THE α-ADRENOCEPTOR-MEDIATED COMPONENTS OF THE RESPONSES TO ADRENALINE AND PHENYLEPHRINE OF RAT ISOLATED ATRIA

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Alpha-adrenoceptor stimulation has been implicated in the positive inotropic responses of phenylephrine (Shibata et al. 1980) and to a lesser extent adrenaline (Schümann,1983). Our previous studies have demonstrated positive inotropic responses of rat left atria mediated via α_1-adrenoceptors (Broadley & Williamson, 1985). This study examines the relative contributions of α- and β-adrenoceptor stimulation to the responses of rat isolated left and right atria to these two agonists. Atria were set up in Krebs-bicarbonate solution at 30°C gassed with 5% CO_2 in O_2. Tension responses of paced left atria (2Hz 5ms pulse width, threshold voltage + 50%) and rate responses of spontaneously beating right atria were recorded. Cumulative concentration-response curves to phenylephrine (PHE) or adrenaline (AD) were constructed in the absence or presence of propranolol (PRO), pindolol (PIND) or prazosin (PRAZ) (after equilibration for 30 min). Metanephrine (10μM) was present throughout and desipramine (1μM) when adrenaline was the agonist.

PHE exerted positive chronotropic responses which were abolished by propranolol (1μM) and thus β-adrenoceptor-mediated. The left atrial positive inotropic responses were, however, unaffected, the EC_{50} values in the absence (2.2(1.55-3.24)μM) and presence of propranolol (3.09(1.0-9.55)μM) not differing significantly($P>0.05$). Prazosin (3nM) had no effect on the right atrial rate responses. The left atrial tension dose-response curve was, however, significantly shifted ($P<0.05$) to the right by prazosin (EC_{50} value 6.03(3.39-10.7)μM), as did prazosin and propranolol combined (EC_{50} value 29.5(20.0-43.6)μM). Thus the inotropic response to PHE appears to be mediated predominantly via α-adrenoceptors.

The EC_{50} values for the rate responses to AD were progressively increased with increasing concentrations of pindolol (Table 1), yielding a pA_2 value of 8.13 ± 0.096. Thus the rate responses to AD were solely β-adrenoceptor mediated.

Table 1. Effects of pindolol and prazosin on adrenaline EC_{50} values (μM)

Antagonist	n	Right Atrium	Left Atrium
None	5	0.068(0.037-0.124)	0.776(0.301-1.99)
PIND 0.3μM	4	1.86(0.13-27.5)	1.17(0.068-20.4)
PIND 1μM	5	11.7(2.39-57.5)	3.39(0.77-14.8)
PIND 3μM	8	24.0(13.1-44.1)	12.3(6.87-22.0)
PIND 10μM	4	148(85.7-255)	10.5(0.98-112)
PRAZ 0.1μM	6	0.135(0.056-0.327)	0.389(0.135-1.12)
PIND 3μM + PRAZ 0.1μM	6	46.8(14.6-150)	81.3(37.5-176)

The left atrial positive inotropic effect was initially antagonized by pindolol, but after 3μM there was no further antagonism. Prazosin, which had antagonized PHE in left atria, had no significant effect ($P>0.05$) on left atrial responses to AD at 0.1μM. A combination of prazosin (0.1μM) and pindolol, at a concentration causing maximal blockade of inotropic responses (3μM), had no further effect on right atria than with pindolol alone. However, there was a significant further increase of EC_{50} value in the left atria. This indicates an α-adrenoceptor-mediated component of the inotropic response to AD, which unlike previous studies, could only be shown in the presence of substantial β-blockade. In the absence of α- and β-antagonists, AD preferentially stimulates β-adrenoceptors in both left and right atria, whereas the inotropic response to PHE is mediated predominantly via α-adrenoceptors.

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THE EFFECT OF INDOMETHACIN AND DICLOFENAC SODIUM ON URETERAL CONTRACTION IN VIVO AND IN VITRO

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Indomethacin inhibits motility in isolated sheep and human ureteral preparations (Angelo-Khattar et al, 1984). The present investigation extends these observations to diclofenac Na found to be effective in treating renal colic (Lundstam et al, 1982). We also present results of in-vivo experiments in sheep with indomethacin and pharmacokinetic data.

