THE BEHAVIOURAL AND BIOCHEMICAL CONSEQUENCES OF DOPAMINE INFUSION INTO THE FRONTAL CORTEX OF RAT BRAIN

Amanda J. Bradbury, Brenda Costall, Annette M. Domeney & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

The infusion of dopamine (DA) into the nucleus accumbens of rat brain causes biphasic changes in locomotor activity during infusion with consequent changes, post-infusion, in locomotor responsiveness to the DA agonist (-)N-n-propylnor-apomorphine [(-)NPA] (Costall et al, 1982). The present study investigated the immediate and long-term changes in locomotor responsiveness following infusion of DA into another DA terminal area, the frontal cortex (FC).

Male Sprague-Dawley rats were selected as low hyperactivity responders to (-)NPA, measured using 'photocell cages' (Costall et al, 1982). They were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for bilateral infusion into the FC (Ant. 11.7, Vert. 4.5, Lat. $\pm$1.6, Pellegrino et al, 1979). Infusion of vehicle or DA commenced 14 days after surgery and was effected by Alzet osmotic minipumps (25 µg/24 h, 0.48 µl/h). 'Spontaneous' locomotor activity was assessed daily during the 13 days of infusion and for 6 weeks after discontinuing infusion. Hyperactivity responding to (-)NPA was assessed on the 2nd post-infusion day and thereafter at weekly intervals for 9 weeks. Rats were then killed by cervical dislocation and the levels of DA, noradrenaline (NA) and 3,4-dihydroxyphenylacetic acid (DOPAC) determined in the anteromedial (FC), suprarhinal (SR), limbic (tuberculum olfactorium plus nucleus accumbens, L) and striatal (ST) DA fibre systems using HPLC and electrochemical detection.

Spontaneous locomotion was not changed by DA or vehicle infusion either during the 13 days of infusion into the FC or the 9 weeks post-infusion. However, DA (but not vehicle) markedly enhanced the hyperactivity responding to (-)NPA, this achieving up to 350% of baseline values by 6 weeks post-infusion. This marked behavioural difference between vehicle and DA infused rats was still apparent at the time of sacrifice for biochemical assessment (9 weeks). The biochemical parameters of vehicle and control animals were indistinguishable but the animals which had received a DA infusion in their FC 9 weeks earlier had raised DA levels in the FC (55 $\pm$ 4 increased to 284 $\pm$ 98 pg/mg, $P < 0.05$) with no significant change in DOPAC levels (36 $\pm$ 6 compared to 55 $\pm$ 26 pg/mg), so reducing the DOPAC/DA ratio from 0.65 to 0.2. In contrast, DA levels were reduced in the SR (31 $\pm$ 2 reduced to 18 $\pm$ 2 pg/mg, $P < 0.01$) but DOPAC levels were too small to reliably determine. The long-term effects of the FC DA infusion was also to reduce NA levels in the SR and FC (345 $\pm$ 11 reduced to 236 $\pm$ 14 pg/mg, $P < 0.01$, 260 $\pm$ 9 reduced to 192 $\pm$ 12 pg/mg, $P < 0.01$, respectively). However, DA,DOPAC and NA levels were not significantly altered in the ST (7.5-8.2 ng/mg, 610-744 pg/mg, 147-177 pg/mg respectively) or L (1.8-2.1 ng/mg, 225-248 pg/mg, 107-115 pg/mg respectively).

Thus, a chronic behavioural change detectable 9 weeks after a DA infusion into the FC of rat brain may be linked with chronic biochemical disturbance detectable in the FC after the 9 weeks. Long-term compensatory changes in the neurochemistry of the SR may also occur.

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CONTROL OF FOREBRAIN DOPAMINE FUNCTION FROM THE MIDBRAIN

Amanda J. Bradbury, Brenda Costall, M. Elizabeth Kelly & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Dopamine (DA) agonists can inhibit the firing of midbrain DA cells (see Bunney, 1979) and DA injected into the midbrain can reduce locomotor activity (Bradbury et al, 1983). Here we report effects of DA agonist-antagonist injections in the midbrain on forebrain DA function.

Male Albino mice were stereotaxically prepared for intra-nigral injection, and spontaneous locomotion measured in photocell cages as described by Bradbury et al (1983). At the time of maximum behavioural change (20 min) animals were killed and levels of DA and dihydroxyphenylacetic acid (DOPAC) determined in the brain areas specified in Table 1 using HPLC with electrochemical detection.

Intra-nigral 2-di-n-propylamino-5,6-dihydroxytetralin (tetralin, 0.0025-0.05 µg) dose-dependently reduced locomotor activity (309 ± 33 - 78 ± 8 counts/20 min respectively, control 352 ± 42 counts/20 min, $P < 0.01$ - $P < 0.001$ at 0.0125-0.05 µg), whilst (-)sulpiride (0.05-0.2 µg) consistently increased locomotor activity (134-177%, $P < 0.001$ at the highest dose. The biochemical consequences are shown in Table 1.

Table 1 Effects of intra-nigral tetralin (0.05 µg) and (-)sulpiride (0.2 µg) on forebrain DA function

Intra-nigral treatment	STRIATUM (ST)			LIMBIC (L)		
	DOPAC(A) pg/mg	DA(B) ng/mg	Ratio A/B x 10^3	DOPAC(A) pg/mg	DA(B) ng/mg	Ratio A/B x 10^3
Control	1665±79	13.66±0.64	0.12	611±50	3.40±0.22	0.18
Tetralin	1099±75**	17.36±1.6	0.06	636±42	5.50±0.36***	0.12
Sulpiride	1567±61	14.39±0.84	0.11	1075±150**	3.59±0.24	0.30
	FRONTAL CORTEX (FC)			SUPRARHINAL CORTEX (SR)		
Control	39±4	0.051±0.004	0.76	91±15	0.058±0.014	1.5
Tetralin	20±2*	0.065±0.008	0.3	38±4*	0.054±0.014	0.7
Sulpiride	101±11**	0.132±0.008***	0.76	118±8	0.131±0.04	0.9

n = 5-6. *P < 0.05, **P < 0.01, ***P < 0.001 (Student's t test).

The tetralin injection is almost certain to diffuse and influence DA cells projecting to the striatal, limbic and cortical regions. The decrease (34-62%) in DOPAC levels in the FC, SR and ST suggests decreased DA release in these areas, supported by reduced DOPAC/DA ratios. However, (-)sulpiride increased (161-259%) DOPAC levels in the L and FC tissues. These findings support the general concept that stimulation of midbrain DA systems may reduce forebrain DA function and that within the midbrain there normally exists a tonic inhibitory DA influence. The specificity of the tetralin and sulpiride effects are being assessed.

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THE COMPARATIVE PHARMACOLOGY OF TWO SELECTIVE INHIBITORS OF MONOAMINEOXIDASE B: DEPRENYL AND MDL 72145

J.R. Fozard, M.G. Palfreyman and M. Zreika, Merrell-Dow Research Institute, Strasbourg Center, 16, rue d'Ankara 67084 Strasbourg-Cedex, France.

Deprenyl and (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) are potent, irreversible inhibitors of monoamineoxidase (MAO) with selectivity for the B form of the enzyme (Knoll & Magyar, 1972; Bey et al, 1984). In man and animals deprenyl is extensively metabolized to (-)-amphetamine and methamphetamine (Reynolds et al., 1978); MDL 72145, in contrast, cannot be metabolized to amphetamine. In this study we have compared in detail the pharmacological properties of deprenyl and MDL 72145 seeking to define which of their effects, if any, can be ascribed solely to inhibition of MAO B.

Male mice (CD_1 albino) and rats (Sprague-Dawley) were used throughout. All drugs were administered i.p. unless otherwise stated. Tissue MAO activities were measured as previously described (Bey et al., 1984).

In the "behavioural despair" swim test in mice (Porsolt et al. 1977) deprenyl, 2.5-30 mg/kg, decreased the immobility score dose-dependently when injected 1h before testing. Dexamphetamine, 1-5 mg/kg, was similarly effective but MDL 72145, 0.5-10 mg/kg, and clorgyline, 5 mg/kg, were inactive under these conditions. Eighteen hours after treatment no significant effects on immobility were seen with deprenyl, 30 mg/kg, or MDL 72145, 10 mg/kg, despite substantial and similar inhibition of MAO B in each case. In mice treated with reserpine, 5 mg/kg i.p. 18h before testing, neither MDL 72145, 1 and 10 mg/kg i.p., nor clorgyline, 5 mg/kg, altered the lowered body temperature 2h after injection. Deprenyl, 10 mg/kg, and dexamphetamine, 5 mg/kg, were effective. On the other hand, all the MAO inhibitors slowed the rate of fall in body temperature when reserpine was given 18h after administration of the inhibitors.

A further difference between deprenyl and MDL 72145 was evident in rats lesioned unilaterally in the ascending nigro-striatal bundle with 6-hydroxydopamine. One month after surgery they were given either deprenyl, 10 mg/kg, dexamphetamine, 5 mg/kg, or MDL 72145, 2.5 mg/kg. Within 30 min of receiving deprenyl or amphetamine, the rats began to turn ipsilaterally, an effect which lasted for up to 6h. Rats given MDL 72145 did not rotate. Both MAO inhibitor treatments produced marked (> 95%) inhibition of MAO B in the striatum.

In pithed rats set up for recording blood pressure (B.P.) and heart rate (H.R.) (Fozard et al. 1980), deprenyl, 0.25-10 mg/kg, or dexamphetamine, 0.01-0.5 mg/kg, injected i.v., produced dose-related and sustained rises in B.P. and H.R. MDL 72145 or clorgyline, injected i.v. at doses from 1-10 mg/kg, had minimal cardiovascular effects. The elevated B.P. and H.R. produced by deprenyl and dexamphetamine were restored to control values by desipramine, 2-4 mg/kg i.v., and substantially reduced in animals pretreated 18 h before testing with reserpine, 5 mg/kg.

The fact that MDL 72145 differed markedly in its pharmacological effects from deprenyl despite producing equivalent MAO B inhibition suggests that many of the properties of deprenyl here described result from its amphetamine-like sympathomimetic properties. MDL 72145 can, therefore, be considered a more reliable tool with which to explore the functional importance of MAO B inhibition in experimental animals and man.

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COMPARISON OF THE RATE OF CEREBROCORTICAL β-ADRENOCEPTOR 'DOWN REGULATION' FOLLOWING RS-21361 AND DESMETHYLIMIPRAMINE

C.M. Brown, A.C. Dye and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

Chronic treatment with antidepressants and electroconvulsive therapy can decrease the β-adrenoceptor density and receptor linked adenylate cyclase activity in rat brain (Sulser, 1979). The findings have led to the suggestion that the therapeutic effect of antidepressants may be due to a progressive desensitization of central β-adrenoceptors (Sulser, 1979).

RS-21361, a selective α_2-adrenoceptor antagonist, has been shown to cause a significant 'down regulation' of β-adrenoceptors in rat cerebral cortex after 7 days thrice daily oral dosing at 100 $mg.kg^{-1}$ (Dye et al, 1983). The present study compares the rate of onset of β-adrenoceptor 'down regulation' after twice and thrice daily administration of RS-21361 and desmethylimipramine (DMI).

Male Sprague Dawley rats were orally dosed with RS-21361 (100 $mg.kg^{-1}$), DMI (20 $mg.kg^{-1}$) or distilled water (10 $ml.kg^{-1}$). The groups were dosed either twice or thrice daily for up to 14 days. The animals were killed 8 h after the last dose and the cerebral cortex removed. The densities of β-adrenoceptor binding sites were determined as described by Dye et al (1983) and the data analysed using the iterative non-linear curve fitting programme LIGAND (Munson & Rodbard, 1980). The results are shown in Table 1.

Table 1 Rate of β-adrenoceptor 'down regulation'

		B_{max} (fmol.mg.protein^{-1}) ± mean		
	DAY	CONTROL	RS-21361	DMI
BID	7	117.99 ± 5.08	113.27 ± 12.74	117.29 ± 10.24
	10	134.06 ± 11.06	*73.87 ± 12.24	125.03 ± 11.84
	14	117.29 ± 7.78	**61.22 ± 4.36	*73.09 ± 10.71
TID	5	138.02 ± 20.84	106.64 ± 10.56	141.66 ± 8.22
	7	135.45 ± 8.45	**83.96 ± 6.55	108.27 ± 7.29
	10	126.41 ± 9.57	**66.41 ± 4.59	**75.41 ± 6.51

*$p < 0.01$ **$p < 0.001$

Statistical analysis shows that RS-21361 produces a rapid onset of 'down regulation' of β-adrenoceptors showing a significant change after 10 days twice daily dosing and after 7 days thrice daily dosing. In comparison DMI did not show the same degree of significance until 3-4 days later. No significant difference was seen in the affinity value of [^{3}H]-dihydroalprenolol between the 3 groups on any day of measurement.

This study supports the proposal that RS-21361, a selective α_2-adrenoceptor antagonist, may be useful as a novel antidepressant with a more rapid onset of activity than standard tricylic agents.

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AUTORADIOGRAPHIC LOCALISATION OF NICOTINAMIDE ADENINE DINUCLEOTIDE BINDING SITES IN THE RAT BRAIN

J.M. Candy, C.R. Snell & P.H. Snell, MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE

Recently nicotinamide adenine dinucleotide (NAD) has been identified as an endogenous depressant of synaptic activity in the dentate gyrus of the rat hippocampus (Richards et al 1983a,b). Evidence has also been presented for the presence of specific binding sites for NAD on synaptosomes prepared from whole rat brain (Richards et al 1983b). Under normal conditions NAD is rapidly degraded by an NADase, however, this enzyme can be inhibited by nicotinamide mononucleotide (Snell, Snell & Richards, submitted) thus making possible the autoradiographic visualisation of NAD binding sites. Here we report the distribution of NAD binding sites using tritium film autoradiography (Palacios et al, 1981; Penney et al, 1981).

Unfixed brains from adult male Wistar rats were divided coronally at the level of the caudal hypothalamus and snap frozen. A series of 10μm coronal cryostat sections were cut at 500μm intervals. The sections were thaw mounted and air dried before storage at -30°C. Sections were preincubated for 10 min in ice cold 50mM tris-HCl buffer pH 7.4, followed by incubation for 30 min either in 14nM (adenosine -2,8-^{3}H) nicotinamide adenine dinucleotide (25.0 Ci/mmol, NEN Inc) in ice cold tris-HCl buffer pH 7.4 containing 5 x 10^{-4}M nicotinamide mononucleotide or in the above incubation medium with the addition of 10^{-4}M NAD to define non-specifically bound ligand. The sections were washed for 1 min in ice cold tris-HCl buffer, dipped in ice-cold water and rapidly dried under a stream of argon. The sections were exposed to tritium sensitive film (Ultrafilm, LKB) for 14 days.

Under the incubation conditions used there was no detectable degradation of NAD as determined using thin layer chromatography (Snell, Snell & Richards, submitted), specific binding represented over 90% of the total bound. A high density of NAD binding sites was present in the hippocampus and dentate gyrus consistent with previous electrophysiological findings (Richards et al 1983a,b). In addition high densities of NAD binding sites were also present in the cerebral cortex, caudate nucleus, nucleus accumbens, septum and cerebellum. Areas with a moderate density of binding sites included the hypothalamus, amygdala, substantia nigra and periaqueductal area. Low densities of NAD binding sites were observed in the globus pallidus and lower brain stem. Specific binding was absent from all white matter tracts eg corpus callosum and anterior commissure.

These results show the presence of high densities of specific NAD binding sites in a number of areas of the rat brain and are consistent with a role of NAD in synaptic transmission.

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INDORAMIN: BRADYCARDIA DUE TO PROLONGATION OF REPOLARISATION TIME

D.W.G. Harron*, W. Kobinger[1] and C. Lillie[1], Department of Therapeutics and Pharmacology, The Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland and [1]Pharmakologische Abteilung, Ernst Boehringer-Institut fur Arzneimittelforschung, Dr. Boehringer Gasse 5-11, A-1121 Vienna, Austria.

