjusted so that a 1-2mV population spike (resulting from simultaneous firing of granule cells) was superimposed on the positive-going evoked potential. Potentials were recorded every 90s on chart paper, and a stable control baseline was established for 18 min before the introduction of DGG (10^{-3}M) into the perfusion medium. Subsequent population spike heights (H) and slopes of the initial synaptic wave (S) were then calculated as a percentage of control.

DGG rapidly reduced H to a plateau level of 27±8% of control (mean±SEM, n=11) after 12 minutes, and had a smaller effect on S, reducing it to 82±5% of control. A high-frequency conditioning train (250 Hz for 500ms, stimulus voltage twice test voltage) which under control conditions usually gives rise to LTP (see Dolphin et al., 1982), had no effect on the evoked potentials when given during their inhibition by DGG. However, when standard medium was reintroduced 1-5 min later, the evoked potentials recovered, and 9 out of 11 animals showed a marked potentiation of H, which reached 205±52% of control (n=11) 16 minutes later. A smaller potentiation of S occurred, and this effect was slower in its development, reaching 125±7% of control 36 minutes after the reintroduction of standard medium. As judged by both parameters (S and H) this potentiation was LTP, since once it was established it remained stable for the rest of the experiment (at least 45 min). In 11 control animals, when DGG was perfused for the same length of time, followed by standard medium, H and S usually returned to the same values as before DGG, but in no case was any form of potentiation observed. Thus the latent LTP was dependent on the high-frequency train being given during perfusion with DGG.

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NEURONAL RESPONSES TO TAURINE ARE DISTINCT FROM THOSE TO γ-AMINOBUTYRIC ACID AND GLYCINE IN RAT CUNEATE NUCLEUS AND OPTIC NERVE

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The receptors for the inhibitory transmitters γ-aminobutyric acid (GABA) and glycine are well-defined and clearly differ from each other pharmacologically. Receptors mediating the inhibitory actions of taurine seem to vary in their pharmacological properties from one area of central nervous system to another, particularly in their susceptibility to the antagonists bicuculline, picrotoxin and strychnine (e.g. Curtis et al, 1971; Sonnhof et al, 1975; Frederickson et al, 1978). There is, therefore, no clear indication from the literature whether taurine activates GABA receptors or glycine receptors or receptors of its own. Evidence will be presented here that taurine acts on receptors distinct from those for GABA or glycine in rat cuneate nucleus and optic nerve.

Slices of rat cuneate nucleus were prepared for superfusion with Krebs bicarbonate medium at room temperature (Simmonds, 1980) such that drug-induced changes in the membrane potential of terminals and fibres of the dorsal funiculus could be recorded. Similar recordings were made from the fibre population in 3-4 mm lengths of rat optic nerve.

The GABA analogue muscimol, glycine and taurine all evoked depolarizations of the dorsal funiculus. The maxima of the dose-response curves for glycine and taurine were only about 20% of the maximum response to muscimol and the concentration ranges for the dose-response curves were 0.5-10 mM for glycine and taurine compared with 2.5-50 μM for muscimol. On the optic nerve, the three amino acids again evoked depolarizations over the same concentration ranges as before but, in this preparation, the maximum of the glycine dose-response curve was about 5 times that for muscimol and the taurine maximum was similar to that for muscimol. The ion-dependence of responses to all three amino acids appeared to be similar in that replacement of 80% of Cl^- with isethionate substantially reduced the responses. These results suggest that there are more GABA receptors than glycine receptors on dorsal funiculus fibres and more glycine receptors than GABA receptors on optic nerve fibres. Taurine receptors appear fairly evenly distributed between the two preparations.

Further distinction between receptors activated by these amino acids was made with the antagonists bicuculline, picrotoxin and strychnine. On both tissues muscimol was substantially antagonized by 3 μM bicuculline (log. dose ratio 0.44-0.61) and 3 μM picrotoxin (log. dose ratio 0.63-0.76) but was unaffected by 1 μM strychnine (log. dose ratio -0.22 - 0.00) (compare Simmonds, 1982). Glycine was substantially antagonized by 1 μM strychnine (log. dose ratio 0.44-0.62) but was unaffected by bicuculline up to 10 μM and picrotoxin up to 100 μM. Taurine was unaffected by 3 μM bicuculline (log. dose ratio -0.01 - 0.03), was moderately antagonized by 3 μM picrotoxin (log. dose ratio 0.23-0.34) and was substantially antagonized by 1 μM strychnine (log. dose ratio 0.63 - reversal).

Thus, in the rat dorsal funiculus and optic nerve, taurine does not activate GABA receptors. A minor effect on glycine receptors cannot be excluded but the major part of taurine's effect involves a unique population of receptors.

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INTERACTIONS BETWEEN NON-SELECTIVE AMINE OXIDASE INHIBITORS (MAOI) AND OTHER ANTIDEPRESSANTS

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Tricyclic antidepressants combined with MAOI are used in treatment of refractory depressions but combination has proved dangerous, the majority of interactions occurring with clomipramine, imipramine, desipramine and amitriptyline and consisting of extensor rigidity, myoclonic twitching, convulsions and fatal hyperpyrexia. While such combinations continue to be advocated clinically, the causation and avoidance of such reactions has been little studied in animals.

Male Wistar rats (200-250g) treated with phenelzine (200μmol/kg, 18h and 50μmol/kg i.p. 90min previously), nialamide (200μmol/kg, 18h and 100μmol/kg i.p. 90min previously) or (±)-tranylcypromine (19.4μmol/kg i.p. 90min previously) were challenged in groups of three (n = 12) with compounds predominantly affecting neuronal uptake of 5-hydroxytryptamine (5-HT) (fluoxetine, paroxetine, clomipramine), 5-HT and noradrenaline (imipramine, amitriptyline), dopamine (benztropine, nomifensine) or noradrenaline (desipramine, maprotiline, nisoxetine) in doses of 31.6μmol/kg i.p., except with nomifensine (2.8μmol/kg i.p.). The phenomena evoked included tremor, head-twitches, forelimb flexor-extensor movements (FFEM), body-twitches, hindlimb abduction, wet dog shakes (WDS) and Straub tail; FFEM and WDS were obtained after fluoxetine, paroxetine, clomipramine and imipramine, WDS being present only with the intensest reactions and FFEM being elicited by amitriptyline, desipramine and nisoxetine. More prominent phenomena were obtained with phenelzine than nialamide in association with decreased locomotor activity; with tranylcypromine they were superimposed on augmented locomotor activity. Inhibition of brain MAO A was > 90% with these MAOI and for MAO B, 70 to 87%. Cortical and hypothalamic 5-HT, and dopamine in the corpus striatum and nuclei accumbens were significantly elevated by phenelzine and tranylcypromine.

For the uptake inhibitors, frequency and intensity of phenomena were of the order paroxetine or fluoxetine > clomipramine > imipramine > amitriptyline, desipramine or nisoxetine > benztropine, maprotiline or nomifensine, which parallels clinical findings. FFEM, WDS, head and body twitches were diminished in frequency by the antagonists methysergide, cyproheptidine, pimozide and clozapine. Similar phenomena, but to a lesser extent body-twitches, were observed after L-5-hydroxytryptophan (450μmol/kg i.p.) but not L-Dopa (450μmol/kg i.p.) combined with clomipramine.

That the interactions were much more likely with the 5-HT than the other uptake inhibitors, that they were present after clomipramine and 5-hydroxytryptophan, that they included WDS thought to involve central 5-hydroxytryptaminergic mechanisms (Bedard & Pycock, 1977) and a constellation of other features which Jacobs (1976) regarded as evidence for selective activation of central 5-HT receptors, points to this system as crucially implicated in the interactions in man. While the phenomena partially mimicked those in man e.g. myoclonic twitching and rigidity, core temperature was unaltered. However, the pyrexia is possibly a species-specific response, since fatal hyperthermia was elicited by imipramine or amitriptyline infused in rabbits pretreated with phenelzine, nialamide or tranylcypromine (Loveless & Maxwell, 1965).

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INTERACTIONS BETWEEN RELATIVELY SELECTIVE AMINE OXIDASE INHIBITORS (MAOI) AND CLOMIPRAMINE

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Although a hydrazide, nialamide, evoked less intense adverse central phenomena with the 5-HT uptake inhibitors than did combination with hydrazine or amine MAOI (phenelzine, (±)-tranylcypromine), there were potential advantages to combining these uptake inhibitors with selective rather than non-selective MAOI, particularly MAO B inhibitors since 5-HT is a preferred substrate for MAO A (Johnston, 1968) rather than MAO B.