In ureteral ring preparations from 60 freshly slaughtered sheep spontaneous rhythmic contractions were recorded in an organ bath. Frequency and amplitude dose dependently declined after administration of dicofenac Na (Geigy) with a threshold of $1.5 \cdot 10^{-8}$M.

In-vivo experiments on ureteral motility were carried out in 12 sheep anaesthetised with 30 $mg.kg^{-1}$ sodium pentobarbital. After abdominal incision one ureter was catheterised and urinary occlusion pressure recorded together with blood pressure from the femoral artery. Indomethacin with sodium dihydrogen phosphate buffer (Dumex Ltd) was administered iv. at a dose of 0.8 mg/kg body weight. A reduction of frequency and urinary pressure amplitude occured 10 minutes after administration as shown in table 1.

Table 1: Ureteral motility before and after indomethacin (mean ± s.e. mean $P < 0.05$ compared to control underlined).

	Control	5 min.	10 min	n
Frequency (c/min)	15.2 ± 1.2	12.0 ± 1.2	11.5 ± 1.4	12
P mean (mm Hg)	17.5 ± 2.2	16.3 ± 1.8	16.2 ± 1.7	12
P ampl.(mm Hg)	20.0 ± 3.5	14.6 ± 3.5	12.3 ± 2.7	12

Indomethacin concentration in serum was determined by HPLC with a method modified after Ou & Frawley (1984). The values 5 and 10 minutes after i.v. injection were 4.52 and 0.95 mg l^{-1}.

Our investigation confirms that indomethacin and diclofenac Na reduce smooth muscle contractions in sheep ureters probably by inhibition of prostaglandin synthesis.

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DESENSITISATION OF RAT PINEAL β-ADRENOCEPTORS

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Rat pineal gland β-adrenoceptors are desensitized *in vivo* during the dark cycle and also upon injection of isoprenaline (Kebabian *et al.*, 1975). We have examined the biochemical characteristics of control and catecholamine desensitized pineal gland β-adrenoceptors. Wistar Kyoto rats, in groups of 10-15 rats (wt. 100-150g) were injected at the same time of the day with isoprenaline 10 mg/kg i.p. or saline and 30 minutes later the animals were decapitated, the pineal glands removed within 25 seconds, and either used immediately or frozen in liquid N_2. Membrane preparations were made according to Sugden & Klein (1984) and binding of ^{125}I-CYP to membranes was performed essentially as described by Engel *et al.*(1981). β-Adrenoceptors were covalently labelled using the photoaffinity ligand ^{125}I-pABC, solubilised and SDS-PAGE was performed. Dried gels were exposed to autoradiography as previously described (Lavin *et al.*, 1982).

Specific ^{125}I-CYP binding (that displaced by 200 μM(-)Isoprenaline) to rat pineal membranes was saturable and of high affinity (K_D = 30 ± 5 pM, n = 4). The B_{max} of control (saline treated) rats was 1020 ± 85 fmoles/mg protein, n = 4. Injection of Isoprenaline resulted in down regulation of β-adrenoceptor number to 651 ± 97 fmoles/mg protein, without any change in the K_D for the radioligand (K_D = 24 ± 4 pM). Maximum Isoprenaline stimulated adenylate cyclase was attenuated by 40% in membranes from treated animals. The pharmacological characteristics of pineal gland β-adrenoceptors was that of a β_1-adrenoceptor. Thus displacement studies revealed an order of agonist potencies Isopren. > NA ≥ Adr. and the antagonist Betaxolol (K_i = 7.1 nM) was more potent than ICI 118,551 (K_i = 169 nM). Isoprenaline displacement of ^{125}I-CYP binding to membranes from control animals exhibited low slope (nH = 0.65) which was shifted to the right and steepened (nH = 0.97) in the presence of the non-hydrolysable GTP analogue Gpp NHp. In contrast, however, membranes from isoprenaline treated animals generated isoprenaline displacement curves which were steep (nH = 1.08) and Gpp NHp produced little change in their characteristics.