Indoramin is a selective α_1 adrenoceptor antagonist which reduces blood pressure in animals and man without an accompanying reflex tachycardia. It has been suggested that the lack of reflex tachycardia is due to myocardial membrane stabilising activity (Alps et al, 1971) but a recent study has indicated that this is unlikely at therapeutic doses (Harron and Shanks, 1982). It has also been suggested that selective α_1-adrenoceptor blockade by indoramin would allow the negative feedback of noradrenaline release, modulated through presynaptic α_2-adrenoceptors, to continue (Archibald, 1980). If this were the case, it is difficult to explain why direct vasodilator drugs (such as hydralazine) produce a tachycardia, as they too leave the negative feedback mechanism intact. In the present study, a possible direct bradycardic effect of indoramin was investigated and compared with the effects of mexiletine (Class I anti-arrhythmic agent, Vaughan Williams, 1974) and sotalol, a β-adrenoceptor antagonist which also prolongs action potential duration (Class III anti-arrhythmic activity).

In isolated guinea-pig atria, 1 μg/ml indoramin reduced spontaneous rate (31 ± 1.0%, mean ± S.E., n=6) and maximal driving frequency (25 ± 1.2%, n=4). Mexiletine 3 μg/ml reduced maximal driving frequency (35 ± 1.4%, n=4) but had less effect on spontaneous rate (-13 ± 2.0%, n=4). In the ECG of isolated perfused electrically driven (2.5 Hz) guinea pig hearts (n=6), indoramin 1 μg/ml mainly increased ST interval (22 ± 1.9%) with no effect on QRS interval, higher concentrations (3 μg/ml) also increased QRS interval (21 ± 4.0%). In anaesthetised cats (n=6) indoramin 6 mg/kg i.v. (infusion 30 min) reduced blood pressure (systolic 36 ± 3.6%) and heart rate (increased cycle length 35 ± 2.2%), increased ST interval (31 ± 5.3%) and effective refractory period (45 ± 3.2%, measured by electrical stimuli from the right ventricle) but had little or no effect on QRS interval (12 ± 2.9%) and diastolic electrical stimulation threshold (7 ± 6.8%). With a dose of 10 mg/kg (n=6) the latter two parameters were increased (20 ± 2.8%, 23 ± 2.5%). Analogous experiments with mexiletine (15 mg/kg i.v. infusion, n=6) showed little changes in cycle length (7 ± 1.3%), effective refractory period (21 ± 2.8%) and the ST interval (6 ± 2.5%); however there was a marked increase in diastolic threshold (91 ± 8.9%). Sotalol (15 mg/kg i.v. infusion, n=6) had a similar cardiac profile as indoramin. For both indoramin and sotalol a significant positive correlation was shown between increase in cycle length and increase in effective refractory period. It is suggested that indoramin exerts Class III anti-arrhythmic activity and that this property is responsible for the bradycardic action of the drug which is seen in doses that already markedly reduce blood pressure. Recent findings with melperone have shown that a drug with Class III actions on myocardial fibres is able to slow the rate of sinus node discharge (Millar and Vaughan Williams, 1983). In higher doses or concentrations indoramin also exerts Class I anti-arrhythmic activity which does not contribute to the bradycardic effect.

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PRE- AND POSTSYNAPTIC ACTIONS OF INDORAMIN ON ISOLATED ARTERY PREPARATIONS

Ove A. Nedergaard, Department of Pharmacology, School of Medicine, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

Indoramin is a clinically effective antihypertensive agent which is considered to be due primarily to competitive postsynaptic α-adrenoceptor antagonism and myocardial membrane stabilizing effect (Archibald, 1980). The aim of the present study was to examine the effects of indoramin on sympathetic neuroeffector transmission and on the vasoconstrictor response evoked by various agonists. The rabbit isolated pulmonary artery and aorta preparations were used. The methods described in detail (Nedergaard, 1980) were used.

Indoramin (10^{-8}-$3x10^{-5}$M) inhibited the contractions of pulmonary artery evoked by selective electrical-field stimulation (150 monophasic pulses; 0.5 msec; 3 Hz; 250 mA) of postganglionic sympathetic neurone terminals. The IC_{50} was $6x10^{-8}$M. In the presence of cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M) + (-)-propranolol (10^{-7}M), the IC_{50} was $4x10^{-7}$M. Within 30 min after addition indoramin (10^{-6}M) caused a submaximal steady state block which was partially reversible.

Indoramin (10^{-8}-$3x10^{-7}$M) caused a slight enhancement (up to 130% of control) of the ^3H-overflow evoked by field stimulation (300 pulses; 0.5 msec; 3 Hz; 250 mA) of pulmonary artery preloaded with (-)-7-^3H noradrenaline (^3H-NA). In the presence of cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M) + (-)-propranolol (10^{-7}M), indoramin also enhanced the stimulation-evoked ^3H-overflow. The effect of indoramin on stimulation-evoked ^3H-overflow depended only slightly on frequency (1-30 Hz): Small enhancement at 1 Hz and no significant change at 3,10 and 30 Hz. Indoramin ($3x10^{-7}$M) did not antagonize the inhibitory effect of clonidine (10^{-7}-$3x10^{-7}$M) on the stimulation-evoked ^3H-overflow. Indoramin ($3x\ 10^{-7}$M) did not prevent the block of the stimulation-evoked ^3H-overflow seen with bretylium (10^{-5}M).

Indoramin (10^{-6}-$3x10^{-4}$M) and desmethylimipramine ($3x10^{-9}$-10^{-4} M) reduced the ^3H-accumulation by aorta preloaded with ^3H-NA (10^{-8}M). The IC_{50} was $3x10^{-5}$M (indoramin) and 10^{-8}M (desmethylimipramine).

Indoramin ($3x10^{-8}$-10^{-5}M) inhibited in a competitive manner the contractions of aorta induced by (-)-noradrenaline ($3x10^{-9}$-10^{-2}M), (-)-phenylephrine ($3x10^{-8}$-$5x10^{-4}$M), histamine (10^{-6}-$3x10^{-3}$M), and serotonin (10^{-8}-$3x10^{-4}$M). The corresponding pA_2-values were: 7.54, 7.98, 7.45, and 6.61. Indoramin ($3x10^{-6}$) did not alter the contractions of aorta evoked by potassium (16-56 mM). At 10^{-5}-10^{-4}M, indoramin caused a non-competitive block.

It is concluded that indoramin is a potent competitive antagonist of postsynaptic α_1-adrenoceptors, while it may have a slight inhibitory effect on presynaptic α_2-adrenoceptors. Indoramin is devoid of adrenergic neuron blocking activity. It is a weak inhibitor of the uptake-1 mechanism. Indoramin has a direct smooth muscle relaxant effect only at a high concentration. It is a competitive antagonist of histamine and serotonin.

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EFFECT OF THE α_1-ADRENOCEPTOR ANTAGONIST INDORAMIN ON BARORECEPTOR REFLEXES IN CONSCIOUS RATS

Vanessa Pierce and N B Shepperson, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire.

The decrease in blood pressure (BP) evoked by the α_1-adrenoceptor blocker indoramin is not accompanied by a reflex tachycardia in either man or animals, and in anaesthetised animals indoramin evokes hypotension and bradycardia. It has been suggested that the inhibition of electrically evoked tachycardia by indoramin in pithed rats is attributable to its local anaesthetic properties (Algate et al, 1981). Experiments in anaesthetised rats, however, have revealed that the bradycardia induced by indoramin is not due to a direct action on cardiac muscle but involves cholinergic pathways (Pierce and Waterfall, 1982). To further investigate this mechanism the effects of indoramin on baroreceptor mediated tachycardia, in the conscious rat, have been examined and compared with those of prazosin.

Groups of 5-6 female rats (250-300g) were anaesthetised with halothane. The left femoral vein and artery were cannulated and exteriorised at the back of the neck. The rats were allowed a minimum of four hours to recover from anaesthesia. BP was recorded from the femoral artery and heart rate (HR) derived from the pulse pressure signal using a tachograph. Drugs were administered via the left femoral vein.

The effects of indoramin and prazosin on the reflexly induced tachycardia, evoked by a series of bolus injections (0.03-100μgkg^{-1} i.v.) of the vasodilator sodium nitroprusside (SN), were examined in the presence and absence of cholinergic muscarinic blockade by atropine or methylatropine ($1mgkg^{-1}$ i.v.). Linear regression analysis was performed on the data pairs (change in HR to change in BP) and the slopes of the regression lines were compared before and after α_1-adrenoceptor blockade (paired t-tests).

Vagal withdrawal was measured as the maximum reflex tachycardia (30-34 $bmin^{-1}$) evoked by a supramaximal dose of SN (30 or $100\mu gkg^{-1}$ i.v.) following β-adrenoceptor blockade (propranolol $3mgkg^{-1}$ i.v.). The effects of cumulative doses of indoramin (0.4-1.6$mgkg^{-1}$ i.v.) and prazosin (0.01-0.04$mgkg^{-1}$) on reflex vagal withdrawal were assessed.

Administration of indoramin ($0.4mgkg^{-1}$ i.v.) or prazosin ($0.01mgkg^{-1}$ i.v.) significantly ($p<0.01$) reduced the slopes of the regression lines from 2.6±0.3 to 1.5±0.2 and 4.1±0.5 to 2.3±0.3 respectively - indicating a reduction in cardiovascular reflex sensitivity. Neither compound induced a significant effect on the sympathetic component of this reflex, as a reduction in sensitivity was not apparent following cholinergic muscarinic blockade with either atropine or methylatropine. Peripheral muscarinicblockade with methylatropine was used to investigate whether blockade of central muscarinic receptors (by atropine) masked a possible reduction in sympathetic tone following α_1-adrenoceptor blockade. In the presence of propranolol, indoramin and prazosin significantly reduced the reflex tachycardia due to vagal withdrawal.

In summary the inhibition of cardiovascular reflex sensitivity by indoramin was mediated via cholinergic pathways resulting in a reduction of reflexly mediated vagal withdrawal. This action is thought to be due to α_1-adrenoceptor blockade as similar results were obtained with prazosin.

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SELECTIVITIES OF IDAZOXAN, WY 26703 AND YOHIMBINE FOR PREJUNCTIONAL α_2-ADRENOCEPTORS IN RATS AND DOGS

T. L. Berridge, A.G.Roach, Diane A. Strachan and Narinder K. Virdee. Department of Pharmacology, Reckitt and Colman, Hull, HU8 7DS.

Idazoxan and Wy 26703 are potent, selective α_2-adrenoceptor antagonists *in vitro* (Doxey et al., 1983a; Lattimer et al., 1982). We have determined the potency of these two compounds as well as the standard antagonist yohimbine at pre α_2- and postjunctional α_1-adrenoceptors in pithed rats and spinal dogs in order to ascertain the selectivities of these antagonists *in vivo*.

Male Sprague-Dawley rats (275-350g) were pithed, vagotomised and prepared for recording of blood pressure (BP) and heart rate (HR). Antagonist potency at cardiac prejunctional α_2-adrenoceptors was determined against the inhibitory effects of UK-14,304 on a continuous stimulation-evoked tachycardia. Antagonism of cirazoline pressor-response curves was used to assess vascular postjunctional α_1-adrenoceptor antagonist potency (Doxey et al., 1983b). Beagle dogs of either sex (8-16kg) were anaesthetised with sodium pentobarbital (30 mg/kg,i.v.), spinalised, vagotomised and prepared for measurement of BP and HR (Cavero et al., 1979). HR was elevated by continuous stimulation (1-2 Hz, 0.1 ms, 10V) of the right ansa subclavia nerve and α_2-induced inhibitory cumulative dose-response curves to UK-14,304 constructed 10 min after either saline or the antagonists. At vascular α_1-adrenoceptors in the dog, cirazoline dose-pressor response curves were constructed 10 min after saline or drug treatment. In both rats and dogs, UK-14,304 ID_{50} values (doses required to inhibit the elevated HR by 50%) were determined. The doses of cirazoline required to increase diastolic BP (DBP) by 50 mmHg in rats (ED_{50}) and 70 mmHg in dogs (ED_{70}) were also calculated. The dose-ratio (DR) shifts produced by the antagonists against UK-14,304 and cirazoline were calculated from the ID_{50}, ED_{50} and ED_{70} values obtained in saline and drug treated animals. The antagonist doses required to produce DR of 2 were then calculated. All animals were pretreated with atropine (1.0 mg/kg) and propranolol (1.0 mg/kg) was given in the cirazoline experiments. Pithed rats used for the UK-14,304 experiments were also given d-tubocurarine (3.0 mg/kg).

Table 1. Antagonist potencies and selectivities in pithed rats and spinal dogs.

ANTAGONIST	PITHED RATS			SPINAL DOGS		
	Antag. Dose for DR2 (μM/kg,iv) HR (α_2) vs UK-14,304	Antag. Dose for DR2 (μM/kg,iv) DBP (α_1) vs CIRAZOLINE	RATIO α_2/α_1	Antag. Dose for DR2 (μM/kg,iv) HR (α_2) vs UK-14,304	Antag. Dose for DR2 (μM/kg,iv) DBP (α_1) vs CIRAZOLINE	RATIO α_2/α_1
IDAZOXAN	0·13 ± 0·02	7·53 ± 2·46	58	0·17 ± 0·06	5·32 ± 1·31	31
WY 26703	0·10 ± 0·01	12·0 ± 1·10	120	0·13 ± 0·01	1·53 ± 0·12	12
YOHIMBINE	0·19 ± 0·04	4·26 ± 1·08	22	0·09 ± 0·03	2·64 ± 0·20	29

All three compounds were competitive antagonists at pre α_2- and post α_1-adrenoceptors and were selective for α_2- receptors in both animal species. Yohimbine was less selective than idazoxan in pithed rats but was similar to idazoxan in dogs. There was a large (10 fold) difference between the α_2-selectivity of Wy 26703 in rats and dogs; Wy 26703 being the most selective compound in rats but the least selective in dogs. This reduced selectivity was mainly due to an enhanced α_1-antagonist potency. The reason for the differences with Wy 26703 in the two species is unknown but may reflect differences in its distribution and/or its metabolism.

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CHRONIC INFUSIONS OF IDAZOXAN AND DESMETHYLIMIPRAMINE ON α_2-RECEPTOR CONTROL OF NORADRENALINE RELEASE

M.Adams and I.Marshall. Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London, W1P 7PN.

It has been suggested that on chronic antidepressant treatment the α_2-adrenoceptor involved in the modulation of noradrenaline release in rat heart becomes subsensitive (Crews and Smith, 1978). To determine whether similar changes are apparent in the mouse vas deferens or cortex the effects of the selective α_2-adrenoceptor agonist clonidine, and antagonist idazoxan (RX 781094), have been measured following chronic infusion of desmethylimipramine (DMI), idazoxan, or DMI plus idazoxan combined.

Male T.O. mice (33-39g) were implanted with Alzet mini-osmotic pumps s.c., delivering either saline, DMI, idazoxan or combined DMI and idazoxan (all at 10 mg/kg/day). Following 7 days infusion mice were killed, one vas deferens and 25-30 mg of left frontal cortex were prepared as previously described (Adams and Marshall, 1983). Tissues from mice receiving each infusion were divided into 3 groups ($n > 4$), to which either no drugs were applied, or the effects of clonidine or idazoxan on stimulated noradrenaline release were determined.