Male Wistar rats (200-250g) tested in groups of three (n = 12) were given an MAO A inhibitor clorgyline (30μmol/kg i.p. 18h and 10 or 20μmol/kg i.p. 90 min previously), or an MAO B inhibitor (-)-deprenyl (30μmol/kg i.p. 18h and 20μmol/kg i.p. 90min previously) or both clorgyline and (-)-deprenyl (each 10μmol/kg i.p. 18h and 90min previously - Schedule 1; or each, 20μmol/kg i.p. 18h and 90min previously - Schedule 2). Mean inhibition of brain MAO A was 63 and 89% with clorgyline 10 and 20μmol/kg respectively and 36% with (-)-deprenyl; mean inhibition of brain MAO B was 25 and 34% with the two clorgyline doses and 66% with (-)-deprenyl. For combined clorgyline and (-)-deprenyl treatment, mean MAO A inhibition was 79 and 95%, and for MAO B 48 and 98% (Schedules 1 and 2 respectively).

Pretreated rats were challenged with clomipramine (31.6μmol/kg i.p.), a compound which evokes dangerous interactions in man when combined with non-selective MAOI. Of the constellation of phenomena encountered with non-selective MAOI pretreatment, only body-twitches were observed following clomipramine in rats pretreated with (-)-deprenyl or the smaller dose of clorgyline; with the larger dose of clorgyline, head and body-twitches and occasional FFEM developed but total incidence scores were significantly less ($P < 0.002$) than for either combined clorgyline and deprenyl, or phenelzine pretreatment. With the larger dose of combined clorgyline and deprenyl (Schedule 2), head and body-twitches, FFEM, Straub tail and WDS were observed after clomipramine, their total incidence as great as with phenelzine and clomipramine. Similar phenomena but without WDS were elicited after the smaller dose of (-)-deprenyl with clorgyline (Schedule 1), total incidence of phenomena being significantly less ($P < 0.02$) than with phenelzine and clomipramine. Rats were also given clorgyline chronically i.e. 20μmol/kg i.p. twice daily for 4 days and 20μmol/kg i.p. 90min prior to clomipramine, after which FFEM,head and body twitches developed but no WDS. Total incidence of these phenomena was increased but not significantly, compared to that obtained after pretreatment with two clorgyline doses and was significantly less than with combined clorgyline and deprenyl pretreatment ($P < 0.02$, Schedule 1; $P < 0.002$, Schedule 2).

Thus clomipramine combined with a relatively selective MAO B or an MAO A inhibitor, evoked significantly fewer adverse phenomena than when administered with a non-selective MAOI which inhibits MAO A and B, or with a combination of MAO A and B inhibitors. Consequently, such a regime would seem substantially safer in the clinical context, with the proviso that MAO A inhibitors dispose to interactions with amine-containing foods.

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TOLERANCE DOES DEVELOP TO DIAZEPAM'S PROTECTIVE EFFECTS AGAINST PENTYLENETETRAZOLE-INDUCED SEIZURES

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Male Swiss grey mice (25-30 g) were challenged with convulsant doses of pentylenetetrazole (PTZ 60-120 mg/kg) or picrotoxin (8 mg/kg), following pretreatment 30 min before test with vehicle or with diazepam (4 mg/kg acute or 5-45 days of treatment). The drugs were dissolved or suspended in distilled water and were given i.p. The mice were observed for 15 min after administration of the convulsant.

Considering the incidence of frank convulsions significant tolerance ($p < .01$) to diazepam's protective effects was evident after 5 days of diazepam treatment if the mice were challenged with PTZ (120 mg/kg), by day 10 if the challenge was picrotoxin (8 mg/kg) and by day 20 if the challenge was PTZ (105 mg/kg). Diazepam retained its protective effects for 45 days against frank convulsions induced by PTZ (60-90 mg/kg), but significant tolerance could be detected as soon as 5 days from the incidence of myoclonic spasms.

Although all the mice in the chronic diazepam groups convulsed to PTZ (120 mg/kg), their mean latencies to convulse were longer ($ps < .05$) than that of untreated mice tested with PTZ (120 mg/kg). Similarly, the mean latency to convulse to picrotoxin (8 mg/kg) was significantly longer ($p < .05$) in the 20 day diazepam group than in untreated mice. This suggests that diazepam still retained some protection against the convulsants.

There is nothing in these results to support the rather widely held notion, based on just two reports (Juhasz & Dairman, 1977; Lippa & Regan, 1977), that tolerance does not develop to the anti-pentylenetetrazole effects of diazepam, whereas it does do so to its protective effects against other convulsants.

Table 1. Numbers of mice showing frank convulsions and myoclonic spasms to PTZ or picrotoxin after acute or chronic pretreatment with diazepam (4 mg/kg)

Diazepam	Acute	5	10	20	30	45 days
a) Convulsions						
PTZ (60 mg/kg)	0/7	0/7	0/7	0/7	0/7	0/7
PTZ (90 mg/kg)	1/8	1/8	0/8	2/8	2/8	2/8
PTZ (105 mg/kg)	1/6	2/6	3/7	5/7		
PTZ (120 mg/kg)	0/8	8/8	7/7	7/7		
Picrotoxin (8 mg/kg)	0/8	3/8	6/7	7/8		
b) Myoclonic Spasms						
PTZ (90 mg/kg)	2/8	7/8	7/8	8/8		
PTZ (105 mg/kg)	4/6	6/6	7/7	7/7		
PTZ (120 mg/kg)	6/8	7/8	7/7	7/7		
Picrotoxin (8 mg/kg)	5/8	3/8	5/7	7/8		

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CARDIOVASCULAR AND PLASMA CATECHOLAMINE RESPONSES TO CENTRAL AND PERIPHERAL MORPHINE IN CONSCIOUS RATS

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Morphine and other opiate agonists have been reported to cause a decrease in blood pressure and heart rate on systemic injection to rats, whereas central administration results more usually in a rise in both parameters (Lang et al, 1982). The opiate peptides β-endorphin and D-ala^2-met-enkephalinamide have also been shown to stimulate plasma catecholamine levels (Van Loon et al, 1981); however no attempt was made to correlate these changes with the cardiovascular actions. In this study we examine the effect of icv morphine on blood pressure and heart rate simultaneously measuring plasma catecholamine levels and in addition compare these effects with those of systemically administered morphine. Narcotic induced respiratory depression has been reported to produce variations in blood pressure (Bellet et al, 1980) so blood gases and pH were determined in some cases.

Male Wistar rats (250-350 g) were prepared under Nembutal anaesthesia (50 mg/kg) with chronically implanted 23 gauge guide tubes for lateral ventricle injections and with carotid artery cannulae exteriorized to the back of the neck for blood pressure and heart rate recordings and blood sampling; 2-3 days were allowed for recovery. Experiments were carried out in freely moving animals. Catecholamine levels were determined by radioenzymatic assay (Brown et al, 1981).

Morphine (105 nmol icv) produced a slowly developing, modest hypertension (117% of control values after 2 h) and tachycardia (120% of control after 3 h). Increases in plasma noradrenaline (NA) and adrenaline (A) were maximal earlier and were more dramatic (NA 214% of control and A 1900% of control after 1 h; control NA .42 $\pm$ .07 ng/ml, A .04 $\pm$ .01 ng/ml, n=8); however NA levels were positively correlated with blood pressure and heart rate changes during the response. Following im injection morphine (35 μmol/kg) induced an initial fall in blood pressure and heart rate (78% and 58% of control respectively after 1 min) followed by a modest hypertension (110% of control at 30 min) and variable heart rate changes. Plasma catecholamine responses were again more marked than the cardiovascular effects (NA 410% of control, A 2800% of control at 1 h). Naloxone (2.2 μmol/kg ia) virtually abolished both cardiovascular and plasma catecholamine responses to the im morphine but did not alter the response to icv morphine. Icv naloxone (300 nmol)was also ineffective. To investigate the naloxone resistance of icv morphine more fully, lower doses (3.5, 10.5, 35 nmol) were administered and plasma catecholamine levels determined at 1 h. The effects were dose dependent and naloxone (300 nmol icv) significantly inhibited the increases in plasma catecholamines at these low doses. Determination of blood gases and pH at 5 and 60 min after morphine indicated that the threshold dose required to produce respiratory depression was 35 nmol.

These results suggest that many of the cardiovascular effects of morphine are due to activation of the sympathetic nervous system although different receptors may be involved in producing these effects depending on the route of administration.

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VASCULAR EFFECTS OF β ANTAGONISTS ON PROCATEROL-INDUCED HYPOTENSION IN THE RAT

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We have previously demonstrated that the phenoxybenzamine-treated, ganglion-blocked, angiotensin II (Ag II)-supported rat may be a suitable model for studying the vascular effects of ß agonists and antagonists (Brown & Worcel, 1982). In this model, i.v. injection of the $ß_2$ agonist procaterol (Yabuuchi, 1977) induces a long lasting, dose-dependent reduction of blood pressure (BP) at doses which do not produce a chronotropic effect upon the heart ($ß_1$ and $ß_2$ mediated, e.g. Minneman et al., 1979) of the phenoxybenzamine-treated, ganglion-blocked animal. Procaterol-induced hypotension has been employed here to investigate the actions of some ß antagonists.