^{125}I-pABC labelled two peptides of pineal gland membranes which exhibited Mr = 64,000 and 41,000 daltons. However, in contrast to other mammalian β-adrenoceptors only incorporation of label into the 41,000 K peptide was inhibited by adrenergic drugs with the characteristics of a β-adrenoceptor. The labelling pattern of the 41 K peptide was not significantly different between membranes of control and desensitized pineals. Thus, desensitization of pineal β_1-adrenoceptors results in uncoupling of receptor from N protein, down regulation of receptor number but no change in the receptors mobility as assessed by SDS-PAGE.

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POTENTIATION BY VERAPAMIL OF GALLAMINE AND PANCURONIUM-INDUCED BLOCKADE AT THE CHICK NEUROMUSCULAR JUNCTION

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Verapamil,an organic calcium antagonist or entry blocker,has been reported to reduce the amplitude of the directly and indirectly-elicited twitch contractions in frog(Bondi,Kirsten & Hofmann,1974;Bondi,1978) and rat skeletal muscle(Williams, Broadbent,Pearce & Jones,1983).In addition,verapamil potentiated the neuromuscular blockade produced by non-depolarizing and depolarizing muscle relaxants in vivo (Durant,Nguyen,Briscoe & Katz,1982;Carpenter & Mulroy,1983) and in vitro(Bikhazi, Leung & Foldes,1982).

In the present investigation,the effects and interactions of verapamil with gallamine and pancuronium were studied in the isolated chick biventer cervicis nerve-muscle preparation(Ginsborg & Warriner,1960) to see if verapamil potentiated the neuromuscular blockade in this preparation. The preparation was set up in an organ bath(20-25 ml) containing Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% carbon dioxide in oxygen.The motor nerve was stimulated,throughout,at 0.18 Hz with 5-10 V(maximum) and 0.2 ms pulse duration.The contractile responses produced by electrical nerve or muscle stimulation or by drug action were recorded isometrically.

Verapamil(2-200 μM) produced concentration-dependent reduction in the amplitude of the direct and indirect twitch tension,the direct twitch being reduced by about 20% of maximum indirect inhibition.The mean IC_{50} values(concentration to produce 50% inhibition) of verapamil-induced inhibition of indirect twitch tension was 106±4.2 μM(n=6,mean±s.e.).In high concentrations,verapamil(200 μM) produced a contracture in the chick skeletal muscle(1.0±0.1 g,n=6).Verapamil(20 μM),which had little effect on the twitch tension,significantly increased the neuromuscular blockade produced by gallamine(28-1280 nM) and pancuronium(18-573 nM) in the chick muscle.The mean IC_{50} values of gallamine inhibition of the indirect twitch tension in the control Krebs solution and in Krebs containing verapamil were 334±5.7 nM and 195±4.9 nM respectively($n=6, P < 0.001$).The corresponding values of pancuronium inhibition of twitch tension were 108±2.8 nM and 61±5.4 nM($n=6, P < 0.001$).A dose ratio(gallamine/pancuronium) of 3.1:1.0 was obtained in the control Krebs solution.Verapamil(20 μM) produced a similar increase in the intensity of the neuromuscular blockade produced by gallamine or pancuronium.A dose ratio (gallamine/gallamine+verapamil) of 1.7:1.0 and (pancuronium/pancuronium+verapamil) of 1.8:1.0 was obtained.

Bondi(1978) has studied the effect of verapamil on excitation-contraction coupling in frog sartorius muscle.Verapamil(1 mM) had two main effects:(a) it depressed the twitch tension without producing membrane depolarization,and(b) it produced a contracture in the frog muscle in a similar manner to that of caffeine.These actions of verapamil have been attributed to(a) a local anaesthetic-like action,in depressing the surface membrane excitability,and(b) an action on intracellular Ca^{2+} stores,in releasing Ca^{2+} from the sarcoplasmic reticulum.Since Ca^{2+} is essential for neuromuscular transmission,it is possible that by blocking Ca^{2+} entry verapamil enhances the blockade produced by gallamine and pancuronium in the chick muscle.