Uptake of ^{3}H-noradrenaline (^{3}H-NA, 590 nM, sp.Act. 36 Ci/mmol) into either tissue following each drug infusion was not significantly altered from uptake into tissues from mice infused with saline (^{3}H-NA uptake on saline infusion; vasa deferentia 124.8 ± 13.9 nCi/mg, mean ± s.e.mean, cortical slices 80.3±10.5 nCi/mg). On stimulation (100 pulses, 1Hz, 2ms) the fractional release of ^{3}H-NA was also not significantly different from saline control values of $7.2 \pm 0.9 \times 10^{-4}$ (vasa deferentia) and $4.0 \pm 0.6 \times 10^{-3}$ (cortical slices). Following saline infusion clonidine (1-100 nM) reduced the ^{3}H-NA fractional release in both vasa deferentia and cortical slices (IC_{50}, concentration causing 50% inhibition, 2.6 ± 0.7 nM and 6.0 ± 0.9 nM respectively), with over 80% maximal inhibition in both tissues. Clonidine potency was not altered in tissues from mice infused with DMI or DMI plus idazoxan. The potency of clonidine in cortical slices was slightly reduced after idazoxan infusion (IC_{50}, 15.0 ± 3.2 nM) although no change was seen in vasa deferentia. Following saline infusion idazoxan (10 nM - 1.0 nM) increased ^{3}H-NA fractional release in vasa deferentia (from $7.3 \pm 0.5 \times 10^{-4}$ to $12.3 \pm 1.9 \times 10^{-4}$ in the presence of 1.0 µM idazoxan). The response to idazoxan was markedly reduced in vasa from mice infused with DMI, idazoxan or DMI plus idazoxan (^{3}H-NA fractional release increased by only 24 ±22%, 34 ± 13% and 6 ± 13% respectively). In cortical slices idazoxan also increased ^{3}H-NA fractional release, from $3.7 \pm 0.8 \times 10^{-3}$ to $8.2 \pm 2.8 \times 10^{-3}$ by 1.0 µM idazoxan. The magnitude of this increase was not altered by drug infusion.

The reduced effect of idazoxan in vasa deferentia from mice infused with DMI, idazoxan or DMI plus idazoxan combined indicates a reduction in feedback at α_2-adrenoceptors. However this is probably not due to a change in α_2-adrenoceptor sensitivity, as neither the potency of clonidine, or ^{3}H-NA fractional release in the absence of drugs was altered. In cortical slices from the same mice no change in the effect of idazoxan was seen. These tissue differences may reflect differences in drug distribution following s.c. infusion.

M.A. holds an SERC CASE award with Reckitt and Colman plc.

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MATURATION OF α-ADRENOCEPTORS IN MALE AND FEMALE RABBITS

C.A. Hamilton, C. Leslie, N. Mishra , J.L. Reid, University of Glasgow Department of Materia Medica, Stobhill General Hospital, Glasgow, Scotland.

Reductions in β adrenoceptor sensitivity and density with age have been extensively reported (Maggi et al., 1979; Bertel et al., 1980). However, there are few studies on the influence of age on alpha adrenoceptors. We have previously reported an age related decrease in alpha$_1$ adrenoceptor density in brain and peripheral tissues in male white New Zealand rabbits (Dalrymple et al., 1982; Hamilton et al., 1983). We have now extended these studies to females. [^{3}H] prazosin and [^{3}H] clonidine binding was studied in peripheral (spleen and heart) and central (forebrain and hindbrain) tissues in young (2-3 months) and adult (6-8 months) female rabbits (Berthelot et al., 1982). The maximum number of specific binding sites, Bmax, and the dissociation constant, K_D, were calculated for each tissue from each rabbit and the results compared using the Generalised Likelihood Ratio test (Hamilton et al., 1983). In the female rabbits Bmax for specific prazosin binding was significantly higher in adult as compared to young animals, whereas in the males binding was significantly higher in the young animals.

Table Maximum number of specific [^{3}H] prazosin binding sites (fmoles/mg protein)

	Female		Male	
Tissue	Young	Adult	Young	Adult
Spleen	45 ± 6^{+*}	88 ± 12	123 ± 15*	80 ± 7
Heart	22 ± 6^{+*}	70 ± 5	127 ± 10*	52 ± 10
Forebrain	63 ± 12^{+*}	140 ± 10^{+}	123 ± 15*	55 ± 8
Hindbrain	75 ± 15^{+*}	207 ± 34^{+}	147 ± 19*	62 ± 15

* Significant difference between young and adult animals.
\+ Females significantly different to males of same age.
n = 6-8 animals per group.

In contrast there was no difference in specific clonidine binding between young males and females. The only significant changes in binding on maturation were a decrease in Bmax in forebrain from 145 ± 27 to 77 ± 28 fmoles/mg protein in male rabbits and from 241 ± 27 to 151 ± 22 fmoles/mg protein in female rabbits, and in spleen of female rabbits only from 140 ± 10 to 111 ± 25 fmoles/mg protein.

Despite the changes in the binding of α adrenoceptor ligands no alteration in in vivo pressor responses to phenylephrine (α_1 selective) or BHT 920 (α_2 selective) were observed with maturation in either males or females. These results suggest a different pattern of maturation in the two sexes. However, the biological significance of these changes is not clear and further studies over a wider age range, in localised brain regions,and with investigation of the functional consequences are needed.

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Dr. Mishra is a Commonwealth Medical Fellow.

COMPARATIVE CARDIOTOXICITY OF IDAZOXAN, AMITRIPTYLINE, MIANSERIN AND YOHIMBINE IN THE RABBIT

A. S. Hersom and J.E. Mackenzie, Reckitt and Colman, Pharmaceutical Division Hull, HU8 7DS.

Cardiovascular toxicity may be a limiting factor in the use of antidepressant drugs, even in therapeutic doses (Lipscomb, 1980). It has been proposed that use of selective α_2-adrenoceptor antagonists may provide a novel approach to the treatment of depression. The following study was therefore performed to compare the cardiotoxicity of idazoxan with that of the less selective α_2-adrenoceptor antagonist yohimbine, the tricyclic antidepressant amitriptyline and the tetracyclic antidepressant mianserin.

The electrocardiogram (ECG) was monitored in conscious restrained male NZW rabbits. Idazoxan (3mg/kg/min) amitriptyline (3mg/kg/min), mianserin (3mg/kg/min) and yohimbine (0.8 mg/kg/min) were each infused via the marginal ear vein, into groups of 6 rabbits. The times to 1st ECG change, severe ECG change (defined by Elonen et al. (1974) as a deepened and broadened S wave with ST changes and severely deformed QRS complexes) were measured. The dose eliciting cardiotoxicity as indicated by severe change in ECG are shown in Table 1.

Infusion (mg/kg/min)	Dose eliciting 1st ECG change mg/kg,i.v.	Dose eliciting Severe ECG change mg/kg,i.v.
idazoxan(3.0)	8.4±0.9	18.5±1.5
amitriptyline(3.0)	5.7±1.2	7.0±1.1
mianserin(3.0)	3.9±0.3	18.5±3.4
yohimbine(0.8)	1.8±0.2	2.9±0.4

Table 1. Mean doses (±s.e.m.) eliciting first changes in ECG and severe changes in ECG indicative of cardiotoxicity in conscious rabbits (n=6).

The rank order of potency with respect to cardiotoxicity was yohimbine > amitriptyline > mianserin = idazoxan, (Table 1). The lower potency of mianserin with respect to amitriptyline is consistent with previous studies (Hughes and Radwan,1979). In contrast, the rank order of α_2-adrenoceptor antagonist potency is idazoxan > yohimbine > mianserin (Doxey et al, 1983), and it is unlikely therefore, that cardiotoxicity is related to α_2-adrenoceptor antagonist properties. Furthermore,it should be stressed that the doses of idazoxan required to induce cardiotoxicity are at least two orders of magnitude greater than those required to produce α_2-adrenoceptor antagonism in this species (Gadie et al, 1983).

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HOW IMPORTANT IS THE PRESYNAPTIC EFFECT OF ANGIOTENSIN II IN MODULATING PRESSOR RESPONSES TO SYMPATHETIC NERVE STIMULATION?

D.R. Bull & G.M. Drew, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Herts, SG12 ODJ, U.K.

Previous studies have shown that the angiotensin converting enzyme inhibitor, captopril, selectively inhibits the vasopressor responses to preganglionic sympathetic nerve stimulation in pithed rats, and that the intravenous infusion of angiotensin II restores the responses (Hatton & Clough, 1982). These findings suggest that, in addition to its established effects on vascular smooth muscle, angiotensin II facilitates sympathetic neurotransmission, probably by stimulating presynaptic angiotensin receptors. This view has recently been challenged by De Jong et al., (1983), who suggested that angiotensin II restored responses to nerve stimulation primarily because it restored the resting blood pressure that had previously been reduced by captopril. We have reinvestigated this phenomenon using enalapril to inhibit angiotensin converting enzyme; enalapril is more potent and longer lasting than captopril (Cohen et al., 1983).

Rats (male, AHA Wistar derived, 200 - 300 g) were pretreated with indomethacin (5 mg/kg i.p. 1 hour before pithing) to inhibit synthesis of endogenous prostanoids. After pithing, preparations were artificially respired and treated with atropine and (+) tubocurarine (1 mg/kg i.v. of each). Pressor responses were elicited to intravenous injection of angiotensin I (1 µg/kg), tyramine (100 µg/kg) or 5-HT (30 µg/kg), or to stimulation of the entire spinal preganglionic sympathetic outflow (1 Hz, 1ms; 15 s). Repeated application of these vasopressor stimuli elicited reproducible responses for at least 2½h.

The intravenous injection of enalapril (50 µg/kg) reduced the vasopressor responses to angiotensin I by 80% within 7½ min. Enalapril also reduced resting diastolic blood pressure from approximately 40 to 30 mmHg and reduced pressor responses to sympathetic nerve stimulation by 50 - 70%; peak effects were seen approximately 15 and 30 min, respectively, after dosing. All these effects of enalapril were sustained for at least 2h. In contrast, enalapril did not reduce the vasopressor responses produced by tyramine or by 5-HT. Thus it appears that enalapril inhibited responses to sympathetic nerve stimulation by an action at the neuronal, rather than the vascular level, and this effect coincided with inhibition of angiotensin converting enzyme activity.

The intravenous infusion of angiotensin II (3, 10, 30 ng/kg/min for 15 min) caused a dose-dependent increase in resting diastolic blood pressure in pithed rats already treated with enalapril (50 µg/kg i.v.). However, the reduction of the pressor responses to sympathetic nerve stimulation was reversed by only about 50% at most.

These results seem to suggest that the effect of enalapril on sympathetic neurotransmission may not only be the result of its ability to decrease endogenous levels of angiotensin II. An alternative explanation is that enalapril inhibits sympathetic neurotransmission by decreasing the level of vascular smooth muscle angiotensin II, rather than plasma angiotensin II, and that infused angiotensin II does not easily penetrate, from the blood, to the same site of action as endogenous vascular angiotensin II.

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ANTAGONISM OF THE CARDIOVASCULAR ACTION OF VASOPRESSIN LOWERS BLOOD PRESSURE IN ISOLATED RATS

T. Bennett & S.M. Gardiner, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH, UK.

Male Wistar rats housed in individual glass metabolism cages have increased arterial pressures whereas rats with a congenital inability to synthesize vasopressin (Brattleboro strain) do not (Gardiner & Bennett, 1983). We have now investigated the effects on blood pressure (BP) of an antagonist specific for the pressor action of vasopressin (/1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),8-D-arginine/ vasopressin; $d(CH_2)_5$DAVP) in male Wistar rats which had been housed individually ("Metabowl", Jencons; n=5) or in groups (n=6) for 7 weeks before the study. On the day of investigation, the animals were anaesthetized (sodium methohexitone, 60 $mg.kg^{-1}$ i.p.) and catheters were implanted in the abdominal aorta for BP recording and jugular vein for drug administrations; heart rate (HR) was derived from the BP recording. The measurements began 5 h later when the animals were fully conscious. During a 30 min control period, BP was significantly higher in the isolated rats (176±1/112±2 mmHg; systolic/diastolic, mean±S.E. of mean) than in the group-housed rats (162±2/104±1 mmHg; $P<0.02$ for systolic; $P<0.05$ for diastolic); there was no significant difference between the HR of the 2 groups. Administration of $d(CH_2)_5$DAVP (10 $\mu g.kg^{-1}$ bolus; 10 $\mu g.kg^{-1}\ h^{-1}$ infusion) caused a sustained tachycardia in the group-housed rats (+48±13 beats min^{-1} within 2 min) with no change in BP. Administration of captopril (2 $mg.kg^{-1}$; 1 $mg.kg^{-1}\ h^{-1}$ infusion) at this stage had no effect on BP although there was a further tachycardia (+55±14 beats min^{-1}).

In the isolated rats, $d(CH_2)_5$DAVP caused a progressive fall in BP (down to 154±5/99±4 mmHg after 20 min) with a sustained rise in HR (+42±16 beats min^{-1}). At this time, administration of captopril caused BP to fall further (down to 149±5/95±5 mmHg after 20 min) and heart rate to increase further (+54±8 beats min^{-1}).

We have shown previously that antagonism of the actions of angiotensin does not lower BP in isolated, otherwise untreated, Wistar rats (Bennett & Gardiner, 1983).

One interpretation of these findings together is that vasopressin plays a role in the maintenance of the elevated BP in isolated rats and that only when this effect is antagonized is there an acute activation of the renin angiotensin system.

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EFFECT OF OUABAIN AND K^+-FREE KREBS' ON HYPOXIC AND RECOVERY RESPONSES OF RAT AORTA TO NORADRENALINE

Downing, O A, Marriott, J F* and Wilson, K A. Department of Pharmacy, University of Aston, Birmingham B4 7ET

Most drug induced contractions of vascular muscle are known to be depressed by hypoxia. In rabbit aorta a biphasic recovery response (relaxation followed by contraction) on return to normoxia has been described by Griesmer and Coret (1960). These workers suggested that the relaxation was dependent upon re-entry of potassium lost during hypoxia. We have found that the isolated rat aorta shows a similar profile of responses on the re-introduction of oxygen after hypoxia (Figure 1). It is likely that the relaxation on re-oxygenation is due to activation of a membrane ion-pump, consequently we have determined the effect of ouabain and K^+-free Krebs' on this relaxation. Both procedures are known to compromise the activity of Na-K ATPase dependent ion pumps in vascular muscle (Fleming, 1980).

Male Wistar rats (200-280 g) were sacrificed by cervical dislocation and 8 mm circular preparations of thoracic aorta were mounted under a resting tension of 3g in Krebs' solution gassed initially with 5% CO_2 in O_2 (Krebs' pO_2 = 380 mm Hg) maintained at 37 °C. After a 1 hr equilibration period, reproducible reponses to 1 μM noradrenaline (NA) were obtained (normoxic response) followed by a 30 min period during which preparations were gassed with 5% CO_2 in N_2 (Krebs' $pO_2 < 70$ mm Hg). Responses to NA were re-elicited and peak contractions measured (after 30 min). The gassing mixture was rapidly switched to 5% CO_2 in O_2 and contractile changes observed measuring the new maximum tension. Responses during hypoxic episodes attained stable maxima measuring 79.6 ± 4.9% (n=6) of the normoxic response. On re-oxygenation a transient loss in tension was seen followed by a slow increase in tension reaching 105.3 ± 6.1% (n=6) of the normoxic response.

0.1 mM ouabain caused an 82.5 ± 10.0% (n=6) inhibition of the transient relaxation but only had small effects on the hypoxic (5.7 ± 1.7% inhibition, n=6) and recovery contractions (0.1 ± 1.4% potentiation, n=6) (Fig 1). Similarly in the presence of K^+-free Krebs' the transient relaxation was reduced by 79.2 ± 5.1% (n=6); the hypoxic contraction was reduced by 22.4 ± 6.7% (n=6) and the recovery contractions increased by 18.7 ± 3.3% (n=6). This selective sensitivity of the transient relaxation to both ouabain and K^+-free Krebs' indicates a dependence on the operation of a membrane ion pump which has been inactivated by hypoxia.

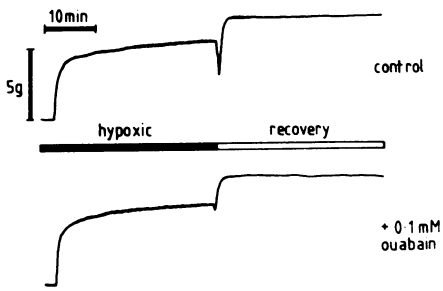

Figure 1. Representative hypoxic and recovery contractions to 1μM NA before and after treatment with 0.1 mM ouabain.