Rats were prepared as previously described (Brown & Worcel, 1982) and ß antagonists perfused (when required) i.v. with the Ag II. The preparations were left 30 min to stabilize before the procaterol dose-response curve was performed. Rats received either procaterol alone, or procaterol in the presence of a ß-antagonist.

Accumulative i.v. administration of procaterol produced a maximum reduction of BP (initial BP 116±4 mmHg, n=40) of 51.1±1.1% (n=8), the EC_{50} being 2.04±0.21 nmole/kg. Increasing doses of propranolol and IPS 339 induced parallel displacements of the procaterol curve without affecting the maximum reduction of BP. Arunlakshana & Schild (1959) plots of the data yielded straight lines with slopes of 0.90±0.15 and 0.95±0.08 (mean±SEM) for propranolol (n=7) and IPS 339 (n=6) respectively. The intercepts on the log-dose axis at log (concentration ratio -1) = 0 were ; propranolol 0.16±0.02 and, IPS 339 0.20±0.04 nmole/kg/min.

Atenolol (3.75-37.5 nmole/kg/min) produced a non-dose-dependent parallel displacement of the procaterol curve without affecting maximum reduction of BP (EC_{50} ; 2.79±0.33 nmole/kg with 37.5 nmole/kg/min, n=10 and 4.14±0.35 nmole/kg with 3.75 nmole/kg/min, n=9, atenolol).

Butoxamine (33-66 nmole/kg/min) induced dose-dependent parallel displacements of the procaterol curve (EC_{50} ; 3.96±0.45 nmole/kg with 33 nmole/kg/min, n=6 and 14.98±1.34 nmole/kg with 66 nmole/kg/min, n=6). Again maximum reduction of BP was not significantly different from the control value.

The results obtained with propranolol and IPS 339 demonstrate the competitive nature of their antagonism, and confirm their considerable potency as $ß_2$ adrenergic antagonists and hence the $ß_2$ adrenergic nature of the procaterol induced reduction of BP. The data give a order of potency on vascular $ß_2$ receptors of : propranolol = IPS 339 $\gg$ butoxamine.

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PLASMA LIPIDS, KETONE BODIES AND INSULIN IN GENETICALLY HYPERTENSIVE RATS OF THE LYON STRAIN

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In a previous work (Sassolas et al, 1981) we reported a spontaneous increase in the hypertensive (LH) compared to normotensive (LN) and low blood pressure (LL) unfasted male rats of the Lyon strains. In the present study we attempted to extend this observation to fasted (17 h) females of the same 3 strains. Body weight (BW), systolic blood pressure (SBP), plasma trigycerides (TG), phospholipids (PL), total cholesterol (TC) radioimmunoassayable insulin and ketogenesis were recorded in the same rats between the ages of 5 and 32 weeks. The results (mean ± s.e. mean) were as follows :

Table 1

Age weeks	Strains (n)	SBP mmHg	BW g	TG mmol trioleine/l	PL g/l	TC mmol/l
5	LL (6)	107 ± 6	94 ± 5	0.9 ± 0.04	1.8 ± 0.08^{a}	2.5 ± 0.06
	LN (6)	116 ± 5^{ab}	88 ± 1^{ab}	0.9 ± 0.07^{ab}	1.6 ± 0.02^{ab}	2.4 ± 0.05^{ab}
	LH (6)	135 ± 2^{ab}	113 ± 3^{ab}	3.7 ± 0.33^{ab}	2.7 ± 0.10^{ab}	3.1 ± 0.09^{ab}
9	LL (11)	124 ± 4^{a}	161 ± 3^{a}	0.7 ± 0.09^{a}	1.5 ± 0.07^{a}	1.1 ± 0.06^{a}
	LN (11)	105 ± 2	147 ± 3	1.2 ± 0.10$_{b}$	1.7 ± 0.09	1.4 ± 0.07$_{ab}$
	LH (11)	124 ± 4^{a}	194 ± 2^{ab}	1.4 ± 0.15^{b}	2.3 ± 0.14^{ab}	1.7 ± 0.08^{ab}
21	LL (12)	115 ± 3	213 ± 2^{a}	0.9 ± 0.08^{a}	1.3 ± 0.03^{a}	1.1 ± 0.03^{a}
	LN (12)	116 ± 3$_{ab}$	191 ± 3$_{ab}$	0.7 ± 0.05$_{ab}$	1.7 ± 0.05$_{ab}$	1.7 ± 0.07
	LH (12)	141 ± 4^{ab}	261 ± 1^{ab}	1.3 ± 0.08^{ab}	2.1 ± 0.04^{ab}	2.0 ± 0.05^{a}
32	LL (12)	113 ± 2	231 ± 3^{a}	0.6 ± 0.04	1.3 ± 0.04	1.1 ± 0.04^{a}
	LN (12)	114 ± 2$_{ab}$	203 ± 4$_{ab}$	0.8 ± 0.09$_{ab}$	1.7 ± 0.06$_{ab}$	1.6 ± 0.06$_{ab}$
	LH (11)	133 ± 3^{ab}	279 ± 2^{ab}	1.1 ± 0.06^{ab}	2.1 ± 0.07^{ab}	2.1 ± 0.07^{ab}

a : $p < 0.05$ vs LN ; b : $p < 0.05$ vs LL

Early and persistent increased BW and plasma lipid concentrations were observed in fasted LH female rats. Plasma insulin did not differ in 5 week-old but was significantly increased in 22 week-old LH rats compared to LL (LH = 10 ± 1.2^{b} ; LN = 8.7 ± 0.9 ; LL = 6 ± 0.4 µU/ml). At the same age the ratio of plasma hydroxybutyrate to acetoacetate concentration was decreased in LH animals (LH = 1.3 ± 0.05^{ab} ; LN = 1.8 ± 0.1 ; LL = 2.7 ± 0.2).

In conclusion, while receiving a standard diet, LH rats spontaneously exhibit an hyperlipidemia with increased insulin secretion and decreased ketogenesis. The mechanism of these alterations remained to be precised, but it can be emphasized that LH rats are the only one to associate a genetic hypertension with increased BW and plasma lipid concentration.

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PROLONGED SUPRAMAXIMAL ANTIHYPERTENSIVE DOSES OF CLONIDINE ARE NEEDED TO CHANGE α_2-ADRENOCEPTORS IN SHR

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The withdrawal syndrome that can follow cessation of chronic clonidine treatment of hypertension has been attributed to changes in α_2-adrenoceptor numbers and sensitivity (see Parker and Atkinson, 1982; Conway and Jarrott, 1982). We have now studied the characteristics of α_2-adrenoceptors in the cerebral cortex and the vas deferens of spontaneously hypertensive rats (SHR) after stopping prolonged dosing of clonidine.

Male SHR (Charles River, France) 3 months of age weighing 210-270g were used. Systolic arterial blood pressure of conscious rats was measured indirectly using a tail cuff method. Clonidine was administered either by single injection (0.1 mg/kg, s.c.) or by i.v. perfusion using an implanted Alzet osmotic minipump. The pumps delivered either saline (at 0.1 µl/hr) or clonidine (at 0.1 mg/kg/24 hr or 0.5 mg/kg/24 hr) for 10 days. At various intervals after clonidine administration, animals were killed and the cortex and vas deferens were removed for studies of ^{3}H-clonidine binding (U'Prichard et al. 1977) and inhibition of the electrically-evoked twitch of the vas deferens (Shepperson et al 1981).

Prolonged administration of 0.1 mg/kg/24 hr clonidine lowered the blood pressure from 210 ± 6 mmHg by 25 ± 5 mmHg (n=8). Sixteen hours after cessation of the clonidine infusion the blood pressure had returned to control values. Clonidine binding studies using these animals gave K_D values that were higher (2.72 ± 0.24 nM, n=6) than in controls (2.04 ± 0.12 nM, n=9; $p<0.05$) but no difference in B_{max} was detected (clonidine 5.5 ± 0.5 pmol/g wet weight of tissue and controls 5.0 ± 0.3 pmol/g wet weight n=8/group). Forty hours after cessation of clonidine (0.1 mg/kg/24 hr, i.v.) no difference in values of K_D or B_{max} was observed. Vas deferens from these animals showed no difference in sensitivity to clonidine (IC_{50} clonidine, 11.9 ± 1.0 nM; control 10.4 ± 1.4 nM, n=6/group) or in the pA_2 value for the α_2-adrenoceptor blocking agent RX 78 1094 (Chapleo et al. 1981; pA_2 control 7.95 ± 0.07; clonidine treated 8.11 ± 0.05, n=6/group). Two and 16 hours after a single dose of clonidine (0.1 mg/kg, s.c.) the K_D values of ^{3}H-clonidine binding were higher (3.56 ± 0.38 nM and 3.30 ± 0.28 nM respectively) than in controls but the B_{max} values were unchanged. There were no differences in values of either K_D or B_{max} 40 hours after the same single dose. After i.v. injection of 0.5 mg/kg/day clonidine for 10 days animals exhibited aggressive behaviour, piloerection and exophthalmos. Forty hours after stopping clonidine administration, binding studies revealed a small increase in K_D (from 2.04 ± 0.12 nM, n=4 to 2.61 ± 0.17 nM, n=4; $p<0.05$) and a pronounced increase in B_{max} (from 5.0 ± 0.04 pmol/g to 7.6 ± 0.3 pmol/g, n=4/group; $p<0.05$). Furthermore, the vas deferens preparations were less sensitive to inhibition by clonidine (IC_{50} controls 10.17 ± 1.68 nM, n=4; clonidine 17.1 ± 1.2 nM, n=4; $p<0.05$) without a significant difference in pA_2 value for RX 78 1094.