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THE EFFECT OF NICARDIPINE ON CARDIAC FUNCTION AND HIGH ENERGY PHOSPHATE METABOLISM IN THE RAT WORKING HEART IN VITRO

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Nicardipine, a calcium entry blocking agent, has been shown to protect the myocardium from infarction by limiting the area of tissue affected and by promoting healing (Alps et al 1983a; Alps et al 1983b). As a prelude to investigation of its effects on ischaemia, we have studied the activity of nicardipine on the normal isolated working heart. This preparation allows the direct cardiac effects of agents to be studied independently of their action on the peripheral vasculature which may also be beneficial in myocardial infarction.

Male rats (Sprague-Dawley) weighing 296± 3 g. were used. Hearts were removed during pentobarbitone anaesthesia and perfused via the pulmonary vein with Kreb's solution (Ca^{++} 2.5 mM) gassed with O_2 95%+CO_2 and heated to 37°C. Cardiac perfusion was maintained at 9 mmHg and the aortic output was opposed by a column of fluid equivalent to an afterload of 59 mmHg. Cardiac function and integrity was assessed from measurements of left ventricular pressure, coronary flow (and its lactate dehydrogenase activity LDH), aortic flow and from calculations of cardiac output (aortic + coronary flows), stroke volume (cardiac output/heart rate), oxygen consumption (O_2 tension of inflow - O_2 tension of coronary outflow), heart work (aortic flow x left ventricular systolic pressure) and cardiac efficiency (heart work/oxygen consumption). The area of the left ventricle served by the descending coronary artery was removed and frozen to allow biochemical measurements of creatine phosphate, adenine nucleotides, glucose and lactate by spectrophotometry. Energy charge was calculated as [ATP] + 0.5[ADP]/[ATP] + [ADP] + [AMP]. Mean (± sem, n= 5)). Nicardipine was infused at concentrations between 1pM to 1uM. Baseline values of the measured variables are shown in the Table.

A. Mechanical function

Coronary flow	Cardiac output	Stroke volume	Systolic pressure	Diastolic pressure	+dp/dt	-dp/dt	Work	O_2 consum.	Effic
ml/min		ml/beat	mmHg		mmHg/sec		Kg.m/min	ul/min	%
13 ±1	53 ±1	0.2 ±0.01	122 ± 4	1 ±1	4283 ±375	3110 ±274	0.09 ±0.003	0.2 ±0.02	21 ±1

B. Biochemical indices

Creatine Phosphate	ATP	ADP	AMP	Glucose	Lactate	Adenylate Charge	LDH release
nM /mg protein						units	uU/min
20 ±3	16 ±2	6 ±1	3 ±0.4	53 ±0.4	22 ±4	0.7 ±0.04	25 ±4

No major changes in cardiac function occurred until 100 nM nicardipine when decreases in cardiac output (-26± 1%), coronary flow (-12± 3%), cardiac work (-38± 5%) and beating rate (-22± 10%) were observed. Ventricular biochemistry was not altered even during perfusion of 100 nM. These findings are compatible with previous conclusions regarding nicardipine's selective action on blood vessel relative to heart muscle function (Armstrong and Dumez, 1984).

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NICARDIPINE, A CALCIUM ENTRY BLOCKER, PROTECTS STRIATAL DOPAMINE IN PERIODS OF CEREBRAL ISCHAEMIA

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The lack of connecting arteries (circulus arteriosus) between the basilar and carotid circulatory systems in the brain of the Mongolian gerbil, Meriones unguiculatus, has made it a suitable model for the study of cerebral ischaemia (Kahn, 1972). Ligation of one common carotid artery has been shown to cause a unilateral infarction (Levine and Payan, 1966), leading to a profound decrease in brain dopamine. We reported previously (Alps et al, 1984a) that the ipsilateral decrease in dopamine was dependent upon the failure of the connecting arteries to form the circulus arteriosus. This study examined the effect of the calcium entry blocker, nicardipine, on the ipsilateral decrease in corpus striatal dopamine after 3 h ligation of the right common carotid artery in the gerbil.