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REDUCED PLATELET FUNCTION IN RATS TREATED CHRONICALLY WITH ETHANOL

D.R. Anderson, L. Caberos, G.C. Fenn & J.M. Littleton, Department of Pharmacology, King's College, London WC2R 2LS.

The prolonged administration of ethanol to rats results in poor platelet aggregation, specifically to collagen, in platelet-rich plasma in vitro (Littleton, et al, 1982). This does not depend on the presence of ethanol or its major metabolite acetaldehyde in plasma during the testing procedure nor does it involve obvious morphological changes in the platelet. These experiments seek to determine whether the platelets themselves are functionally damaged or whether some inhibitory factor is present in the plasma.

Male Sprague Dawley rats, 400-450 g, were exposed to intoxicating concentrations of ethanol by inhalation for periods of 5-7 days. They were then anaesthetized lightly with ether and exsanguinated from the abdominal aorta using 3.8% trisodium citrate as anticoagulant. Platelet-rich plasma was prepared as described previously (Littleton et al, 1982) and centrifuged gently (500 x g for 20 min) to form a platelet pellet and the plasma removed. Control animals were treated similarly and then control rat platelets were resuspended, either in the plasma from ethanol-treated rats or in that from other control rats. Platelets from ethanol-treated rats were resuspended in the plasma from control rats. Platelet aggregation was studied turbidimetrically in a Payton dual channel aggregometer and the release reaction was studied by following the release of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5HT) induced by 10 μM ADP in a platelet superfusion system (Patrikiou-Caberos et al, 1983).

Control platelets resuspended in the plasma from ethanol-treated rats required about six times the concentration of collagen to induce aggregation compared to that required for control platelets resuspended in control plasma. The aggregation induced by ADP was inhibited very little. The inhibition of the collagen responses by plasma from ethanol-treated rats was much greater than could be explained by the presence of ethanol (50-75 mM) in the plasma. It was noted that about 3-6 h after removal of blood the inhibitory activity of plasma from ethanol-treated animals began to wane and platelet aggregation then recovered rapidly toward control levels. Platelets from ethanol-treated rats which were resuspended in control plasma showed some inhibition of aggregation to collagen compared to control platelets but this was rapidly lost on continued testing, making the alteration in sensitivity difficult to quantify. There was again no alteration in ADP-induced aggregation.

In contrast to the lack of effect of ethanol treatment on ADP-induced platelet aggregation, the release of [^{3}H]-5HT by ADP was significantly inhibited both from ethanol-treated platelets resuspended in control plasma 30 min before superfusion (27% control release) and from control platelets resuspended in ethanol-treated plasma before superfusion (50% control release).

The experiments strongly suggest that collagen-induced aggregation of rat platelets is inhibited by some labile factor in the plasma of ethanol-treated rats. In addition this factor can inhibit the ADP-induced release reaction of platelets and this reaction is markedly deficient in the platelets from ethanol-treated rats. The mechanism by which ethanol produces these changes in vivo may be of relevance to diseases of the cardiovascular system in which alcohol may play either a protective or a predisposing role.

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THE EFFECT OF PARASYMPATHETIC DENERVATION ON (^{3}H)-QNB BINDING IN THE RAT PAROTID GLAND

N. Adham and D. Templeton, Department of Physiology and Pharmacology, University of Southampton, Southampton, Hampshire, SO9 3TU

Sympathetic denervation of the salivary glands results in supersensitivity to adrenoceptor agonists and this has been shown to be partly due to an increase in the number of alpha and beta adrenoceptors (Pointon and Banerjee, 1979). Similarly the increased responsiveness to cholinergic muscarinic agonists following parasympathetic denervation (Ekström, 1980) may be the result of an increase in the number and possibly a change in the properties of the muscarinic cholinergic receptors. This possibility was tested using the radioligand [^{3}H]-QNB.

The postganglionic parasympathetic nerve (auriculo-temporal) to the parotid gland was sectioned unilaterally in pentobarbitone anaesthetised rats. The contralateral nerve was not sectioned thus each animal provided a control and a denervated gland. To be certain that the possible changes resulting from denervation were specific to the parotid gland, binding was also investigated in the submaxillary gland. Six weeks after denervation the salivary glands were removed, weighed and the tissue homogenised in Tris-HCl, pH 7.8. After a slow speed spin the supernatant was centrifuged at 20,000 g for 20 min and the final pellet resuspended in Tris-HCl, pH 7.8. Assays were performed in triplicate at 37 °C for 90 min and bound radioactivity separated by filtration through GF/C filters. The specific binding was determined in the presence of 10 μM atropine.

The specific binding of [^{3}H]-QNB (1 nM) represented $\geq$ 75% of the total binding and was found to be linear with membrane protein concentration within the range (25 - 450 μg protein) in the parotid and (25 - 150 μg protein) in the submaxillary gland. Binding was optimal at pH 7.8 and temperature at 37 °C.

Parasympathetic denervation resulted in a significant increase in Bmax of the parotid gland (160 ± 8 fmol/mg protein) compared to the control gland (78 ± 3 fmol/mg protein; n = 3; $p \geq 0.001$). However, since denervation causes a 30% decrease in gland wet weight without any cell death (Poat and Templeton, 1982) it is appropriate also to consider the total number of binding sites per gland. This was not altered by denervation, the values being 116 ± 8 and 130 ± 32 fmol/gland ($p = 0.6$) for denervated and control tissue respectively. K_D was also unaffected by denervation (0.77 ± 0.11 nM and 0.6 ± 0.006 nM, $p \leq 0.1$ in denervated and control respectively). Investigation of the association and dissocation rates also revealed no change following denervation. Confirmation that denervation was selective is provided by the fact that there was no change in the binding of ^{3}H-QNB by the submaxillary gland on the denervated side compared with the control.

The only effect of denervation measured this far is an increase in the number of binding sites/mg membrane protein, i.e. an increase in the density of binding sites, without changing their total number. How this is coupled to increased responsiveness of the tissue requires further investigation, especially of the second messenger systems involved.

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NORADRENALINE AND ANGIOTENSIN II-STIMULATED RELEASE OF PROSTACYCLIN FROM THE MESENTERIC ARTERIAL BED OF HYPERTENSIVE RATS

E. Anne Lennon & N.L. Poyser, Department of Pharmacology, Edinburgh University, Edinburgh, EH8 9JZ

Prostacyclin (PGI_2) is released from the perfused rat mesenteric arterial bed following stimulation with noradrenaline (NA) and angiotensin II (AII) (Pipili & Poyser, 1982; Desjardins-Giasson et al, 1982). We have previously observed a difference in basal vascular PGI_2 production between normotensive Wistar rats and rats of the New Zealand genetically hypertensive strain (GH) (Lennon & Poyser, 1983) and we were interested in determining if there was a similar difference in stimulated PGI_2 release. This study has investigated NA- and AII-stimulated release of PGI_2 from the perfused mesenteric arterial bed of male and Day 4 (pro-oestrus) female GH rats and Wistar rats of a similar age (n=4 per group).

The mesenteric arterial bed was prepared according to McGregor (1965), was perfused at 4 ml/min with McEwen's solution and allowed to equilibrate for 30 min. NA and AII (0.1 µg and 1.0 µg) were given as a bolus injection in a random order. Samples were collected at 1 min intervals immediately before and after stimulation. Pressor responses to the two hormones were recorded. 6-Oxo-$PGF_{1\alpha}$ in the perfusion fluid was measured by radioimmunoassay, without extraction.

In the mesenteric bed from male rats, NA, at both doses caused a significant increase in 6-oxo-$PGF_{1\alpha}$ release in normotensive and hypertensive animals ($P < 0.05$, paired t-test). However, no significant increase in 6-oxo-$PGF_{1\alpha}$ release was seen in the mesenteric bed from male GH rats at either dose of AII, whereas 2 out of 4 control animals showed a marked increase in 6-oxo-$PGF_{1\alpha}$ release after 0.1 µg AII, and all 4 animals showed a significant increase in 6-oxo-$PGF_{1\alpha}$ release after 1.0 µg AII ($P < 0.05$). GH males showed a significantly greater ($P < 0.05$) pressor response to the 1.0 µg dose of NA and AII compared to control males. There was no significant stimulation of 6-oxo-$PGF_{1\alpha}$ release with either dose of NA or AII in the mesenteric bed from both control and GH female rats. Pressor responses to NA and AII were similar in normotensive and GH female rats. There was no difference in the basal release of 6-oxo-$PGF_{1\alpha}$ from the mesenteric bed of males compared to females in either GH or control rats.

It has been suggested previously that PGI_2 released from blood vessels following stimulation by NA may attenuate the pressor response (Pipili & Poyser, 1982). However, in the present study, there was a greater pressor response to NA in GH rats than in control rats indicating that PGI_2 alone is not controlling the extent of the pressor response. If the stimulation of PGI_2 release by AII is important in attenuating the pressor response to AII, the findings that GH males do not respond by releasing PGI_2 and show an increased pressor response to AII, may reflect a deficiency in this proposed aspect of the control of vascular tone in the GH male rat. However, in female normotensive and GH rats, PGI_2 release from the mesenteric vascular bed does not appear to be involved in the attenuation of pressor responses to NA and AII.

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THE HYPOTHALAMIC SITE AND MECHANISM OF ACTION OF METOCLOPRAMIDE TO FACILITATE GASTRIC EMPTYING

Brenda Costall, S.J. Gunning & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Gastric emptying can be facilitated by the intrahypothalamic injection of metoclopramide and clebopride (Costall et al, 1984). The present study investigates the topography of this action and the mechanisms involved.

Starved and fed Dunkin-Hartley guinea-pigs (500 ± 50 g) were subject to standard stereotaxic surgery for the injection of drug or vehicle via chronically implanted guides at 12 different locations in the hypothalamus (Table 1). Gastric emptying was determined by non-invasive fluoroscopic measurement of the passage of barium sulphate spheroids from the stomach 0.5-4.0 h after intrahypothalamic injection (Costall et al, 1983).

Table 1 Topographical analysis of the hypothalamic sites at which metoclopramide facilitates gastric emptying

Metoclopramide 1 µg, 1 h				Metoclopramide 0.2 µg, 30 min	
Lat. ±1.0 Vert. -10.5	Gastric emptying*	Lat. ±1.0 Ant. 8.9	Gastric emptying	Ant. 8.9 Vert. -9.5/-10.5	Gastric emptying
Ant. 9.6	80	Vert. -8.5	250†	Lat. ±1.0	195†/134**
Ant. 8.9	550†	Vert. -9.5	460†	Lat. ±1.6	420†/400†
Ant. 8.0	130	Vert. -10.5	550†	Lat. ±2.2	120/55

Anterior and lateral coordinates are according to the zero of the Kopf stereotaxic instrument. The vertical coordinates indicate depth below the dura.

*% control. **Values for Vert. -9.5/Vert. -10.5. n = 5-7. S.E.M.s (original data) <12%. †P < 0.05 - P < 0.001 (Mann Whitney U test).

Maximal increase in gastric emptying of starved guinea-pigs was caused by 1 µg metoclopramide injected into the perifornical area of the hypothalamus at Ant. 8.9, Lat. ±1.6 and Vert. -9.5 and -10.5 (Table 1). This action was dose-dependent with 25% emptying at 0.1 µg, 35% at 0.2 µg and 57% at 1.0 µg (control emptying 16%). Atropine injected into the perifornical area of the hypothalamus reduced the increased emptying of fed guinea-pigs (from 80% at 2 h to 67 and 38% at 0.2 and 1.0 µg atropine respectively) and dose-dependently antagonised the action of metoclopramide at the same site in starved guinea-pigs (71% emptying to metoclopramide alone at 2 h reduced to 62, 45 and 27% by 0.02, 0.2 and 0.4 µg atropine respectively). Hexamethonium (1 µg) did not antagonise the action of metoclopramide but carbachol did mimic its action by facilitating gastric emptying of starved guinea-pigs after 30 min from 25% to 44 and 68% at 0.2 and 2.0 µg respectively.

It is concluded that the perifornical area of the hypothalamus is an important locus of action for metoclopramide to facilitate gastric emptying. This action may involve an enhancement of cholinergic activity via a muscarinic mechanism.

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A FURTHER EXAMINATION OF INHIBITORY α_1-ADRENOCEPTORS OF RAT VAS DEFERENS

J. R. Docherty, L. Hyland & P. Warnock, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2.

Recent evidence suggests that inhibitory alpha-adrenoceptors on adrenergic nerve terminals, while predominantly of the $alpha_2$-subtype, may contain a proportion of $alpha_1$-receptors in pithed rat heart and in the rat isolated vas deferens (Docherty, 1983a). Difficulties are met in identifying presynaptic $alpha_1$-receptors : in the pithed rat heart $alpha_1$-agonists increase basal heart rate thus altering nerve-evoked responses indirectly (Docherty, 1983b); in the vas deferens $alpha_1$-agonists potentiate nerve-evoked responses by a postsynaptic action (Docherty, 1983a). The object of the present study was to obtain further evidence for presynaptic $alpha_1$-receptors in the rat vas deferens.

Epididymal portions of vasa deferentia were obtained from young adult wistar rats (2-3 months old), and isometric contractions were obtained to single pulse field stimulation at intervals of 5 min. Cumulative concentration/response curves were obtained to the $alpha_1$-agonist amidephrine in the presence of either the calcium entry facilitator Bayer K 8644 (10uM) (Schramm et al., 1983) or to the calcium entry blocker nifedipine (10uM); in some experiments prazosin (0.1uM) or yohimbine (0.1uM) was additionally present from 30 min prior to agonists. Isotope experiments were carried out as described elsewhere (Docherty et al., 1984).

In isotope experiments, the $alpha_2$-agonist xylazine (1uM) significantly reduced the transmitter overflow in response to stimulation for 3 min at 5 Hz to 42.2±3.2 % of control (n=4), but the $alpha_1$-agonists amidephrine (10uM) or cirazoline (10uM) had no significant effect on the stimulation evoked overflow. Effects of $alpha_1$-agonists on stimulation-evoked contractions were complicated by spontaneous contractions produced by the agonists.

In the presence of Bayer K 8644 (10uM), the isometric contraction to a single stimulus pulse was potentiated to 228.8±48.2 % of control (n=4), and amidephrine produced a concentration-dependent inhibition of this response. The inhibitory IC_{30} of amidephrine (concentration producing 30 % inhibition of contraction) in the presence of Bayer K 8644 was 6.02±0.31 (-log M, n=4), and this IC_{30} was shifted significantly by prazosin (0.1uM) to 4.36±0.23 (n=4, $P<0.01$) but not by yohimbine (0.1uM). In the presence of nifedipine (10uM), which prevents the postsynaptically mediated potentiation of nerve-evoked contractions by amidephrine, amidephrine had an inhibitory IC_{30} of 6.82±0.06 (n=4) and this IC_{30} was not shifted significantly by rauwolscine (0.1uM).

In conclusion, the inhibition by amidephrine of the contraction to a single pulse in the presence of Bayer K 8644 was $alpha_1$-mediated since the IC_{30} was shifted significantly by prazosin but not by yohimbine. The failure of $alpha_1$-agonists to inhibit transmitter overflow to trains of pulses at 5 Hz may mean that the inhibitory actions of amidephrine against single pulses is postsynaptic in origin or that there are too few presynaptic $alpha_1$-receptors to inhibit transmission with long trains of pulses. Overall, it is more likely that the inhibitory $alpha_1$-effects are presynaptic since they occurred both when amidephrine caused contractions of the vas (in the presence of Bayer K 8644) and when it did not (in the presence of nifedipine).