These findings indicate that changes in α_2-adrenoceptors can be produced in SHR after stopping prolonged administration of clonidine in a supramaximal dose. Therefore, the cardiovascular effects observed during the withdrawal syndrome after antihypertensive doses of clonidine are not associated with changes in binding parameters of α_2-adrenoceptors.

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PHYSIOLOGICAL ROLE OF PREJUNCTIONAL α_2-ADRENOCEPTORS IN THE HEART OF SPONTANEOUSLY HYPERTENSIVE RATS

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The observation that α-adrenoceptor agonists can inhibit the release of noradrenaline from sympathetic nerve endings by a prejunctional mechanism is well documented (Langer, 1974; Starke, 1977). However, the physiological significance of the prejunctional α_2-adrenoceptor mediated feedback is still a subject of debate (Angus & Korner, 1980). Webb et al (1981) demonstrated that in contrast with young spontaneously hypertensive rats (SHR), noradrenergic nerve terminals in blood vessels of adult SHR can modulate the junctional concentration of noradrenaline. Although the observed modulation merely resulted from an increased neuronal uptake of noradrenaline in the blood vessels of adult SHR, the question arises whether the prejunctional α_2-adrenoceptor mediated feedback may play a more important role in adult SHR as well. In the present study we investigated the presynaptic modulation of heart rate responses to cardiac nerve stimulation in young (7 weeks) and adult (20 weeks) SHR and also in comparable Wistar Kyoto normotensive rats (WKY).

The animals were pithed, artificially ventilated and prepared for electrical stimulation (rectagular pulses, 2 ms, 50 V) of the spinal cardioaccelerator nerves (Th1-C7). The maximal increase in heart rate (beats/min) to trains of stimuli (25s) was measured. In all animals, B-HT 920 (100 µg/kg, i.v.) produced a maximal inhibition of the cardiac response to electrical stimulation, which was significant ($P < 0.05$) up to 1.0 Hz of stimulation frequency in young SHR and WKY and up tp 2.0 Hz in the adult animals. Rauwolscine (1 mg/kg, i.v.) produced a maximal potentiation of the cardiac response to electrical stimulation in SHR which was significant in the range 0.2-10 Hz of stimulation frequency in young SHR and from 0.1-10 Hz in adult SHR. In young WKY, rauwolscine did not potentiate the increase in heart rate to electrical stimulation, whereas in adult WKY α_2-adrenoceptor blockade by rauwolscine produced a small, but significant potentiation of the cardiac response to electrical stimulation from 0.2-10 Hz of stimulation frequency. In SHR, the control cardiac response to nerve stimulation (saline treated animals) was virtually identical to that observed in WKY. However, after rauwolscine the increase in heart rate to electrical stimulation was generally larger in SHR than in the corresponding WKY.

It is concluded that in adult animals the prejunctional α_2-adrenoceptor mediated feedback is more developed than in young rats. In contrast with young WKY, a significant endogenous feedback can be demonstrated in adult WKY. In SHR, however, the physiological significance of prejunctional α_2-adrenoceptors seems much more pronounced.

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28P

BETAXOLOL INHIBITS PRESSOR RESPONSES TO ELECTRICAL STIMULATION OF SPINAL CORD, IN SPONTANEOUSLY HYPERTENSIVE RATS

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Betaxolol is a β-adrenoceptor antagonist as potent as propranolol, but which exhibits a high degree of selectivity toward the β_1-adrenoceptor subtype (Boudot et al, 1979). Because this compound was found to lower aortic blood pressure in spontaneously hypertensive rats (Cavero and Lefèvre-Borg, 1983), the present study was designed to gain information on possible peripheral or central sites which can account for the mechanism of this action.

Spontaneously hypertensive rats (SHR), aged from 23 to 30 weeks, were orally given saline (5.0 ml/kg) or betaxolol (5.0 mg/kg), on the morning and the afternoon of the first day and on the morning of the second day , 2 hrs before they were pithed during a brief period of ether anesthesia. The animals were subsequently adrenalectomized. Arterial pressure was measured directly from a cannulated carotid artery. Responses to electrical stimulation of the spinal cord (0.5 Hz, 50.0 V, 1.0 msec), noradrenaline (0.5 μg/kg, i.v.) and tyramine (300.0 μg/kg, i.v.) were studied in saline and betaxolol pretreated rats, before and 10 min after starting an i.v. infusion of angiotensin II (0.05-0.1 μg/kg/min).

The dose regimen of betaxolol used in this investigation produced a significant antihypertensive effect in SHR, as reported elsewhere at this meeting (Cavero & Lefèvre-Borg, 1983). In pithed adrenalectomized SHR, betaxolol inhibited significantly ($p<0.05$, t-test) the pressor response to electrical stimulation of the spinal cord (betaxolol: 29.7 $\pm$ 1.4 mmHg, n=6; control: 49.2 $\pm$ 1.3 mmHg, n=5) but not those elicited by noradrenaline or tyramine. This inhibition was no longer present when the stimulation of the spinal cord was again performed during the infusion of a small dose of angiotensin II (betaxolol: 58.0 $\pm$ 3.6, n=6; control: 59.4 $\pm$ 3.2, n=5). The latter procedure failed to influence the magnitude of pressor response to i.v. noradrenaline.

These results indicate that betaxolol,in the dose regimen used, inhibits the pressor responses evoked by stimulation of the peripheral sympathetic nervous system. Although this effect is antagonized by angiotensin II, it remains to be assessed whether the antagonism is specific and, then, whether betaxolol interferes directly with the renin-angiotensin system.

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EARLY AND LATE ADMINISTRATION OF A PGI_2-ANALOGUE, ZK 36374: EFFECTS ON CARDIAC PRESERVATION, COLLATERAL BLOOD FLOW AND INFARCT SIZE

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ZK 36374 was shown recently to protect the myocardium from acute ischemic damage when infused 0.5 h after left anterior descending coronary artery (LAD) occlusion (Schrör et al., 1981). However, there is no information as to whether ZK 36374 would preserve myocellular integrity when administered later than 0.5 h and if the protective effects are due to increased collateral perfusion.

Pentobarbital anaesthetized adult cats were subjected to 5 h of LAD occlusion at time 0. I.v. infusion of ZK 36374 (1.19 µg/kg x min) was started at times 0.5, 2 or 4 h. As compared to myocardial ischemia (MI)-vehicle cats, ZK 36374 prevented the decrease in creatine kinase-specific activity, the loss of free amino nitrogen and the fall in percent bound cathepsin D in the ischemic area when infusion was started at times 0.5 or 2 h ($P < 0.05$) and the first two parameters also with infusion starting at time 4 h ($P < 0.05$).

For measurement of regional myocardial blood flow (RMBF) 15 µm radioactive microspheres were injected at times 0, 0.5 and 5 h. In sham control cats, ZK 36374 starting at 0.5 h increased LAD area epicardial RMBF by 100% at 5 h compared to vehicle animals ($P < 0.05$) while endocardial RMBF remained unchanged ($P > 0.05$). RMBF in the area at risk or the infarct area were not altered by ZK 36374 (Table 1).

TABLE 1: Regional myocardial blood flow [ml/min x 100 gww]

area	group	n	Epicardial 0h	Epicardial 5h	Epicardial %0h	Endocardial 0h	Endocardial 5h	Endocardial %0h
Control	Sham-vehicle	6	175 ± 23	134 ± 22	- 23	166 ± 11	159 ± 29	- 4
	Sham-ZK 36374	6	200 ± 28	263 ± 31	+ 32	166 ± 20	231 ± 27	+ 39
Risk	MI-vehicle	6	156 ± 24	66 ± 11	- 58	141 ± 17	68 ± 12	- 52
	MI-ZK 36374	5	156 ± 10	69 ± 11	- 56	149 ± 6	77 ± 27	- 48
Infarct	MI-vehicle	6	144 ± 23	15 ± 4	- 90	117 ± 12	11 ± 2	- 91
	MI-ZK 36374	5	147 ± 4	18 ± 7	- 88	130 ± 7	6 ± 2	- 95

The area at risk and infarct size were determined by injecting Evans blue into the left atrial catheter and by staining left ventricular tissue with nitro blue tetrazolium, respectively. Left ventricular and area at risk weights were similar between the two MI groups. In MI-vehicle cats the infarcted area was 9 ± 1% of the total left ventricle and 44 ± 7% of the area at risk and 8 ± 2% and 42 ± 7%, respectively, in MI-ZK 36374 cats ($P > 0.05$).