Thirty five male gerbils (60 - 80 g) were pretreated with 50 $\mu g.kg^{-1}$ nicardipine (i.p.) or saline, 30 min before being anaesthetised with 6 mg pentobarbital. The right common carotid artery was exposed in the paratracheal region. The artery was dissected free and doubly ligated. In sham operated controls the carotid artery was exposed and ligatures put in place but not tied. After 3 h the animals were decapitated, the corpus striatum separated into left and right hemispheres and stored under liquid nitrogen until analysed for catecholamine content by high performance liquid chromatography coupled to electrochemical detection. Chromatographic conditions for the separation and measurement of dopamine were as described by Alps et al (1984b).

Table 1 shows the level of dopamine in the affected hemisphere of the corpus striatum expressed as a percentage of that in the unaffected hemisphere.

Table 1

	Treatment Group		
	Sham operated	Ligated	Nicardipine pre-treated
Dopamine Level	89.99 ± 3.18 (10)	43.41 ± 3.05 (12)#	58.22 ± 5.91 (13)#*

#Statistical significance $p < 0.001$ relative to sham operated animals
*Statistical significance $0.05 < p < 0.02$ relative to ligated animals.
Each value represents the mean ± SE for the number of determinations in brackets.

The results indicate that pretreatment with 50 $\mu g.kg^{-1}$ nicardipine (i.p.) 30 min before ligation of the common carotid artery causes a signficant protection of dopamine as indicated by the reduced ipsilateral fall in the affected hemisphere. This preliminary study suggests that nicardipine may be of benefit in the treatment of cerebral ischaemia.

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COMPARATIVE INOTROPIC EFFECTS OF CALCIUM MODULATORS ON GUINEA-PIG AND RAT MYOCARDIUM

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There has been recent interest in the different effects of ryanodine on cardiac muscle from rat and guinea-pig. Ryanodine has been demonstrated to act by affecting the release of calcium from intracellular stores and selectively inhibits contractility in rat cardiac muscle (Mitchell et al, 1984; Clarke & Patmore, 1984). Calcium entry via inward calcium currents is much larger (≈7x) in guinea-pig than rat ventricle. Thus the contribution to contractile calcium from calcium entry and intracellular release is different, the rat being more dependent on intracellular release. The present study seeks to identify agents which affect intracellular release by comparing inhibitory potencies on rat and guinea-pig papillary muscle.

Right ventricular papillary muscles from rat or guinea-pig were used as described by Patmore & Whiting (1982). Contractility was recorded from 20 mmol.l^{-1} K^+ depolarised fibres. The inotropic effects of concentrations of each compound were measured over 15 min periods. Results were calculated as % of control contractility; pIC_{50} values are compared in Table 1

Table 1 Comparison of inotropic potencies

Compound	Rat	Guinea-pig
Nicardipine	7.32 (7.24 - 7.41)	8.08 (8.00 - 8.17)
Nifedipine	6.96 (6.91 - 7.03)	7.43 (7.36 - 7.51)
Verapamil	7.60 (7.47 - 7.80)	7.82 (7.71 - 7.96)
Diltiazem	6.83 (6.82 - 6.84)	6.92 (6.88 - 6.95)
Lidoflazine	5.55 (5.49 - 5.63)	5.44 (5.36 - 5.54)
Cinnarizine	5.35 (5.25 - 5.48)	5.46 (5.38 - 5.56)
pr-MDI	4.73 (4.66 - 4.82)	5.40 (5.27 - 5.60)
Dantrolene	4.12 (3.96 - 4.38)	4.94 (4.77 - 5.19)
Ryanodine	7.08 (7.01 - 7.16)	<4.00 [65% of control]
Trifluoperazine	4.61 (4.37 - 5.13)	<4.00 [286% of control]

Data shown are pIC_{50} values with SD range (n = 3 - 6). Values within [ ] are % of control contractility attained at highest concentration tested.