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ACCUMULATION OF (^{3}H)-ADRENALINE BY RABBIT ISOLATED AORTA

J. Abrahamsen & O.A. Nedergaard, Department of Pharmacology, School of Medicine, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

The uptake of adrenaline by isolated blood vessels has been the concern of only a few reports (Osswald & Guimarães, 1983). The importance of adrenaline as a circulating hormone has recently gained new interest, since increased plasma levels of adrenaline may represent the primary "abnormality" in essential hypertension (Brown & Macquin, 1981). The aim of the present investigation was to study the accumulation of (-)-^{3}H-adrenaline (^{3}H-A) by a blood vessel in vitro.

The ring preparation of rabbit isolated aorta was used. In all experiments, monoamine oxidase and catechol-O-methyltransferase were inhibited by treatment with pargyline and 3',4'-dihydroxy-2-methyl-propiophenone (U-0521), respectively. The methods described in detail (Nedergaard, 1980) were used.

The relationship between accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M) and duration of incubation (0-3 hr) was linear. The ^{3}H-accumulation after 3 hr incubation was 22.5 ml·g^{-1}. In reserpine-treated tissue, the ^{3}H-accumulation levelled off after 30 min and was 8.5 ml·g^{-1} after 3 hr. Concentration of ^{3}H-A or (-)-^{3}H-noradrenaline (^{3}H-NA) and ^{3}H-accumulation (ml·g^{-1}) was inversely related. At 10^{-8}M, the 1 hr accumulation of ^{3}H derived from ^{3}H-A and ^{3}H-NA was 7.8 and 15.2 ml·g^{-1}, respectively. With increasing concentrations ($3x10^{-8}$-10^{-4}M) the accumulation values approached each other. At 10^{-4}M, the accumulation was 2.3 and 2.8 ml·g^{-1} for ^{3}H-A and ^{3}H-NA, respectively. The accumulation of ^{3}H derived from ^{3}H-A (10^{-8}-10^{-4}M) by reserpine-treated tissue also showed an inverse relationship with concentration: 5.4 ml·g^{-1} (10^{-8}M) and 2.6 ml·g^{-1} (10^{-4}M). The accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr) was dependent on bath temperature (1-37°C). The accumulation increased continuously from 1.1 ml·g^{-1} (1°C) to 11.1 ml·g^{-1} (37°C).

Storage of tissue (0-5 days in salt solution without equilibration with 95% O_2/5% CO_2; 4°C) did not affect the accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr). Thereafter (7-14 days), the accumulation decreased.

The inhibitory potency (ID_{50}; -log M) of desmethylimipramine, cocaine, (-)-propranolol, (-)-isoprenaline, and (-)-normetanephrine on accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr) was found to be 8.26; 6.50; 5.48; 4.88 and 4.02, respectively. The maximal degree of inhibition was almost the same for these drugs, while that of clonidine and corticosterone was 50 and 20%, respectively. In the presence of desmethylimipramine (10^{-6}M), either clonidine (10^{-5}-10^{-3}M), corticosterone (10^{-6} -10^{-4}M) or (-)-isoprenaline (10^{-5}-10^{-3}M) reduced the accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr).

Ouabain ($3x10^{-4}$M) and iodoacetic acid (10^{-3}M), but not sodium cyanide (10^{-3}M) and 2,4-dinitrophenol (10^{-3}M) reduced accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr). Anoxia (95% N_2/5% CO_2; 37°C; 1-24 hr) did not alter accumulation of ^{3}H derived from ^{3}H-A (10^{-8}M; 1 hr). D-(+)-glucose deprivation alone or combined with anoxia markedly reduced ^{3}H-accumulation.

These results suggest that adrenaline is transported into neuronal and extraneuronal sites via a carrier mechanism which is energy-dependent.

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THE EFFECT OF ACUTE ADMINISTRATION OF DILTIAZEM AND NIFEDIPINE ON THE FUNCTION OF THE DENERVATED RAT KIDNEY

E. J. Johns. Department of Physiology, The Medical School, Birmingham, B15 2TJ.

There is increasing evidence that the calcium entry blocking drugs have an effect on the kidney to increase the excretion of sodium and water in man (Lederballe Pedersen, 1983) and animals (Brown & Churchill, 1983). The aim of this study was to examine more closely the renal actions of both diltiazem and nifedipine at dose levels which had either no or modest effects blood pressure.

Sprague-Dawley rats (345-425 g) were anaesthetised with sodium pentobarbitone, 240μmol/kg i.v., and a carotid artery and jugular vein cannulated for blood pressure measurements and infusion of saline (6.6ml/h), inulin and drugs, respectively. The left kidney was exposed via a ventral mid-line incision, the renal nerves were sectioned, the ureter cannulated and a flow probe placed on the renal artery. Experiments were begun 2h after the completion of surgery and consisted of twenty minute clearance periods, two before and two 30min after beginning drug administration. The mean values of the periods before and after the drug were compared.

Infusion of 5 μg/min/kg diltiazem (6 rats) had no effect on the blood pressure of 121±7mmHg. Renal blood flow (RBF), 17.86±1.64 ml/min/kg, did not change but there was a 24% increase in glomerular fltration rate (GFR) from 3.30±0.20 ml/min/kg ($p<0.05$). Urine flow (UV, 28.5±3.2 μl/min/kg), increased but not significantly, while absolute sodium excretion ($U_{Na}V$, 4.33±0.83 μmol/min/kg) and fractional sodium excretion (FE_{Na},1.21±0.19%) increased significantly (both $p<0.05$) by 154 and 77% respectively. Diltiazem 10 μg/min/kg (6 rats), decreased blood pressure by 14±3 mmHg from 121±5 mmHg ($p<0.01$), did not change either RBF, or GFR, but increased UV by 20% from ($p<0.05$),$U_{Na}V$ by 20% ($p<0.02$) and FE_{Na} by 24% ($p<0.02$). Administration of diltiazem at 20 μg/min/kg into 6 rats significantly ($p<0.001$) decreased blood pressure from 118± mmHg to 97+4 mmHg and did not change RBF, GFR, UV, $U_{Na}V$ or FE_{Na}. Nifedipine infused at 0.5 μg/min/kg into 5 rats decreased blood pressure significantly($p<0.01$) from 125+6 to 115+4 mmHg, did not change RBF or GFR but significantly increased UV by 127%($p<0.01$), $U_{Na}V$ by 96% ($p<0.02$) and FE_{Na} by 90%($p<0.02$). Administration of 1.0 μg/min/kg nifedipine into 6 rats decreased blood pressure significantly ($p<0.02$) from 114+5 mmHg, increased RBF by 7% ($p<0.05$), did not change GFR but increased UV by 127%($p<0.01$) from $U_{Na}V$ by 197% ($p<0.02$) and FE_{Na} by 194%($p<0.02$). Infusion of 2.0 μg/min/kg nifedipine in 5 animals decreased blood pressure from 116±4 to 93± 4mmHg ($p<0.001$), had no effect on either renal haemodynamics or excretion. Basal values of all variables were similar in all groups of animals.

These results show that acute administration of diltiazem and nifedipine over these dose ranges had minimal effects on renal haemodynamics. Both compounds, in doses which caused no or small reductions in blood pressure produced marked increases in the excretion of both sodium and water. At higher dose rates, which cause moderate reductions in blood pressure, the natriuretic and diuretic activities are then not apparent. These data indicate that diltiazem and nifedipine have a direct action on the sodium reabsorptive processes of the nephron.

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EXAMINATION OF RESERPINE- AND HYPOTHERMIA-INDUCED SUPERSENSITIVITY TO β-ADRENOCEPTOR AGONISTS BY USE OF FORSKOLIN

K.J. Broadley, R.G. Chess-Williams, P.F. Grassby and M.H. Hawthorn, Division of Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF1 3NU

Isolated cardiac tissues exhibit a specific supersensitivity to β-adrenoceptor agonists after chronic pretreatment of animals with reserpine (Hawthorn & Broadley 1982) and when tissues are cooled (Broadley 1980). This study examines whether these examples of supersensitivity are due to increases in adenylate cyclase sensitivity by use of forskolin, a direct stimulant of this enzyme (Seamon et al. 1981).

Tension increases of guinea-pig paced left atria and papillary muscles (2Hz, threshold voltage + 50%, 1ms pulse width) and rate increases of spontaneous right atria were recorded. Tissues were suspended in Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in O_2 and containing phentolamine (5μM) and metanephrine (10μM).

To examine reserpine-induced supersensitivity, guinea-pigs receiving reserpine for 3 days (5mgkg^{-1} at 72h, 3mgkg^{-1} at 48 and 24h) or 7 days (0.1mgkg^{-1} daily i.p.) were compared with untreated animals. A cumulative dose-response curve to isoprenaline was followed by one to forskolin and geometric mean EC50 values calculated ($n \geq 4$). The sensitivity of left atria to isoprenaline was increased by 3 and 7 day reserpine pretreatment, the EC50 values falling from 10.2 to 3.8 and 3.3nM respectively. However, forskolin was not affected, the EC50 values for 3 day (0.75μM) and 7 day pretreatments (0.44μM) not differing significantly from controls (0.68μM). Similarly, in papillary muscles, the EC50 values for isoprenaline were significantly less after 3 and 7 day pretreatments (4.2 and 5.6nM) than in untreated tissues (13.9nM), but no difference occurred between pretreated tissue (0.53 and 0.85μM respectively) and controls (0.75μM) with forskolin.

Hypothermia-induced supersensitivity was examined by obtaining a dose-response curve to isoprenaline (or forskolin) at 38°C, and repeating after cooling to 30°C. Supersensitivity to isoprenaline was exhibited by significant leftwards shifts of the curves in left (EC50; 38°C, 26.5μM, 30°C, 0.63μM) and right atria (EC50; 38°C, 12.2μM, 30°C, 0.55μM). There was also a small but significant reduction of EC50 values on cooling for forskolin, from 0.50 to 0.23μM in right atria and 1.19 to 0.38 M in left atria. The dose-ratios for this change with forskolin (3.1±0.3 and 2.5±0.9) were, however, significantly less than for isoprenaline (43.0±5.2 and 15.1±3.9).

No β-adrenoceptor-mediated components of the responses to forskolin were found since propranolol (10μM) failed to displace the curves.

The site of hypothermia- and reserpine-induced supersensitivity does not therefore appear to be directly on adenylate cyclase but at a preceding stage such as the coupling mechanisms.

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THE EFFECT OF EXPERIMENTAL DIABETES ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO OXYTOCIN AND PROSTAGLANDINS

J.M. Foy, Judith Senior & Rose Zeko, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP

Pregnancy in diabetic subjects is associated with an increase in intrauterine and neonatal mortality, congenital abnormalities and an increased incidence of spontaneous abortion (Chamberlain, 1977). Experimental diabetes may be induced in animals using streptozotocin, thus it is possible to study mechanisms whereby diabetes affects pregnancy (Golob et al., 1970). In the current study the effect of streptozotocin induced diabetes in the rat was used to investigate uterine responsiveness in vitro to oxytocic agents using tissue from pregnant and non-pregnant animals.

Virgin female rats (Sprague-Dawley derived) weighing between 250-350g were used. Pregnant rats were housed in single cages after mating, the day of finding the vaginal plug was taken as day 1 of pregnancy. Diabetes was induced by injection of streptozotocin 50 mg kg^{-1} i.v. Pregnant rats were treated on day 12 of gestation and were used on day 20 or 21. Non-pregnant rats were given streptozotocin at least 48h before the experiment. Control rats received injections of vehicle only. All non-pregnant rats were given stilboestrol 1 mg kg^{-1} s.c. 18h before the experiment to ensure a similar hormonal pattern in the uterine tissue. Blood was collected from all animals at the start of the experiment and the blood glucose concentration was measured using a YSI Glucose Analyser (Model 23AM). Animals were considered to be diabetic if the blood glucose was higher than 8 mmol l^{-1}. The uterine tissue was removed and superfused with Krebs' solution maintained at 37°C at a rate of 2 ml min^{-1}. The Krebs' solution was aerated with 95% O_2 5% CO_2. Uterine strips (2mm x 20mm) were used from pregnant rats, the whole uterine horn was superfused from non-pregnant animals. Dose-response curves were established to oxytocin, prostaglandin E_1 (PGE_1), PGE_2, and $PGF_2\alpha$. Tension changes were analysed in terms of area of the response using an integrator. Group size = 6.

In the non-pregnant rats, the uteri from diabetic animals were less sensitive to the PGs ($P < 0.05$). Oxytocin showed different results, with the uteri from diabetic animals appearing to be more sensitive than the tissue from normal rats, though failing to show a statistically significant difference. The uteri from pregnant rats which were diabetic were more sensitive to the PGE's and oxytocin when compared to the results from normal pregnant animals ($P < 0.001$-0.05). PGs of the E series and oxytocin were more active on the uterine strips from pregnant animals than on the whole uterine horns from non-pregnant rats. $PGF_2\alpha$ responses were not significantly different on tissue from pregnant and non-pregnant animals. The order of potency of the oxytocic agents tested in normal and diabetic non-pregnant rats was: oxytocin >$PGF_2\alpha$ >PGE_2 >PGE_1. In the normal pregnant animals the order changed to: oxytocin >PGE_1 >PGE_2 >$PGF_2\alpha$, whereas in the diabetic pregnant rats it was: oxytocin >PGE_2 >$PGF_2\alpha$ >PGE_1.

It would appear from the results that the presence of diabetes further sensitizes the uterus of the pregnant rat to oxytocics and this may be a factor involved in the complications associated with pregnancy in the diabetic subject.

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EFFECTS OF PRAZOSIN AND NICERGOLINE ON NORMAL AND MILDLY DEPRESSED SHEEP PURKINJE FIBRE ACTION POTENTIALS

K.A. Kane, J.R. Parratt & A.L. Rothaul. Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, U.K.

In this study, the effects of prazosin and nicergoline, both α, adrenoceptor blocking agents, on the intracellular action potential characteristics of sheep Purkinje fibres superfused in vitro with a normal or an altered physiological salt solution (P.S.S.) were examined. The P.S.S. (normal composition in mM:NaCl 125, KCl 5.4, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.1, $CaCl_2$ 1.8, glucose 5) was altered to mimic some of the conditions occurring during ischaemia by raising the potassium concentration to 6 mM, lowering sodium bicarbonate to 1.9 mM and by gassing with 95 N_2% 5% CO_2 rather than 95% O_2 5% CO_2. This yielded in normal and modified P.S.S. a pH of 7.3±0.03 and 6.9±0.08 and a PO_2 of 419±31 and 122±15 mmHg respectively. The preparations were stimulated at a frequency of 1.5 Hz. In the experiments using normal P.S.S., the drug was added cumulatively and readings taken from 10 cells before and after a 30-40 min exposure period. A different set of preparations was exposed to either altered P.S.S. alone or that containing drug for a period of 1h. Table 1 summarises the results obtained.

Table 1. The effect of prazosin and nicergoline on the resting membrane potential (RMP), maximum rate of depolarisation of phase 0 (MRD) and the action potential duration of 90% repolarisation (APD_{90}) of sheep Purkinje fibres superfused with normal or modified P.S.S.

	Prazosin				Nicergoline			
P.S.S.	Concentration μM	RMP mV	MRD v/s	APD_{90} ms	Conc. μM	RMP mV	MRD v/s	APD_{90} ms
normal	0	90±1	359±19	247±3	0	89±1	322±11	259±6
	2	89±1	351±14	243±2*	0.1	88±1	285±8*	253±6
	16	88±1	248±6*	214±6*	1.0	88±1	202±7*	271±2
	64	84±1	140±1*	181±1*	2.0	87±1	151±7*	250±7
modified	0	77±2*	242±22*	201±13*	0	77±1*	242±22*	201±13*
	2	73±1	173±8‡	193±13	1.0	75±2	130±14‡	220±20

*$P<0.05$ ‡$P<0.05$ significantly different from control in normal and modified P.S.S. respectively. 5 preparations were used in each set of experiments.