In conclusion, ZK 36374 exerted a consistent cardioprotective effect, as determined by biochemical indices after 0.5, 2 and 4 h of MI. This confirms previous findings with PGI_2. Myocellular protection by ZK 36374 does not result from increased perfusion of the risk or infarct areas and after 5 h is not associated with a smaller size of these zones. Previous studies have reported that at 24 h 19-33% of the left ventricle becomes infarcted with LAD occlusion in cats (Ritchie et al., 1981, Smith et al., 1982). Thus, ZK 36374 may still limit infarct size expansion at later times.

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THE EFFECTS OF IBOPAMINE, AN ORALLY ACTIVE DOPAMINE ANALOGUE, ON THE GUINEA-PIG ISOLATED WORKING HEART

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Ibopamine (IB), the di-isobutyric ester of N-methyl dopamine, increases cardiac contractility, renal blood flow and diuresis (Casagrande et al., 1980; Merlo et al., 1982). We have compared the cardiac effects of IB with those of dopamine (DO), epinine (N-methyl dopamine, EP) and isoprenaline (ISO).

Guinea-pig isolated working heart preparations were established (Flynn et al., 1978) and in each heart single cumulative concentration-response curves obtained for IB, DO, EP and ISO. Responses of sinus rate, dLVP/dt max, coronary flow, cardiac output, external work and stroke volume were measured as absolute changes from pre-drug control values. Responses to IB, DO and EP were also obtained in hearts pre-incubated (15 min) with propranolol 10^{-6}M and in hearts from reserpine-treated guinea-pigs (reserpine 1 mg/kg i.p. 24 hours before sacrifice).

IB (10^{-6}-10^{-5}M, DO and EP (3.16 X 10^{-6}-10^{-4}M) and ISO (10^{-10}-10^{-6}M) caused cardiac stimulation with concentration dependent increases in all measured parameters except stroke volume (which decreased). Compared with ISO, DO and EP, IB had a slower onset of action and took longer to achieve the maximum response to a given concentration.

For ISO, the maximum responses and EC_{50} values respectively were for dLVP/dt max 1815 ± 145 mmHg/sec and 2.2 X 10^{-9}M (range 1.0 - 3.7 X 10^{-9}M); and for sinus rate 152 ± 7 beats/min and 2.0 X 10^{-9}M (1.8 - 2.3 X 10^{-9}M) (n=3). On all parameters, IB was less potent than ISO but of similar potency to DO and EP as shown for dLVP/dt max and sinus rate in Table 1. The maximum responses caused by DO and EP respectively were for dLVP/dt max 1335 ± 342 and 1521 ± 342 mmHg/sec; and for sinus rate 123 ± 13 and 144 ± 13 beats/min. IB, at 3.16 X 10^{-5}M, caused an immediate, transient, depression of each heart followed by further stimulation. Under these *in vitro* conditions, IB 10^{-4}M, caused an initial depression of cardiac function followed by stimulation in 2 of 3 hearts.

Propranolol or reserpine abolished the stimulation caused by IB. Propranolol virtually abolished responses to both DO and EP whereas reserpine pre-treatment only reduced responses (see Table 1).

Table 1. Concentrations of ibopamine, dopamine and epinine causing 50% of isoprenaline maxima (all n=3)

	Control		Reserpine-treated	
	dLVP/dt max	Sinus rate	dLVP/dt max	Sinus rate
Ibopamine	7.2 X 10^{-6}M	1.0 X 10^{-5}M	-	-
Dopamine	7.1 X 10^{-6}M	2.5 X 10^{-6}M	2.4 X 10^{-4}M	6.6 X 10^{-4}M
Epinine	6.9 X 10^{-6}M	4.1 X 10^{-6}M	4.3 X 10^{-5}M	1.8 X 10^{-4}M

These results indicate that IB causes cardiac stimulation, like ISO, DO and EP, via β-adrenoceptors and that the agonist activity of IB like DO and EP includes an indirect component (release of cardiac noradrenaline). The slow onset of action and long duration required to achieve maximal response suggests that the cardiac stimulation observed following administration of IB may require hydrolysis of IB to form EP.

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DIFFERENT EFFECTS OF INTRAVENOUS AND INTRACORONARY PROSTACYCLIN AND ZK36374 ON CARDIAC ARRHYTHMIAS IN ANAESTHETIZED DOGS

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There have been contradictory reports regarding the effects of prostacyclin on cardiac arrhythmias induced by coronary artery ligation (e.g. Au et al, 1979; Coker & Parratt, 1981). It is possible that the systemic hypotensive action of prostacyclin may be a contributory factor in some of the adverse reports. We have therefore compared the effects of administering prostacyclin or a more stable analogue, ZK36374 (Ciloprost), either intravenously (100 ng $kg^{-1}min^{-1}$) or directly into the coronary circulation (5 ng $kg^{-1}min^{-1}$).

Chloralose anaesthetised, open-chest greyhounds were prepared for ligation of the left anterior descending coronary artery (Coker et al, 1982). Catheters were placed in the coronary sinus (CS), draining the essentially normal myocardium, and in a local coronary vein draining the area rendered ischaemic by coronary artery occlusion. Plasma concentrations of thromboxane (Tx) B_2 and 6-keto $PGF_{1\alpha}$ (a prostacyclin metabolite) were measured by radioimmunoassay. In dogs receiving intracoronary (i.c.) drug infusions a catheter was placed in the left circumflex coronary artery, under fluoroscopic control. The number of ventricular ectopic beats (VEBs) that occurred during the first 30 min of coronary artery occlusion was counted, along with the incidence of ventricular fibrillation (VF).

The results are summarised in Table 1.

Table 1 Effects on haemodynamics, 6-keto $PGF_{1\alpha}$ and arrhythmias

	Controls	Prostacyclin		ZK36374	
		i.v.	i.c.	i.v.	i.c.
Heart rate : pre-drug	141±5	158±8	127±5	148±5	144±6
(beats/min): post-drug	141±5	167±9*	129±3	173±10**	147±7
Mean BP : pre-drug	130±9	154±13	112±7	124±15	149±9
(mmHg) : post-drug	127±11	142±12*	106±6*	97±16*	141±10*
6-keto $PGF_{1\alpha}$ (pg/ml)					
Aorta : pre-drug	204±57	215±83	206±50	290±81	215±50
Aorta : post-drug	250±82	1705±604	298±68	235±73	240±49
CS : pre-drug	341±99	413±129	257±42	552±156	317±67
CS : post-drug	302±58	2066±363	1945±532	613±157	374±95
Incidence of VF	1/10	4/8	0/8φ	3/6	1/8
VEBs in survivors	720±136	536±407	327±167	537±164	307±110†

Values are mean±s.e.mean, *P<0.05 **P<0.01 compared with pre-drug value, † compared with controls, φ compared with prostacyclin i.v.

Neither drug altered the release of TxB_2 from the acutely ischaemic myocardium. The increase in heart rate observed in the dogs receiving i.v. drugs suggests that in these groups, systemic hypotension may have been at least partially offset by a reflex increase in sympathetic drive. It is possible that increased sympathetic nervous activity could account for the higher incidence of VF in dogs treated with i.v. prostacyclin or ZK36374.

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CALCIUM ANTAGONISTS AND MYOCARDIAL PROTECTION DURING CARDIOPLEGIC ARREST

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We have investigated whether the addition of three different calcium antagonists (verapamil, nifedipine, diltiazem) to a chemical cardioplegic solution can increase myocardial protection above that afforded by the cardioplegic solution alone. For this study we selected the isolated 'working' rat heart preparation as a model of cardiopulmonary bypass and ischaemic arrest. Isolated rat hearts were subjected to 5 min aerobic Langendorff perfusion; 20 min aerobic 'working' perfusion, 3 min cardioplegic infusion (37°C or 20°C) with the St. Thomas' cardioplegic solution (NaCl 110.0, KCl 16.0, $MgCl_2$ 16.0, $CaCl_2$ 1.2, $NaHCO_3$ 10.0 mM pH 7.8, 324 mOsm/Kg H_2O) containing various concentrations of calcium antagonists; 35 min (37°C) or 150 min (20°C) ischaemic arrest (in the hypothermia series the cardioplegic solution was reinfused at 30 min intervals); 15 min aerobic Langendorff reperfusion (37°C); 20 min aerobic working reperfusion (37°C). The perfusion fluid was Krebs-Henseleit bicarbonate buffer (pH 7.4 gassed with 95% O_2: 5% CO_2). The recovery of aortic flow, coronary flow, aortic pressure and heart rate were measured and cardiac output and stroke volume were calculated. In addition, the leakage of creatine kinase into the coronary effluent during the Langendorff reperfusion period was measured as an index of tissue damage.

Dose response curves for the protective effects of calcium antagonists in the cardioplegic solution showed that all drugs possessed 'bell-shaped' dose response curves. Optimal additive concentrations were: 1.2 μM for verapamil, 0.075 μM for nifedipine and 0.5 μM for diltiazem. Under these optimal conditions, the percentage post-ischaemic recovery of aortic flow was improved from its control value of 65.3 ± 2.6 to 77.5 ± 2.7 by verapamil ($p < 0.01$) 47.9 ± 5.2 to 76.7 ± 2.9% by nifedipine ($p < 0.001$) and 53.2 ± 43 to 79.2 ± 4.4% by diltiazem ($p < 0.01$) and post-ischaemic creatine kinase leakage was reduced by approximately 30%, 50% and 40% by verapamil, nifedipine and diltiazem respectively. Despite the marked additional protection under conditions of normothermic ischaemic arrest, all three drugs failed to improve contractile recovery after a period of hypothermic ischaemic arrest.

In order to investigate the loss of protective properties under hypothermic conditions and also to determine the exact temperature dependency characteristics of calcium antagonists, hearts were subjected to hypothermic arrest at various temperatures between 20°C and 37°C with and without verapamil (1.0 mM included in the cardioplegic solution (single dose cardioplegia)). The duration of each period of arrest was varied so as to ensure an approximate recovery of 45-55% in the control group. By subtracting the post-ischaemic recovery (cardiac output) in the control and verapamil groups, the percentage improvement attributable to the inclusion of verapamil could be calculated. The values were 28.4 ± 4.0% at 37°C (35 min ischaemia), 38.9 ± 4.7% at 34°C (50 min ischaemia), 17.3 ± 4.6% at 31°C (55 min ischaemia), 15.7 ± 3.6% at 29°C (60 min ischaemia), 13.8 ± 4.5% at 27°C (80 min ischaemia), 6.9 ± 5.2% at 25°C (100 min ischaemia), 7.9 ± 3.4% at 20°C (130 min ischaemia).

From these results it is clear that verapamil effectively loses its protective properties at temperatures below 25°C.

THE EFFECTS OF NIFEDIPINE, TETRODOTOXIN AND BEPRIDIL ON REPERFUSION-INDUCED ARRHYTHMIAS IN THE RAT ISOLATED PERFUSED HEART

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Bepridil (B) is an antianginal agent (Cosnier et al, 1977) which possesses additional antiarrhythmic properties. B inhibits both the fast and slow inward ion currents in mammalian myocardium (Vogel et al, 1979; Kane and Winslow, 1980) and both of these actions could contribute to its antiarrhythmic activity. The aim of the present study was to compare the effects of bepridil with those of the specific fast sodium channel blocking agent, tetrodotoxin (T) and the specific calcium antagonist, nifedipine (N) on reperfusion induced arrhythmias in the isolated rat heart.

Hearts were perfused at constant pressure (9.3 kPa) with Krebs Henseleit solution containing 4.5 mM K^+. The main left coronary artery was ligated for 20 min and the ischaemic area then perfused for 15 min. Drug or vehicle perfusion was begun 10 min before ligation and was continued for the duration of the experiments. Solutions of nifedipine (in ethanol and polyethylene glycol) were protected from light.

The percent incidence of reperfusion induced premature ventricular systoles (PVS), ventricular tachycardia (VT) and ventricular fibrillation (VF) are shown in Table 1.

Table 1 Antiarrhythmic and electrocardiographic effects of bepridil, tetrodotoxin and nifedipine n=6-19 **P<0.001 *P<0.05

		% incidence of			QTc (s)	
Treatment	μM	PVS	VT	VF	predrug	20' postligation
Control (Krebs)		100	100	100	0.26±0.01	0.27±0.01
Bepridil	1.0	100	100	*57	0.25±0.01	0.27±0.01
	2.0	*75	*63	**13	-	-
	4.0	**33	**17	**0	0.26±0.01	**0.35±0.02
Tetrodotoxin	0.16	100	83	*67	0.32±0.01	0.27±0.02
	0.63	100	100	**33	0.30±0.01	0.29±0.02
	1.57	*67	**33	**0	0.30±0.01	0.23±0.04
Nifedipine Solvent		100	100	*57	0.28±0.02	0.26±0.02
Nifedipine	0.02	100	100	100	0.30±0.01	0.28±0.02
	0.2	100	100	100	0.28±0.01	0.28±0.01

Both B and T antagonised the development of these arrhythmias in a concentration-dependent manner, whereas N was inactive even at a concentration (0.2 μM) which reduced contractility by 73±6% within 10 min of perfusion. Both B and T also reduced contractility (59±6 and 49±15% respectively at the highest concentrations used). In the high concentration B and T groups, heart rate fell from predrug values of 229±7 and 265±17 to 123±17 and 129±34 beats/min respectively at the end of the occlusion period, whereas N was without effect on cardiac rate. The PR interval was unchanged by N whereas B and T caused a prolongation which was particularly marked at concentrations of 4 and 1.57 μM respectively. B also prolonged the QTc interval (Table 1) whereas T and N did not.

Both B and N (but not T) produced increases in coronary flow. It is concluded the antiarrhythmic effects of bepridil in this preparation are more probably related to its inhibitory effect on the fast inward sodium current rather than to its calcium antagonistic effect.

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THE PHARMACOLOGY OF THE TWO STEREOISOMERS OF PRIZIDILOL

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Prizidilol (SK&F 92657) is an arterial vasodilator and β-adrenoceptor antagonist in animals (Taylor et al, 1981) and in man (Collier & Pitcher, 1980) which causes a sustained fall in systemic blood pressure, particularly in hypertensive subjects, without a corresponding increase in heart rate or cardiac output (Lund-Johansen & Omvik, 1982). The two stereoisomers (R and S) of prizidilol have been prepared and tested for vasodilator, antihypertensive and β-adrenoceptor blocking activity and the findings compared with the results obtained with the racemic mixture.

Both R and S prizidilol 10, 20 and 40 μmol/kg s.c. (10 μmol = 4 mg) caused similar dose related decreases in BP in conscious spontaneously hypertensive rats (n = 6-9 per dose) which were maximal at about 3-5 h after dosing. The doses of R and S prizidilol required to cause a 40 mmHg fall in mean BP were 13.5 and 18.5 μmol/kg respectively. Under similar conditions the value for RS prizidilol was 13 μmol/kg. There was no statistically significant difference between these values. Similarly, the three compounds caused falls in BP of anaesthetised, normotensive rats with concomitant and parallel increases in blood flow to the hindquarters (max. increase about 100%) autoperfused at constant pressure using a servo controlled peristaltic pump via a double cannulation of the abdominal aorta caudal to the renal arteries. The doses of R, S and RS prizidilol needed to give a 50% increase in hindquarter blood flow were 15.7, 18.1 and 11 μmol/kg i.a. respectively (compounds injected into arterial inflow to hindquarters).

Although both stereoisomers were β-adrenoceptor antagonists *in vivo* (anaesthetised cat) and *in vitro*, most activity resided in the S isomer which was about twice as potent as the racemic mixture and about 25-50 times more active than the R isomer (Table 1).

Table 1. β-adrenoceptor blockade in vivo and in vitro: agonist - isoprenaline

Compound	ED_{50} (μmol/kg i.v.) in anaesthetised cats		pA_2 g. pig right atrium
	Tachycardia (β_1)	Vasodilator (β_2)	(95% C.L.) and slope
R prizidilol	1.3 ± 0.09	0.68 ± 0.1	5.96(5.41-6.96):0.90±0.14
S prizidilol	0.025 ± 0.002	0.028 ± 0.003	7.28(6.92-7.96):1.06±0.35
RS prizidilol	0.058 ± 0.005	0.062 ± 0.005	7.16(6.14-8.87):1.14±0.31
Propranolol	0.08	0.1	7.87(6.87-8.60):1.26±0.41
Practolol	1.1	>10	6.93(4.58-7.29):0.62±0.26

Conclusions: Both R and S stereoisomers of prizidilol are equiactive as vasodilator/antihypertensives but β-adrenoceptor blocking activity is mainly associated with the S isomer.