Both α-adrenoceptor blocking agents caused a dose-dependent reduction in the rate of depolarisation of phase 0 of the normal action potential (MRD) without altering the resting membrane potential (RMP); the threshold concentrations being 8 and 0.1 μM for prazosin and nicergoline respectively. Prazosin also significantly shortened the action potential duration (APD_{90}). Exposure to the altered P.S.S. caused a fall in RMP and MRD and a shortening of APD_{90}. The sodium channel blocking properties of prazosin appeared to be potentiated in the presence of the altered P.S.S. since a concentration of 2 μM reduced MRD and concentrations of 8 μM or above rendered the fibres inexcitable. On the other hand, nicergoline (1 μM) had a similar effect on mildly depressed and normal action potentials.

In conclusion, therefore, both α-adrenoceptor blocking drugs studied, prazosin and nicergoline, possess sodium channel blocking activity; this might contribute to their antiarrhythmic effects in vivo.

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CORONARY ARTERY OCCLUSION-INDUCED ARRHYTHMIAS IN THE ANAESTHETISED RAT AND MYOCARDIAL LEVELS OF CYCLIC AMP AND CYCLIC GMP

K.A. Kane, E.J. Mercillo*, I.W. Rodger, M. Shahid. Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.
*Department of Pharmacology, University of Alcala de Henares, Spain.

One of the aims of this study was to determine whether a relationship exists between the occurrence of coronary artery occlusion-induced arrhythmias in the anaesthetised rat and the levels of cyclic AMP and cyclic GMP in both normal and ischaemic myocardium. Furthermore, we investigated whether such arrhythmias were modified by pretreatment with two phosphodiesterase inhibitors, quazodine and isobutyl methylxanthine (IBMX) or with butyryl derivatives of cyclic AMP and GMP.

Pentobarbitone-anaesthetised male rats were subjected to occlusion of the left main coronary artery as previously described (Clark et al. 1980). The severity of the resultant arrhythmias, which occurred within the first 30 min post-occlusion, was assessed in control and drug pretreated animals. Drugs were administered intravenously either 15 or 30 min prior to ligation in the following doses. Quazodine 1 mg kg^{-1} bolus followed by an infusion of 1 mg kg^{-1}, IBMX, 0.1 mg kg^{-1} and 0.1 mg kg^{-1} min^{-1}, dibutyryl cyclic AMP and cyclic GMP as an infusion only of 10 µg kg^{-1} min^{-1}. In a separate series of experiments, cyclic AMP and cyclic GMP content of the right and left ventricular free wall was measured, as described by Rodger & Shahid (1984), in both sham operated hearts and in those ligated for periods of 5,10 and 30 min. The cyclic nucleotide levels of hearts pretreated with IBMX were also measured.

Prior to the occurrence of marked arrhythmic activity, at 5 min post-ligation, cyclic AMP levels were elevated in both normal and ischaemic myocardium, respective values in pmol/mg wet weight being 0.55±0.02 sham vs. 0.76±0.05 and 0.63±0.01 sham vs 0.69±0.03. In these hearts cyclic GMP levels in normal myocardium were also significantly higher when compared with sham-operated animals (0.037±0.003 vs. 0.029±0.002 pmol mg^{-1} wet weight) whereas in ischaemic tissue the levels were similar (0.033±0.003 and 0.034±0.004 pmol mg^{-1}). However, at the peak of the arrhythmias (10 min post-ligation) both cyclic AMP and cyclic GMP levels had fallen in ischaemic, althought not in normal tissue, to below those observed in sham-operated animals. A similar pattern of cyclic nucleotide levels was observed at 30 min post-ligation.

The severity of occlusion-induced arrhythmias was exacerbated by prior administration of all the drugs. All pretreated animals fibrillated compared with 58% of control animals and mortality was also significantly increased in those given quazodine or IBMX. There were also more ventricular extrasystoles in those drug-treated animals which survived the first 30 min of occlusion. IBMX, in this arrhythmogenic concentration, significantly increased both cyclic AMP (0.63±0.01 sham vs 0.92±0.03 pmol mg^{-1}) and cyclic GMP (0.034±0.004 sham vs 0.052±0.006 pmol mg^{-1}) content of left ventricular tissue.

Thus, we conclude that in the pentobarbitone-anaesthetised rat subjected to coronary artery occlusion there is no evidence to support the hypothesis suggested by Podzuweit et al. (1976) that a rise in cyclic AMP content of the ischaemic myocardium occurs at the same time as the resultant arrhythmias. We did, however, observe that drug intervention designed to raise the myocardial levels of both cyclic AMP and cyclic GMP exacerbated ischaemia-induced arrhythmias, suggesting that both of these nucleotides may induce arrhythmic activity during ischaemia.

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(^{3}H)-DOPAMINE UPTAKE INTO RAT SUBSTANTIA NIGRA MAY INVOLVE 5-HT NEURONES

P. Jenner, E. Kelly & C.D. Marsden, University Department of Neurology, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

The uptake of ^{3}H-dopamine into and release from slice and synaptosomal preparations of substantia nigra may be an index of dendritic dopamine function (Geffen et al, 1976; Silbergeld & Walters, 1979). However the specificity of these indices for nigral dopamine neurones remain unclear. We now present evidence to suggest that the accumulation of ^{3}H-dopamine by P_2 synaptosomal preparations of rat substantia nigra may, at least in part, not reflect uptake into nigral dopamine dendrites.

P_2 pellets were prepared from rat substantia nigra and striatum and finally suspended in 20 volumes of Krebs bicarbonate buffer according to original tissue weight. Aliquots (100 ul) were incubated in 1.4 ml oxygenated buffer at 37°C and ^{3}H-dopamine (9.7 Ci/mmole) added to give a final concentration of $5x10^{-8}$M. After 5 min the process was terminated by vacuum filtration onto Whatman GF/C filters. Blank values were determined by incubation of synaptosomes on ice at 0°C. Nomifensine hydrogen maleate, fluoxetine HCl or desipramine HCl were incorporated in the incubation medium as required.

In striatal P_2 preparations, the inclusion of nomifensine ($5x10^{-7}$M) inhibited ^{3}H-dopamine uptake whereas fluoxetine ($5x10^{-7}$M) and desipramine ($5x10^{-7}$M) were without effect (Table 1). In contrast, in substantia nigra P_2 preparations nomifensine ($5x10^{-7}$M), fluoxetine ($5x10^{-7}$M) and desipramine ($5x10^{-7}$M) all inhibited ^{3}H-dopamine uptake (Table 1).

Unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle 3 weeks previously markedly reduced ipsilateral striatal ^{3}H-dopamine uptake but nigral uptake was unaffected (Table 1). In contrast, bilateral electrolesions of medial and dorsal raphe nuclei 1 week previously reduced nigral ^{3}H-dopamine uptake but striatal ^{3}H-dopamine uptake was unchanged (Table 1).

Table 1 ^{3}H-Dopamine uptake into P_2 preparations of rat striatum and substantia nigra

Treatment	Striatum	Substantia nigra
Control	60.2±2.1	3.1±0.2
Nomifensine ($5x10^{-7}$M)	16.1±2.1*	1.9±0.3*
Fluoxetine ($5x10^{-7}$M)	59.0±2.6	1.9±0.4*
Desipramine ($5x10^{-7}$M)	59.6±3.8	1.8±0.1*
6-OHDA lesioned side	11.8±1.1*	3.2±0.4
Non-lesioned side	59.8±3.2	2.8±0.3
Electrolesion raphe	63.3±2.2	1.9±0.4*
Sham lesioned	61.3±3.0	2.9±0.3

Values are p moles ^{3}H-dopamine taken up/0.1mg protein/hr ± 1 SEM. n=4-8 for each manipulation, nigral tissue being pooled from 6-8 rats for each n.
* $p < 0.05$ compared to control for striatum or substantia nigra respectively.

These results suggest that major differences may exist between the uptake of ^{3}H-dopamine into nigral and striatal P_2 preparations. In the substantia nigra, at least a proportion of ^{3}H-dopamine appears to be accumulated by non-dopaminergic neurones, particularly 5-HT neurones.

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CARDIOVASCULAR EFFECTS OF WY 26392 AND RX 781094 IN ANAESTHETISED CATS

Margaret E. Philpot and N.B. Shepperson, Department of Pharmacology, Wyeth Institute of Medical Research, Taplow, Maidenhead.

The benzoquinolizine Wy 26392, has previously been reported to be a selective α_2-adrenoceptor antagonist in-vivo (Pierce and Waterfall, 1982). We have now compared some of the cardiovascular effects of Wy 26392 with those of RX 781094 (Idazoxan), a selective α_2-antagonist (Chapleo et al, 1981).

Female cats (2.3-4.0kg) were anaesthetised with sodium pentobarbitone (30mg/kg i.t. and 6mg/kg/hr i.v.) and ventilated artificially ($25min^{-1}$; S.V. 10ml/kg). Cannulae were placed in a brachial vein, for drug/saline administration, and in a femoral artery, for blood pressure (BP) recording. Heart rate was derived from the blood pressure signal using a tachograph. An indirect estimate of left ventricular contractility (dP/dt/P) was obtained from the first differential of left intraventricular pressure, measured via a catheter passed down the left carotid artery into the left ventricle. Wy 26392 or RX 781094 (0.001-30.0mg/kg i.v.) or saline (1ml) were administered cumulatively every fifteen minutes.

In further experiments, cats were bivagotomised and ganglion-blocked (chlorisondamine 1.0mg/kg). Phenylephrine (PE) (1-30µg/kg) and UK14,304 (1-30µg/kg) were administered intravenously and inhibition of the pressor responses to these agonists by Wy 26392 and RX 781094 was used as an index of α_1 and α_2 adrenoceptor blockade.

Wy 26392 had no significant cardiovascular effects at doses of 0.1mg/kg and below, although a dose of 0.01mg/kg was sufficient to produce marked α_2 adrenoceptor blockade. At higher doses ($\geq$1.0mg/kg), significant falls in BP were obtained; systolic and diastolic BP fell by 26±3% and 44±3% respectively with 1.0mg/kg Wy 26392, a dose which produced marked inhibition of responses to PE and UK 14,304. Heart rate and dP/dt/P also fell significantly with doses of 1.0mg/kg and above, maximum falls of 43±5% and 34±4% being observed with 10.0mg/kg Wy 26392.

RX 781094 (0.01-0.1mg/kg) increased systolic BP, which rose significantly by 27±6mmHg following 0.03mg/kg RX 781094. Diastolic pressure and heart rate also rose with 0.01 and 0.03mg/kg RX 781094 whereas dP/dt/P did not change over this dose range. A dose of 0.01mg/kg RX 781094 produced only moderate α_2-adrenoceptor blockade.

RX 781094 ($\geq$0.1mg/kg) evoked marked dose-dependent decreases in dP/dt/P which fell maximally by 46±6% following 10.0mg/kg; heart rate fell significantly following 1.0 and 3.0mg/kg RX 781094. There were no significant effects on blood pressure at high doses, despite significant inhibition of the pressor responses to PE and UK14,304 with 1.0mg/kg RX 781094.

It is unlikely that the qualitative differences in the cardiovascular effects of Wy 26392 and RX 781094 can be explained solely by their α_2-adrenoceptor antagonist properties. In rats, the transient pressor responses produced by RX 781094, seen also in the present study, have been shown to be due to partial agonist properties at α_1 adrenoceptors (Paciorek & Shepperson, 1983). This property may explain why large falls in BP are not observed following high doses of RX 781094.

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A MOUSE IN VIVO MODEL TO DETECT DUAL INHIBITORS OF ARACHIDONIC ACID METABOLISM

A. Blackham & A.M. Woods, Department of Biochemistry, Fisons plc., S & T Laboratories, Loughborough LE11 ORH

The release of SRS from resident peritoneal cells of actively and passively sensitised rats has been widely reported (Jancar et al 1976; Ross et al 1976; Burka & Flower 1979) and attributed to anaphylactic disruption of mast cells (Lewis & Austen 1981). Immune complexes stimulate SRS release in vitro from mouse peritoneal macrophages (Humphray et al 1981). We describe the production of SRS and dye extravasation into the mouse peritoneum after intraperitoneal injection of preformed insoluble ovalbumin/anti-ovalbumin (OA/antiOA) immune complexes.

Female LACA mice (20-30g) received 0.25ml of 0.5% pontamine sky blue in saline i.v. immediately before i.p. injection of 0.75ml of a suspension of washed OA/antiOA (200µg OA + equivalence of rabbit antiserum). Groups of 6 mice were killed at intervals up to 5hrs later and the peritoneal washouts (2ml) were examined for the presence of SRS (bioassays, guinea-pig ileum superfusion), dye intensity (optical density at 630nm) and cell infiltration. Maxima, SRS: 5-10 min (60ng LTC_4 equivalents/mouse approximately), dye: 30mins, cells: 4hr (mainly PMN).

The effect of oral BW755C, phenidone and indomethacin given 30min before OA/anti-OA on SRS formation and dye extravasation is shown in Table 1. The total inhibition of SRS production by BW755C and phenidone 50mg/kg, associated with partial inhibition of dye extravasation at both times, indicates a causal relationship between these events. Indomethacin inhibited dye extravasation at the earlier time (dose response 0.3-1mg/kg) but not at 30mins and increased SRS formation at both times.

Table 1 Drug effects on extravasation of dye and SRS release (n = 4-6)

		% Inhibition			
	Oral dose	7.5 min		30min	
Compounds	mg/kg	Dye	SRS	Dye	SRS
BW 755C	25	24	77	46*	100
	50	68*	100	38*	100
	200	77*	100	46*	100
Phenidone	25	22	85	0	67
	50	27	100	40	100
Indomethacin	0.3	19	0(+14)	0(+13)	0(+23)
	1	49*	0(+62)	0(+ 7)	0(+41)
	3	54*	0(+57)	0(+19)	15

* Student's 't' test $p<0.05$

	O.D.	LTCng	O.D.	LTCng
Control values from 5 assays (range)	.346 (.236 - .545)	87 (49 - 113)	1.002 (.807 - 1,054)	30 approx.

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ISOPRENALINE ACTION ON TETANI OF SLOW CONTRACTING SKELECTAL MUSCLE

Karen L. Cheesebrough, R.W. Marshall, A. Richens & J.F. Wilson, Department of Pharmacology & Therepeutics, Welsh National School of Medicine, Heath Park, Cardiff. CF4 4XN U.K.

Isoprenaline has been shown to reduce the degree of tetanic fusion in the cat soleus muscle produced by electrical stimuli, (Bowman, W.C. et al 1970). This effect is thought to be due to an increase in the rate of relaxation of the muscle, thus shortening the time course of the mechanical response. We have investigated the mechanism of this effect on the guinea-pig soleus muscle using stimuli consisting of either single or pairs of square (0.1 ms) pulses.

Female guinea-pigs were spinalised under chloral hydrate anaesthesia. The soleus muscle was dissected free from surrounding muscle bundles and its tendon attached to an isometric force transducer. Contractions of the muscle were elicited by electrical stimulation of the sciatic nerve. The output from the force transducer was digitsed to 8-bit resolution at a sampling frequency of 500 Hz and stored on a floppy disc under the control of a microcomputer. The muscle was stimulated at 30 second intervals with a single pulse followed by 10 seconds later by a pair of pulses. The repsonses to single pulses were analysed to extract a range of parameters (Marshall et al., 1981). Each was then subtracted from the next twin pulse response to obtain the response to the second pulse of the pair which was analysed in the same way. The percentage difference between responses to first and second pulse was calculated by scaling both responses relative to the peak responses from the single pulse and subtracted one from the other over the time course of the response. Isoprenaline was given as an intravenous infusion.