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EFFECT OF NEOMYCIN ON MEMBRANE POTENTIALS ACROSS THE BRUSH BORDER OF RAT SMALL INTESTINE

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The addition of neomycin to the mucosal fluid of rat small intestine has been reported to increase sugar absorption measured both in vitro (Rogers et al, 1968) and in vivo (Broitman et al, 1967). Since it has been suggested that the potential difference across the brush border membrane is an important driving force for Na^+-hexose entry into the epithelial cell (Schultz, 1977) we have examined the effect of neomycin on the mucosal membrane potential difference ($p.d._m$) of enterocytes in the rat small intestine.

Rats were anaesthetised with pentobarbitone and 1 cm. sections of upper jejunum were removed. The tissue was cut longitudinally and rapidly mounted as a flat sheet on the base of a Perspex chamber using tissue adhesive (R.S. Components) applied to the muscle side. The preparation was superfused at 35 ± 1°C with bicarbonate saline (2 $ml.min^{-1}$) and $p.d._m$ was monitored using 3M KCl filled glass microelectrodes connected to a WPI electrometer (Model KS 700). Neomycin when present in the mucosal fluid caused a dose dependent increase in $p.d._m$ (Table 1).

Table 1 Effect of neomycin on $p.d._m$

	$p.d._m$	
Control	-45.5 ± 1.4 (99)	
Neomycin (10^{-5}M)	-51.5 ± 1.6 (35)	$P < 0.02$
Neomycin (10^{-4}M)	-58.7 ± 1.6 (35)	$P < 0.001$
Neomycin (10^{-3}M)	-58.7 ± 1.8 (25)	$P < 0.001$

Results are given in mV and expressed as Mean ± S.E. with number of impalements in brackets.

The addition of galactose (4 mM) resulted in $p.d._m$ values of -40.3 ± 1.9 mV (50) and -44.8 ± 2.1 mV (29) in the absence and presence of neomycin (10^{-4}M) respectively ($P < 0.05$; $P < 0.001$ compared to appropriate values given in Table 1).

As active hexose entry requires the presence of Na^+, an increase in $p.d._m$ would be expected to increase the driving force for transport across the brush border. Since neomycin causes an increase in $p.d._m$ it is likely that this hyperpolarisation is responsible, at least in part, for the enhancement of sugar absorption across rat small intestine.

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THE EFFECTS OF CHANGING SURFACE CHARGE WITH SALICYLATE ON THE POTENCY OF CALCIUM-ANTAGONISTS IN SMOOTH MUSCLE

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The potency of cinnarizine and flunarizine, but not diltiazem, verapamil or nifedipine, as antagonists of Ca^{++}-induced contractions in K^{+}-depolarized smooth muscle is reduced if Ca^{++} (100μM) is included in the medium during the incubation period (Spedding, 1982a). This protective effect of Ca^{++} may be due to neutralization of membrane negative surface charge. I have, therefore, tested the potency of these drugs in the presence of salicylate ion which is absorbed onto cell membranes, thereby increasing the number of negative charges (McLaughlin, 1973 ; Cohen et al., 1979).

Taenia preparations from the guinea-pig caecum were set up in Ca^{++}-free Tyrode solution containing K^{+} (40 mM) and the isotonic (1 g load) responses to cumulative addition of Ca^{++} (0.1-30 mM) were recorded at 30 min intervals (Spedding, 1982a). Control preparations where the antagonist was incubated for 20 min in the absence of salicylate were run in parallel with preparations where the antagonist was incubated with sodium salicylate (10 mM). Sodium salicylate did not reduce the sensitivity of the preparations to Ca^{++}, although the contractions were less well sustained.

Salicylate reduced the inhibitory effects of diltiazem and verapamil, did not change the effects of nifedipine and increased the effects of cinnarizine and flunarizine (Table 1).

Table 1. Antagonist effects of calcium-antagonists in the absence or presence of sodium salicylate (10 mM)

Antagonist	Control	Salicylate	n
Diltiazem, 1 μM	25.1 (20.9-30.2)	6.5 (4.8-8.7)**	5
Verapamil, 0.2 μM	20.0 (14.4-27.5)	5.5 (3.5-6.9)	5
Nifedipine, 3 nM	2.5 (2.3-2.6)	2.8 (2.3-3.5)	5
Nifedipine, 10 nM	64.7 (49.9-82.4)	47.0 (39.8-74.9)*	8
Cinnarizine, 1 μM	2.5 (2.1-2.9)	8.5 (6.0-12.9)**	7
Flunarizine, 1 μM	2.8 (2.3-3.4)	10.2 (6.1-17.4)	5

Values are mean Ca^{++} dose-ratios with standard error ranges. * $p < 0.05$

These results show that the effects of some calcium-antagonists may be influenced by surface charge. The protective effect of Ca^{++} against cinnarizine and flunarizine (Spedding, 1982a) is therefore likely to be due to neutralisation of negative surface charges. Negative surface charges increase the membrane permeability to cationic drugs such as cinnarizine and flunarizine (McLaughlin, 1973) thereby increasing access to intracellular sites (Spedding, 1982b). The lack of effect of salicylate on nifedipine indicates that this drug interacts with Ca^{++} channels by a mechanism independent of surface charge and perhaps independent of Ca^{++} gating. Nifedipine therefore blocks Ca^{++} channels in smooth muscle by a mechanism which is distinct from that of verapamil, as is the case in the myocardium (Bayer & Ehara, 1978). These findings provide further evidence supporting the classification of calcium-antagonists into three distinct subgroups (Spedding, 1982a).

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EVIDENCE FOR THE PRESENCE OF α_2-ADRENOCEPTOR ON THE RENAL VASCULAR SMOOTH MUSCLE OF THE RABBIT

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The existence of post-synaptic α_2-adrenoceptors on the vascular smooth muscle of various species is now well documented (Timmermans & van Zwieten, 1981; McGrath, 1982) and have been demonstrated in the renal circulation of the rat and dog but not in the cat (Drew and Whiting, 1979). It was the aim of this study to determine whether α_2-adrenoceptors were present on the renal vascular smooth muscle of the rabbit.

The left kidney of sodium pentobarbitone anaesthetized Carolina rabbits (2.0 - 3.5 kg) was exposed retroperitoneally, the renal nerves sectioned and dissected to give sufficient length for stimulation. Renal blood flow was measured using a non-cannulating probe and electromagnetic flowmeter. α-Adrenoceptor agonists and antagonists were administered directly into the renal artery via a cannula placed in a lumbar branch of the renal artery. The peak renal vasoconstrictor effects of 2 min periods of direct renal nerve stimulation (15v, 0.4 msec) and bolus doses of phenylephrine, which caused roughly equivasoconstrictor responses were determined before and following administration of either prazosin or phentolamine at a number of dose levels.

Prazosin and phentolamine inhibited the renal vasoconstrictor responses to phenylephrine in a dose dependent manner, causing a 50% inhibition at 0.031 ± 0.006 and 1.7 ± 0.6 μmol/kg respectively. Phentolamine similarly inhibited the nerve mediated vasoconstriction with 50% of the original response being blocked by 4.4 ± 1.8 μmol/kg. The inhibition by prazosin of the nerve mediated vasoconstriction was very different, being only modestly inhibited (20-40%) over most of the dose range studied and 50% inhibition was achieved by 1.1 ± 0.1 μmol/kg prazosin, some 40 times that required to block a similar phenylephrine induced vasoconstriction. These results indicate that endogenous released noradrenaline, acted on α_1-adrenoceptors, sensitive to blockade by prazosin and phentolamine, and also on other adrenoceptors, sensitive to blockade by phentolamine but not prazosin, which are most probably α_2-adrenoceptors.

The ability of yohimbine to inhibit equivalent renal vasoconstrictor responses to clonidine (9 to 30 nmole), phenylephrine (2.5 to 7.5 nmole), noradrenaline (0.1 to 0.6 nmole) and renal nerve stimulation ($2H_7$), was determined. Over the dose range 77 to 770 nmol/kg, yohimbine antagonised the clonidine induced vasoconstriction in a dose related fashion and a similar pattern of antagonism was observed with the noradrenaline responses. However, yohimbine failed to antagonise the phenylephrine induced vasoconstriction except at the highest dose. The renal nerve induced vasoconstriction tended to be potentiated at the lower doses of yohimbine but were not attenuated by even the highest dose of yohimbine tested.

Taken together the results of these studies provide evidence for the presence of post-synaptic α_2-adrenoceptors on the renal vascular smooth muscle of the rabbit.

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A MICROPUNCTURE STUDY OF THE RENAL HANDLING OF SODIUM DURING CHRONIC HYDROCHLOROTHIAZIDE ADMINISTRATION IN THE RAT

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Clearance and micropuncture experiments have indicated that thiazides exert their diuretic effect mainly by inhibiting salt reabsorption in the distal tubule (Seely & Dirks, 1977). These findings, however, apply to the acute situation and may not necessarily reflect the actions of thiazides in the clinical setting where they are given chronically. The present study reports the effects of chronic hydrochlorothiazide administration on the renal handling of sodium.

Twenty-six male Long Evans rats (200-260g) were placed in metabolism cages. After initial habituation and control periods, fourteen rats were given hydrochlorothiazide in their food (350 mg/kg dry weight; ~5mg/day) for 1-2 weeks. The other animals remained on the control diet. Hydrochlorothiazide induced a significant increase in Na excretion only on the first day of treatment; thereafter Na excretion in control and thiazide-treated rats was almost identical. At the end of the period in metabolism cages, each rat was anaesthetized with Inactin (120 mg/kg body weight) and prepared for micropuncture (Walter et al, 1979). Collections were made from late proximal convoluted tubules and from early and late distal tubules. Results from each of these nephron segments were expressed as an average value per rat.

Sodium excretion during micropuncture was similar to that before anaesthesia and was not significantly different between the 2 groups of animals (32.5 ± 5.9 and 37.1 ± 3.4 μmol/h per g kidney in control and thiazide-treated rats respectively). Total and single nephron glomerular filtration rates also were not significantly different between the 2 groups. The fraction of filtered Na reabsorbed in the proximal convoluted tubule was significantly increased in the thiazide-treated animals (0.659 ± 0.013 vs 0.567 ± 0.020, $P < 0.001$) and there was a reduction in the delivery of Na to the pars recta (Table 1). Sodium delivery to the early part of the distal tubule was also reduced in thiazide-treated rats, but by the end of the distal tubule there was no significant difference between the groups (Table 1).

Table 1 Sodium delivery (pmol/min per g kidney) to different nephron sites

	Glomerular filtrate	Late proximal convoluted tubule	Early distal tubule	Late distal tubule
Control	7016 ± 230	3013 ± 144	531 ± 50	195 ± 32
	P<0.05	P<0.001	P<0.05	NS
Hydrochlorothiazide	6330 ± 224	2153 ± 80	403 ± 28	172 ± 25

The results indicate that the return to normal rates of Na excretion during chronic hydrochlorothiazide treatment is due at least in part to a reduction in the amount of Na delivered to the distal tubule, where thiazides exert their major effect.

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THE EFFECTS OF LEAD ON THE RENIN-ANGIOTENSIN SYSTEM

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Studies in man of the effects of chronic lead exposure on the renin-angiotensin system are contradictory. Decreased plasma renin activity has been reported (McAllister et al, 1971) whilst other workers have found no change in renin activity (Campbell et al, 1979). In animal studies Goldman et al, (1981) demonstrated that acute lead exposure resulted in a sharp rise in plasma renin activity. They postulated that this might be due to both reduced clearance of renin and an inhibition of angiotensin converting enzyme. We have investigated the effects of lead on components of the renin-angiotensin system both in vivo and in vitro.

Male New Zealand white rabbits (2-2.5 kg) were treated acutely with a single intravenous infusion of lead (as acetate) at a dose of 3 mg/kg and compared with a control infusion of sodium acetate in the same animals. Mean arterial pressure (MAP) and heart rate were measured in the conscious animal and blood samples were withdrawn at frequent intervals for 6 hours post infusion. Plasma renin activity (PRA) was determined by radioimmunoassay and plasma angiotensin converting enzyme (ACE) activity was measured by an HPLC assay based on the determination of hippurate produced by the action of the enzyme on the tripeptide hippuryl-histidyl-leucine.

Lead treatment was associated with a small but significant ($p < 0.05$) rise in MAP as assessed by repeated measures analysis of variance of test and control days. The elevation was significant ($p < 0.01$) from 10 to 120 minutes post infusion. Heart rate was not altered by lead treatment. The PRA increased throughout the period of study in the lead treated group, and at 360 minutes PRA in the lead treated animals was 70.3 ±26.2 ng/ml/h compared to 3.8 ± 2.0 ng/ml/h in control animals ($p < 0.01$). The activity of ACE was not altered by lead treatment when compared to control.

The effect of lead on renin release in vitro was studied using slices of rabbit renal cortex (Derkx et al, 1982). Four slices (15-20 mg dry weight) 0.5-1.0 mm thick were prepared from the outer cortex of each kidney. The slices were incubated in a shaking water bath at 37°C in 5ml of a medium containing NaCl 119 mmol/l, $NaHCO_3$ 25 mmol/l, KCl 4.7 mmol/l, Na_2HPO_4 1.2 mmol/l, $MgCl_2$ 1.2 mmol/l, $CaCl_2$ 2.75 mmol/l, glucose 5.65 mmol/l, and ascorbic acid 6 mmol/l. The pH of the medium was adjusted to 7.4 and the slices were incubated for four successive 20 minute periods. Lead or anion control were added during the third and fourth period. Renin release did not change during periods 1 to 4 in untreated slices nor in slices treated with anion control (sodium acetate). Lead at a concentration of 2 µmol/l resulted in a 160 ± 22% increase in renin release, whilst lead at a concentration of 10 µmol/l increased renin release by 215 ± 40%.

It is apparent that the in vivo rise in renin activity associated with lead treatment is not mediated by an inhibition of angiotensin converting enzyme but by a direct action of lead on renin release.

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PRESYNAPTIC α_2-ADRENOCEPTORS AND THE EFFECT OF PHENOXYBENZAMINE ON (^{3}H)-NORADRENALINE RELEASE BY A SINGLE ELECTRICAL PULSE

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Phenoxybenzamine increased the release of tritium from guinea-pig vasa deferentia preloaded with (^{3}H)-noradrenaline following a single electrical pulse (Kalsner, 1979). It was concluded that the presynaptic α_2-adrenoceptor hypothesis of the feedback control of noradrenaline release should be reconsidered. Other experiments with the selective α_2-adrenoceptor antagonists yohimbine and RX 781094 did not find an increased release of (^{3}H)-noradrenaline following one pulse in the mouse vas deferens (Baker & Marshall, 1982 a & b). The reasons for this difference (e.g. species, combination in phenoxybenzamine of uptake blockade in addition to α-receptor antagonist properties) have now been investigated.

Whole vasa from Dunkin-Hartley guinea-pigs or T.O. mice were incubated for 30 min in 1-7,8-(^{3}H)-noradrenaline (Sp. Act. 34 Ci/mmol; 0.59 µM). After washing for 90 min the vasa deferentia were field stimulated (2 ms pulse width) with single pulses spaced at least 15 min apart. The tritium overflow was separated into (^{3}H)-noradrenaline and several (^{3}H)-metabolite fractions.

In the guinea-pig vas deferens the basal release of tritium was increased by more than 2 and 6 fold after 30 min incubation with phenoxybenzamine 10 and 30 µM respectively. The control fractional release of (^{3}H)-noradrenaline with a single pulse was $9.0 \pm 2.1 \times 10^{-6}$ (mean ± s.e. mean) which rose more than 2 and 5 fold in the presence of phenoxybenzamine 10 and 30 µM respectively. In the mouse vas deferens the basal release of tritium and the fractional release of (^{3}H)-noradrenaline (control value, $8.9 \pm 1.6 \times 10^{-6}$) were increased by phenoxybenzamine 10 and 30 µM by more than 2 and 5 fold respectively.

The phenoxybenzamine result might be due to antagonism at presynaptic α_2-adrenoceptors in the presence of neuronal and extra-neuronal uptake blockade. Therefore an attempt was made to reproduce the effect of phenoxybenzamine using more selective drugs to block uptake and α_2-adrenoceptors. After loading mice vasa deferentia with (^{3}H)-noradrenaline, cocaine 10 µM and 17-β-oestradiol 10 µM were added to the Krebs solution. This increased the fractional release of (^{3}H)-noradrenaline by one pulse to $22 \pm 4.6 \times 10^{-6}$. When the α_2-adrenoceptor antagonist RX 781094 (Chapleo et al, 1981) 30, 100 or 300 nM (20 min equilibration) was added to the cocaine and oestradiol the fractional release of (^{3}H)-noradrenaline was not significantly altered ($P > 0.05$, paired t-test).

These results confirm that phenoxybenzamine increases the release of (^{3}H)-noradrenaline following a single pulse in the guinea-pig vas deferens. This effect also occurs in the mouse vas deferens and in both species phenoxybenzamine induces a marked rise in the basal release of tritium. Selective α_2-adrenoceptor antagonists do not alter basal tritium efflux (Baker & Marshall, 1982 a & b). The release of (^{3}H)-noradrenaline with 1 pulse is unaltered by the α_2-adrenoceptor antagonist RX 781094 either on its own (Baker & Marshall, 1982 b) or after blockade of neuronal and extra-neuronal uptake mechanisms. In conclusion there is no evidence to associate the increased (^{3}H)-noradrenaline release by a single pulse with phenoxybenzamine with an action at presynaptic α_2-adrenoceptors.

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