The action of isoprenaline (5-2000 ng/min)on the single pulse response of the muscle was to produce variable increases in the peak tension (max 20%), and the maximum rates of contraction (max 15%) and relaxation (max 75%). The contraction-relaxation time and the time to 75% recovery of the response were reduced, and these actions though more consistent were small (max 15%). Isoprenaline produced qualitatively similar effects on the response of the muscle to the second pulse, the magnitude of the effects being slightly altered by changing the interval between the two pulses of the paired stimuli. At intervals of 26 and 76 ms between the stimuli isoprenaline increased the peak tension and the rates of contraction and relaxation. These changes were not different between the two intervals, nor were they different from those seen to the single pulses. The action of isoprenaline on the contraction-relaxation time and the time to 75% recovery at the two intervals were similar. At both intervals this action was greater than the corresponding effect on the single pulse response (25-30%). The effect of isoprenaline on the difference between the first and second pulse response of a pair varied considerably with changes in the interval between the paired pulses. At 26 ms the differenace was multiphasic, being positive in nature up to 96 ms after the stimilus and then negative for the rest of the response (approx. 200 ms). The area of positive phase increased by up to 700% and the area of negative phase up to 1500% at this interval. With a 76 ms interval the difference between the two pulses was almost monophasic. This phase peaked at 100 ms after stimuli and lasted for the duration of the response. Isoprenaline produced a 2000% change in the area of this phase. The changes produced by isoprenaline on responses to first and second pulses were small when assessed by conventional techniques such as twitch height. However, the overall difference between these responses which is a measure of the interaction between successive pulses was very substantially altered by isoprenaline. This suggests that the dominant drug effect on partially fused tetani is due to an alteration in the interaction between successive pulses of the train.

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APOMORPHINE MODIFIES THE VOLUME AND ACID CONCENTRATION OF GASTRIC SECRETION IN THE RAT VIA TWO DISTINCT MECHANISMS

Brenda Costall, R.J. Naylor & Connie C.W. Tan, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

Dopamine infusion can inhibit gastric acid secretion in man and dogs (Caldara et al, 1978, Valenzuela, 1976) although the precise catecholamine receptor type(s) mediating the responses is not clear. In the present study the ability of apomorphine to modify gastric acid secretion in the rat is investigated to clarify a possible role of dopamine receptors in gastric secretion.

Male Sprague-Dawley rats (300 $\pm$ 50 g) were anaesthetised and a stainless steel gastric cannula was introduced using a modification of the method of Lane et al (1957). Two weeks after surgery, and after fasting for 18 h but with free access to water, the stomachs were rinsed with 6 x 5 ml warm normal saline followed by 3 x 5 ml warm distilled water and the animals placed in individual cages. Gastric secretion collected over the first 15 min was discarded; samples were then collected hourly for 4 h. The volume, H^+, Cl^-, Na^+ and K^+ ion concentrations were determined.

The administration of apomorphine (0.25, 0.35 and 0.5 mg/kg s.c.) caused dose-related reductions (22-68%) in the volume of gastric secretion and also a reduction (44-69%) in gastric acid concentration when assessed after 1 h. The Cl^- ion concentration was also reduced, significantly at 0.5 mg/kg apomorphine from 170.0 $\pm$ 13.0 to 102.0 $\pm$ 17.0 µM/ml, whereas the Na^+ and K^+ concentrations were unchanged (normal values 42.0 $\pm$ 3.7 and 11.0 $\pm$ 1.5 µM/ml respectively). All values had returned to control levels at 2 h (1.9 $\pm$ 0.3 ml/h, 87.3 $\pm$ 6.1 µM/ml H^+).

Catecholamine receptor antagonists were used at doses that did not modify gastric acid secretion and were administered i.p. 45 min before apomorphine. Haloperidol (0.5 mg/kg) enhanced the duration of action of apomorphine (0.5 mg/kg) to reduce the volume of gastric secretion but abolished the apomorphine-induced changes in gastric acid concentration and Cl^- ion concentration. In contrast, dl-propranolol (5.0 mg/kg) prevented the apomorphine-induced reductions in gastric secretary volume whilst failing to antagonise the reduction in gastric acid concentration caused by apomorphine. Prazosin (0.5 mg/kg) and yohimbine (5 mg/kg) failed to antagonise the apomorphine effects.

Thus, apomorphine is shown to modify gastric secretion via two distinct mechanisms. Reductions in gastric volume can be achieved via a ß-adrenoceptor stimulation (propranolol-sensitive) whilst reductions in acid concentration of gastric secretions are mediated via haloperidol-sensitive (dopamine) receptors. A further characterisation of these receptors and their location is being established.

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LEUKOTRIENE D_4 DOES NOT STIMULATE ^{45}Ca UPTAKE INTO GUINEA PIG TRACHEAL SMOOTH MUSCLE

D. Raeburn* & I.W. Rodger, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Leukotriene D_4 (LTD_4)-induced contractions of guinea pig isolated airways are only partially inhibited by high concentrations of nifedipine and verapamil but completely inhibited by the intracellular Ca^{2+} ion antagonist TMB-8 (Weichmann et al. 1983). These observations suggest that LTD_4 may utilise Ca^{2+} from an intracellular source to initiate contraction. We have investigated this possibility by comparing the effects of LTD_4 and KCl on ^{45}Ca uptake and tension responses in guinea pig tracheal smooth muscle.

Spirally cut preparations of trachea were suspended in Krebs-Henseleit solution (KHS) at 37°C and bubbled with 5% CO_2 in O_2. Isometric tension changes in response to each agonist were measured by conventional means. Lanthanum-resistant ^{45}Ca content (in Tris buffered KHS) was measured using the method of Van Breemen & McNaughton (1970) as modified by Godfraind (1976) and Deth (1978). Some experiments were performed in the presence of flurbiprofen($1x10^{-6}$ mol l^{-1}) to inhibit formation of cyclo-oxygenase products . The results for ^{45}Ca uptake are shown in table 1.

Table 1 Effects of LTD_4 and KCl on the La^{3+}resistant ^{45}Ca content of tracheal smooth muscle (mean ± s.e. mean; n=3-10)

	La^{3+}-resistant ^{45}Ca content (nmol g^{-1})		
	Control (no flurbiprofen)	Control	Verapamil ($1x10^{-6}$ mol l^{-1})
Basal	143 ± 4	143 ± 3	143 ± 9
LTD_4 ($3x10^{-9}$ mol l^{-1})	144 ± 5	147 ± 5	147 ± 1
LTD_4 ($5x10^{-8}$ mol l^{-1})	136 ± 5	142 ± 7	133 ± 8
KCl ($9x10^{-2}$ mol l^{-1})	229 ± 21*	217 ± 24*	136 ± 4

* significantly different from basal value $P<0.001$

LTD_4 failed to stimulate ^{45}Ca uptake at either of the concentrations used. In contrast KCl markedly increased ^{45}Ca uptake, an effect that was blocked by verapamil. The contractile responses induced by both LTD_4 and KCl were inhibited by verapamil ($1x10^{-6}$ mol l^{-1}); LTD_4 (low concn.) 5±1 to 3±1 mN, LTD_4 (high concn.) 24±3 to 16±2 mN, KCl 22±3 to 14±2 mN.

These data are consistent with the hypothesis that LTD_4 utilises an intracellular source of Ca^{2+} to initiate contraction. KCl on the other hand is dependent upon the uptake of Ca^{2+} from the extracellular compartment, an effect known to be inhibited by verapamil (Foster et al. 1984). At present we can offer no suitable explanation for the inhibitory effects of verapamil upon the LTD_4-induced contractions other than to invoke an intracellular mechanism of action.

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THE EFFECT OF ENALAPRIL AND BILATERAL NEPHRECTOMY ON SYMPATHETIC FUNCTION IN NORMOTENSIVE RATS

D. Angell, D.P. Clough, R. Hatton and Alison J. Roberts, Bioscience II Dept., ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG.

An in-vivo interaction between angiotensin and the sympathetic nervous system occurs in the periphery. Antonaccio and Kerwin (1981) and the authors (Hatton and Clough, 1982; Clough et al, 1982) showed that the angiotensin converting enzyme (ACE) inhibitor captopril attenuated the evoked pressor response to spinal sympathetic nerve stimulation and exogenous noradrenaline in pithed rats. Typically this response occurred during both oral and i.v. administration and the effect on noradrenaline was confirmed by Timmermans et al (1982). Antonaccio and McGill (1982) subsequently reported that enalapril given orally did not reduce responses to either stimulus in the pithed rat raising the possibility that the observed actions of captopril do not depend upon the ability to inhibit ACE. Clough et al (1983) recently reported that enalapril interferes with adrenergic neurotransmission in the pithed rat upon i.v. dosing.

In this present study we have confirmed these findings for enalapril and investigated the angiotensin dependency of its effect. Female wistar rats (230-250g) were pithed as previously described (Gillespie et al, 1970; Hatton and Clough, 1982). Vasoconstrictor-responses to electrical stimulation of the spinal cord (1-30Hz, 0.5ms, 60v, 10s duration) were significantly attenuated by enalapril (1.0 mg/kg i.v., -15 min). The pressor response to noradrenaline (0.04-2.0µg/kg, i.v.) was also reduced. Similar results were obtained when saralasin, an angiotensin II antagonist, was infused (4µg/kg/min) in a separate group. In rats which had undergone bilateral nephrectomy 3h previously, this dose of enalapril was now without effect. In these animals the responses to nerve stimulation and noradrenaline before enalapril were not significantly different from those in rats with kidneys after treatment with enalapril. Infusion of angiotensin II at a threshold dose for direct vasoconstrictor effects (50ng/Kg/min) failed to reverse the effects of enalapril alone, however in nephrectomised rats it potentiated responses to both nerve stimulation and noradrenaline in the presence or absence of enalapril.

The results confirm a very pronounced effect of enalapril on adrenergic vasoconstrictor-responses as we previously reported for captopril, teprotide and saralasin. The absence of effect in nephrectomised rats (-3h) favours blood borne rather than locally-generated angiotensin facilitating the adrenergic responses. This is supported by the similar effect of saralasin in this model. The presence of the kidney appears to oppose the action of exogenous angiotensin in its ability to potentiate responses after enalapril as previously reported for captopril and this may involve prostaglandin release. We could find no evidence from i.v. dosing of the inhibitors that interference with adrenergic neurotransmission was due to anything other than inhibition of ACE.

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GABA MODULATES THE HEAD-TWITCH INDUCED BY L-5-HTP

S.L. Handley and L. Singh, Pharmacological Laboratories, Department of Pharmacy, Aston University, Birmingham.

A recent study has shown that systemic administration of GABA caused head-twitching and other behavioural changes in rats and rabbits but that similar doses in mice failed to induce the head-twitch response (Smialowski et al, 1980). We have investigated the effect of centrally administered GABA and of drugs which affect brain GABA concentrations on head-twitch behaviour produced by L-5-hydroxytryptophan (L-5-HTP).

Male MF1 mice (20-30g) received carbidopa (9 mg/kg s.c.) 15 min before L-5-HTP (200 mg/kg i.p.). Head-twitches were counted for 5 min starting 20 min after L-5-HTP. Each mouse was observed in parallel with a control animal (saline / carbidopa / L-5-HTP). GABA was administered i.c.v. (Brittain and Handley,1967); control groups for GABA received saline i.c.v. L-2,4-diamino-n-butyric acid (DABA) and amino-oxyacetic acid (AOAA) were administered 24 and 6 hours before L-5-HTP respectively. 3-Mercaptopropionic acid (3MPA) was given simultaneously with L-5-HTP. The two antagonists, picrotoxin and bicuculline were given (i.p.) 15 min before L-5-HTP; control groups for bicuculline received 0.01N HCL vehicle (1ml/100g).

Table 1. Head-twitch counts following L-5-HTP/carbidopa (mean ± s.e.mean).

Test Treatment	Head-twitches	Control Treatment	Head-twitches
picrotoxin (0.5 mg/kg)	35.5 ± 6.3*	saline	24.8 ± 4.7
picrotoxin (0.5 mg/kg) + GABA (36.0 ug i.c.v.)	10.1 ± 2.3*	GABA (36.0 ug i.c.v.)	4.5 ± 1.5
bicuculline (1.0 mg/kg)	16.8 ± 5.6*	vehicle	31.0 ± 12.7

n/group = 5. * $P < 0.05$ Wilcoxon matched-pair signed-ranks test.

GABA itself, DABA (Sutton and Simmonds, 1974) and AOAA (Wallach, 1961) which increase brain GABA concentrations inhibited the L-5-HTP head-twitch (ID_{50} i.e. dose to halve the control response :- GABA 35.5 [57.3-21.9] ug i.c.v.; DABA 22.3 [37.1-10.4] mg/kg i.p.; AOAA 10.9 [24.2-8.4] mg/kg s.c.) while 3MPA which inhibits GABA synthesis (Horton and Meldrum, 1973) increased it (ED_{200} i.e. dose to double the control response :- 6.2 [18.3-2.1] mg/kg i.p.). In line with this picrotoxin which probably acts at a site related to but not identical with the GABA sites (Simmonds, 1980), also increased head-twitching and prevented the GABA-induced reduction (Table 1). However bicuculline, a selective antagonist at $GABA_a$ sites (Hill and Bowery, 1981), antagonised the L-5-HTP head-twitch (Table 1). This suggests the possibility that the inhibitory effect of GABA in the mouse might be related to an effect at $GABA_b$ receptors.

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EFFECT OF DRUGS ACTING AT α_1-ADRENOCEPTORS ON GELLER-SEIFTER CONFLICT

S.L. Handley & S. Mithani, Pharmacology Laboratories, Department of Pharmacy, Aston University, Birmingham, B4 7ET.

It has been postulated that central noradrenergic pathways are involved in fear and anxiety (Redmond and Huang, 1979); α and β-adrenoceptor antagonists and clonidine also modulate the potentiated startle model of anxiety (Davis et al. 1979). Furthermore, prazosin evoked a marked increase in punished drinking of a similar magnitude to diazepam in a conditioned drinking conflict test (Gardner and Piper, 1982). We have previously investigated the effect of α_2-adrenoceptor ligands on operant conflict behaviour (Handley and Mithani, 1984) and the present study was undertaken to examine the effects of α_1-adrenoceptor agonists and antagonists on operant conflict (Geller and Seifter, 1960) .

Two groups of male Lister hooded rats were trained on a 52 min Variable Interval (VI-2 min) schedule in which 3min periods of continuous reinforcement with contingent footshock (CRFs) were interposed every 10mins (see Handley and Mithani, 1984). Footshock intensity was titrated to >75% suppression for Group 1 and <25% for Group 2 rats. The reward was sweetened condensed milk and drugs were injected (ip) immediately before placement in the Skinner box. Both groups of rats were benzodiazepine experienced.

Table 1 Lever presses/rat during the third CRFs (punished) periods (mean ± sem) (*p < 0.05 Wilcoxon matched pairs-signed ranks test).

Drug (mg/kg)	Saline	Drug	Drug (mg/kg)	Saline	Drug
Prazosin (0.05)	1.8±0.9	3.4±0.9*	Phenylephrine (0.25)	10.3±1.4	5.0±2.1*
Prazosin (0.1)	1.0±0.8	2.8±1.1*	Phenylephrine (0.5)	8.5±2.0	1.0±0.4*
Prazosin (0.25)	1.8±0.6	2.0±0.6	Phenylephrine (1.0)	5.5±1.0	1.5±0.8*
			Phenylephrine (2.5)	10.0±2.6	1.0±0.3*
			St 587 (1.0)	8.2±1.9	1.8±0.7*

Table 1 shows the effect of α_1-adrenoceptor ligands on the third CRFs period, since maximal effects were obtained during this period. Phenylephrine and St 587 decreased responding, although effects were poorly dose-related. Phenylephrine only reduced unpunished responding significantly at the two highest doses. Prazosin (0.05 and 0.1 mg/kg) increased punished responding. The effect of prazosin was much smaller than on punished drinking (Gardner and Piper, 1982) perhaps due to the different nature of the reward involved. The results suggest that α_1-adrenoceptors may be involved in the modulation of anxiety.

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(^{3}H)KETANSERIN-BINDING TO 5-HT$_2$ RECEPTORS IN RAT BRAIN: EFFECTS OF CHRONIC NEUROLEPTIC ADMINISTRATION

N.J. Garrett, P. Jenner[1], C.D. Marsden[1], G.P. Reynolds*and N.M.J. Rupniak[1], MRC Neurochemical Pharmacology Unit, Brain Tissue Bank, Addenbrooke's Hospital, Cambridge CB2 2QQ and [1]Department of Neurology, Institute of Psychiatry, Denmark Hill, London SE5.

Clozapine is an effective antipsychotic drug which we have previously shown (Reynolds, 1983) to produce a decrease in the binding of (^{3}H)ketanserin to cortical 5-HT$_2$ receptors after long-term administration to rats. This apparent down-regulation of receptors is anomalous in that clozapine, like several other neuroleptic drugs, is a powerful antagonist of (^{3}H)ketanserin binding (Reynolds et al., 1983). We wish to report effects of the administration of two other neuroleptic drugs on (^{3}H)ketanserin binding.

Male Wistar rats were treated for a period of seven days (short-term) or 18 months (long-term) with either haloperidol or trifluoperazine administered in their drinking water. Control animals received distilled water alone. A further group were killed after (three months) withdrawal from 15 months trifluoperazine administration. Tissue homogenates of the caudate-putamen were prepared and incubated with radiolabel in Tris buffer (50 mM, pH 7.7) using displacement by 10^{-6} M d-LSD to define specific binding. Two concentrations of (^{3}H)ketanserin were employed in order to obtain an approximate assessment of receptor density as well as an indication of any changes in apparent affinity which might contribute to changes in binding. The results are summarized in Table 1.

Table 1. Specific (^{3}H)ketanserin binding at 1.8 nM to rat striatum after neuroleptic administration

	Control	Haloperidol	Trifluoperazine	Trifluoperazine withdrawn
Short-term	5.09 ± 0.77	5.28 ± 0.64	4.21 ± 0.65*	-
Long-term	2.61 ± 0.48	3.71 ± 0.58**	1.79 ± 0.73*	2.72 ± 0.62

Values are mean ± S.D. in pmol/g tissue for 6-8 animals.
* $p < 0.05$ vs controls ** $p < 0.01$ vs controls.

No significant changes in the ratio of binding at 0.4 and 1.8 nM (^{3}H)ketanserin was observed indicating the results were independent of changes in apparent affinity. The decrease in binding due to trifluoperazine is present even after short-term treatment, persists and then reverses to control values after withdrawal. This is consistent with the down-regulation of 5-HT$_2$ receptors observed after both acute and chronic treatment by the neuroleptic loxapine (Helmeste and Tang, 1983). Haloperidol, which has a greater affinity for dopamine D$_2$ sites than for the 5-HT$_2$ receptor, affects a relative increase in these receptors which only reaches significance after long-term treatment.

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BENZODIAZEPINES POTENTIATE THE EFFECT OF MUSCIMOL ON PROLACTIN SECRETION IN VITRO

R.A. Anderson and R. Mitchell (Introduced by B.L. Ginsborg)
MRC Brain Metabolism Unit, 1 George Square, Edinburgh, EH8 9JZ.

We have recently demonstrated that muscimol causes a transient increase in prolactin secretion in vitro (Anderson and Mitchell, 1984) in addition to its recognized inhibitory effect (Grandison and Guidotti, 1979). This biphasic influence is very similar to that of GABA on α MSH release (Tomiko et al. 1983). The presence of populations of both central- and peripheral-type benzodiazepine binding sites has been demonstrated in rat pituitary (Anderson and Mitchell, 1983) and we have, therefore, investigated whether benzodiazepines can potentiate the effects of muscimol on prolactin secretion.

Anterior pituitary glands from male Wistar rats (200-300g) were chopped into 500μm prisms and incubated for 60 min at 37°C in oxygenated Krebs bicarbonate, pH 7.4, containing 0.1% BSA, 2g/l glucose and 30mg/l bacitracin. Tissue from two rats was then superfused at 0.5ml/min and prolactin was measured in 2 min fractions by radioimmunoassay. After 90 min, muscimol was introduced into the medium and produced two effects: a transient stimulation which peaked within 6-8 min followed by a decline to a steady inhibited rate of release within 30 min. Both effects were concentration-dependent from 10nM-100μM, and were inhibited by both (+) bicuculline methiodide (BMI) and picrotoxinin. 10μM muscimol caused + 65 ± 10% stimulation and - 51 ± 3% inhibition: these effects were reduced to + 24 ± 5% and - 34 ± 2% by 10μM BMI ($p < 0.05$, $n = 5$ in each case).

When clonazepam (a benzodiazepine with high affinity for central type receptors) was introduced with muscimol, the stimulation produced by 10nM and 100nM muscimol was significantly increased: 100nM muscimol caused 11 ± 1% stimulation alone and 27 ± 3% in the presence of 1μM clonazepam, ($p < 0.05$, $n = 5$). Clonazepam was not seen to potentiate the inhibitory effect of any concentration of muscimol.

The specific benzodiazepine antagonist Ro15-1788 was found to inhibit the clonazepam potentiation of the stimulation produced by 100nM muscimol in a concentration-dependent manner. In the presence of 10μM Ro15-1788, (introduced into the medium 30 min previously), 100nM muscimol with 1μM clonazepam caused only 12 ± 2% stimulation ($p < 0.05$ vs. muscimol with clonazepam, $n = 5$).

In contrast, Ro5-4864, which has high affinity for peripheral-type benzodiazepine receptors, did not potentiate either the stimulatory or the inhibitory effect of 100nM muscimol: + 11 ± 1% and - 16 ± 2% respectively with 100nM muscimol alone; + 9 ± 1% and - 18 ± 1% in the presence of 1μM Ro5-4864 ($n = 5$).

These results demonstrate that the effect of low concentrations of muscimol on prolactin release can be potentiated by central- but not peripheral-type benzodiazepine receptors. Although the failure of benzodiazepines to potentiate the inhibitory effect of muscimol on prolactin release has been described previously (Grossman et al., 1981), it appears that the stimulatory effect is more sensitive to such modulation.

R.A.A. is a Houldsworth Scholar of the University of Edinburgh.

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IS THE "Ca^{++} -FREE" α_1-ADRENOCEPTOR-MEDIATED RESPONSE OF SMOOTH MUSCLE LIMITED TO FULL AGONISTS?

J.C. McGrath, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ.

In rat anococcygeus, if Ca^{++} is omitted from the saline, noradrenaline (NA) produces an initial, transient contraction (ITC), which peaks within 1 min. Stepwise re-addition of Ca^{++} produces graded contraction. Similarly, amidephrine and xylazine produce contraction on re-addition of Ca^{++} but xylazine does not produce a response before re-addition of Ca^{++} (McGrath, 1983). This is paralleled in rat aortic strips (Godfraind et al, 1982).

This study tested more alpha-agonists for induction of an ITC in conditions which favour a "Ca^{++}-free" component. Preparations were isolated in Krebs' bicarbonate saline at 37°C, initially containing [Ca^{++}] & [Mg^{++}] at 1.25mM, with [EDTA] = 23μM throughout, and bubbled with 95%O_2 5%CO_2. Isometric contractions to agonists were examined in "Ca^{++}-free" saline, either in the presence (MgP) or absence (MgA) of added Mg^{++}. $CaCl_2$ was then added stepwise (0.04 to 5mM) to gain an index of the Ca^{++}-present response (CPR).

(MgP) Phenylephrine (PE) (0.3-3.0μM) produced an ITC with a potency, height and time course equivalent to those of NA. The other agonists did not. Amidephrine produced relatively slow contractions throughout its range for CPR (0.03-3μM); oxymetazoline did so only at 0.3μM (CPR: 0.003-0.3μM); methoxamine only at 3μM (CPR: 0.03-3μM); xylazine only at 300μM (CPR: 0.3-300μM); Sgd 101/75 only at 30μM (CPR: 0.3-30μM). These slow responses, and a slow phase following the ITC to PE or NA, were blocked by nifedipine (0.1-10μM) and potentiated by BAY K 8644 (0.1-10μM). The latter compounds shifted the Ca^{++} concentration/response curve, to each agonist, to the right and left, respectively, suggesting that the slow "Ca^{++}-free" responses are due to the "Ca^{++} contamination" of the extracellular space (McGrath et al, 1984). The CPR to KCl (30mM), which was affected like the agonist responses by nifedipine & BAY K 8644, produced no "Ca^{++}-free" response since its threshold for Ca^{++} remains higher. Amidephrine was an exception, having a slow response which was partly resistant to nifedipine.
(MgA) All agonists produced larger, slow "Ca^{++}-free" responses but Ca^{++}-buffering suggested that this was due to an increase in the Ca^{++} contamination level.

Thus, NA, PE and, to a lesser extent, amidephrine, produced an ITC in low concentrations of Ca^{++}. The failure of the other compounds, at concentrations which produce adequate CPR's, suggests that they lack some critical property. With the possible exception of methoxamine, this latter group act in some preparations as partial agonists. Failure to initiate the ITC may contribute to the "partial" nature of their agonism (providing a further expanation for efficacy). Conversely, low efficacy may disqualify them from producing a vigorous enough biological stimulus to satisfy the demanding requirements of the ITC. Varying Ca^{++} contamination and pD_2's for re-added Ca^{++} explain differences in duration of "Ca^{++}-free" responses.

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MUSCARINIC ANTAGONIST PROPERTIES OF SECOVERINE AND ATROPINE ON GUINEA PIG ILEUM AND ATRIA IN VITRO

R.U. Clague, R.M. Eglen, A.C. Strachan and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

Muscarinic receptors have been classified into three subtypes - those in the CNS, those in the myocardium and those in smooth muscle of the G.I. tract (Birdsall & Hulme, 1983). In addition, Barlow et al (1980) have proposed that the atria contain two distinct muscarinic receptors mediating negative inotropic and negative chronotropic actions respectively. Secoverine, a neuroleptic spasmolytic, has been reported (Zwagemakers & Claassen, 1980) to exhibit selective antagonism of muscarinic receptors in the G.I. tract. However, quantitative data, concerning its antagonist actions on other muscarinic receptor subtypes is unavailable. This study concerns the muscarinic antagonist profile of secoverine on ileum and on both paced (inotropic effects) and spontaneously beating (chronotropic effects) atria of the guinea pig.

Dose response curves to carbachol, both in the absence and presence of either atropine or secoverine (10^{-8} - 10^{-6} mol.litre^{-1}) were undertaken using paired atria (paced or unpaced) or ileum from Dunkin Hartley guinea pigs (female, 250 g b.w.). Tissues were suspended under 1.5 g (atria) or 0.5 g (ileum) tension in Krebs solution at 30^{0}C. Atria were paced using punctate electrodes (4 Hz supramaximal voltage). After control curves were obtained, each tissue was exposed to only one antagonist (30 min incubation at each concentration) prior to subsequent agonist exposure. Antagonist potencies were determined by the method of Arunlakshana & Schild (1959).

Atropine exhibited no selectivity in any of the 3 preparations which is in agreement with previous literature (Barlow et al, 1980), Table 1. Schild analysis showed that secoverine antagonism in the ileum and unpaced atria exhibited a slope significantly different from unity (ileum slope = 1.2 ± 0.04, atria slope = 1.4 ± 0.09, mean ± sem. Even after imposing the unity constraint secoverine still showed a higher affinity for the atria (paced or unpaced) than the ileum (Table 1). Secoverine had a similar affinity for the receptors mediating either rate or force of atrial contraction (Table 1).

Table 1 pA_2 values (with 95% confidence limits) in guinea pig ileum and atria (n = 4 in all preparations).

	ILEUM		ATRIA (RATE)		ATRIA (FORCE)	
	pA_2	Slope	pA_2	Slope	pA_2	Slope
Atropine	8.69 (8.47 - 8.91)	1.0	8.37 (7.88 - 8.86)	1.0	8.76 (8.62 - 8.90)	1.0
Secoverine	7.81 (7.58 - 8.04)	1.2	8.90 (8.48 - 9.32)	1.4	8.76 (8.53 - 8.99)	1.0

In conclusion, secoverine has a greater affinity for muscarinic receptors in atria than ileum. This data provides further evidence for the existence of multiple subclasses of muscarinic receptors in the atria and ileum.

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LACK OF DOPAMINE RECEPTOR BLOCKADE IN THE MESOLIMBIC AREA DURING CHRONIC NEUROLEPTIC TREATMENT OF RATS

S.Fleminger, M.D.Hall, P.Jenner, E.Kelly, G.Kilpatrick, C.D.Marsden & N.M.J. Rupniak, University Department of Neurology, Institute of Psychiatry & The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5,U.K.

During chronic administration of neuroleptic drugs to rodents, dopamine receptor blockade is not maintained in the striatum, and reversal of dopamine receptor blockade may also occur in the mesolimbic area (Clow et al, 1980). We now examine the effects on mesolimbic dopamine function of chronic treatment with haloperidol, sulpiride or clozapine since these drugs may act selectively on mesolimbic dopamine systems (Costall & Naylor, 1976).

Male Wistar rats received either haloperidol (1.4–1.6 mg/kg), sulpiride (102–109 mg/kg) or clozapine (24–27 mg/kg) via their daily drinking water for up to 20 months. Age-matched control rats received distilled water alone. After 1,3,6,9 or 12 months specific mesolimbic dopamine receptor numbers (Bmax) identified by ^{3}H-spiperone (SPIP, 0.1–4.0 nM; defined using 10^{-5} M (-)-sulpiride, ^{3}H-piflutixol (PFT, 0.08–1.3 nM; defined using 10^{-6} M cis-flupenthixol in the presence of $3x10^{-5}$ M (+)-sulpiride) or ^{3}H-N,n-propylnorapomorphine (NPA, 0.05–2.0 nM; defined using 10^{-6} M (+)-6,7-ADTN) did not differ from control values in rats treated continuously with haloperidol, sulpiride or clozapine (Table 1). Mesolimbic dopamine (50 uM)-stimulated adenylate cyclase (AC) activity was not altered by haloperidol, sulpiride or clozapine treatment for up to 12 months (Table 1). Spontaneous locomotor activity (SLA) in haloperidol-treated rats was reduced for the first 3 months, was normal at 6 and 9 months, and again reduced at 12 months by comparison to control animals. Sulpiride treatment did not alter SLA at any time. Clozapine treatment caused a decrease in SLA after 3 months and an increase at 6 months; thereafter, activity was normal (Table 1). After 20 months drug treatment, bilateral intra-accumbens injection of dopamine (12.5–12 ug) caused equivalent increases in locomotor activity in control rats and in animals receiving haloperidol, sulpiride or clozapine (Table 1).

Table 1 Specific mesolimbic ^{3}H-SPIP, ^{3}H-PFT and ^{3}H-NPA binding, dopamine (50 uM)-stimulated AC activity, spontaneous locomotor activity (SLA) and hyperactivity induced by intra-accumbens injection of 25 ug dopamine (ALA) in rats treated continuously for 12 or 20 months with haloperidol, sulpiride or clozapine

Treatment	Bmax (pmoles/g tissue) ^{3}H-SPIP	^{3}H-PFT	^{3}H-NPA	AC (pmoles) cAMP/2.5 min/ 2 mg tissue)	SLA (counts/ 30 min)	ALA (counts/ 2 h)
Control	5.3±1.0	95±13	3.9±0.9	36.6±3.4	656±53	5395±754
Haloperidol	7.0±1.3	80± 7	4.5±0.9	41.0±1.9	451±65*	5823±882
Sulpiride	6.0±0.4	90± 7	6.5±0.7	42.8±2.8	631±65	4270±590
Clozapine	4.0±1.0	68± 8	2.1±0.5	46.7±2.6	591±81	4531±982

* $p < 0.05$ compared to control rats, Student's t-test. N = 3–8.

Our findings indicate that dopamine receptor blockade is not maintained in the mesolimbic area during chronic neuroleptic treatment and that sulpiride and clozapine may not be selective mesolimbic agents.

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