A good correlation (r = 0.97) exists between potencies of agents known to block calcium channels (nicardipine, nifedipine, verapamil, diltiazem and lidoflazine). Cinnarizine, pr-MDI and dantrolene show no selectivity between the two tissues suggesting that their predominant action is not the inhibition of release of stored calcium. Ryanodine is a selective inhibitor of contractility of rat cardiac muscle indicating inhibition of the release of intracellular calcium. Trifluoperazine (TFP), a potent inhibitor of calmodulin, has been reported to influence calcium transport in cardiac muscle (Caroni & Carafoli, 1981). In rat tissues TFP was a weak inhibitor of contractility. In guinea-pig at 10^{-4} mol.l^{-1} TFP exerted a marked positive inotropic effect which suggests that TFP does not antagonise intracellular release of calcium. These dissimilar sensitivities to calcium modulators emphasise the differences in calcium transport between these species.

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TOLERANCE TO SALBUTAMOL ON THE RAT UTERUS IN VIVO COMPARED WITH NIFEDIPINE AND DILTIAZEM

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Dose-dependent inhibition of uterine contractions was observed following bolus i.v. administration of salbutamol, nifedipine or diltiazem in the conscious post-partum ovariectomised rat (Abel & Hollingsworth, 1985).

The objective of this study was to observe the effects of long-term infusion of these drugs in this model. The sensitivity of the uterus was assessed by bolus i.v. injections of salbutamol, nifedipine or diltiazem (day 1). The test drug was then infused i.v. at a dose rate to just completely suppress uterine contractions (salbutamol - 2µg/kg/min, nifedipine - 25µg/kg/min, diltiazem - 200µg/kg/min, solvent - 0.3ml/h) for 20h. Where possible the sensitivity of the uterus was assessed on day 2 on cessation of infusion. Saline was then infused for 20h and sensitivity tests repeated on day 3.

Continuous infusion of salbutamol was associated with initial suppression of uterine contractions but with their reappearance within 4 to 8h and eventual return, despite continued infusion, to a magnitude seen on day 1. The dose-response curve to salbutamol on day 2 was shifted more than 100-fold to the right compared to day 1. By contrast nifedipine produced sustained inhibition of uterine contractions for the 20h of infusion which was reversed during the saline infusion. Sensitivity to nifedipine on day 3 was not significantly different from day 1; sensitivity on day 2 could not be tested. Partial return of uterine contractions was seen during continuous infusion of diltiazem in 4 out of 12 animals. In these 4 rats there was a 25-fold rightward shift of the dose-response curve to diltiazem on day 2 compared to day 1.

Therefore, there is evidence of a rapid onset of marked tolerance to the uterine inhibitory actions of salbutamol in vivo. Also, there was some tolerance to diltiazem, a member of one suggested sub-class of calcium antagonists (Granger et al, 1985; Spedding, 1982), but not to nifedipine, a member of another proposed sub-class.

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MYOCARDIAL REOXYGENATION DAMAGE CAN BE CIRCUMVENTED

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Reoxygenation/reperfusion damage in heart muscle (contracture with loss of contractile function) is dependent on extracellular Ca^{2+}. Whether it is an irrevocable consequence of the preceding hypoxia/ischaemia or whether it is amenable to intervention is unknown but therapeutically important.

Using an established model of reoxygenation contracture in isolated cat or rabbit papillary muscles we investigated whether it can be circumvented by interventions added at the end of the hypoxic period.

Right ventricular papillary muscles were mounted (Lewis et al, 1980) in a 50 ml bath containing Krebs-Henseleit buffer at pH 7.4 gassed with 95% O_2 : 5% CO_2 ($pO_2 > 750$ mmHg) at 29°C, streched to Lmax, stimulated to contract isometrically using suprathreshold voltage at frequency of 0.2 Hz and equilibrated for 3 hrs. Hypoxia was induced with 95% N_2 : 5% CO_2 ($pO_2 < 45$ mmHg) for 60 min in cat muscles and 30 min in rabbit muscles. Muscles were preequilibrated with ouabain to shorten the hypoxic period (otherwise > 8 hr) needed for reoxygenation contracture to occur (Lewis et al, 1980). Ouabain (5×10^{-6}M for cat, 2×10^{-6}M for rabbit) was added 30 min before reoxygenation. All other interventions were added at the end of the hypoxic period, 3 min before reoxygenation. The rise in resting force (% control resting force) was measured after 10 and 30 min of reoxygenation to compare the effects of interventions on reoxygenation contracture.

'0' mM extracellular Ca^{2+} prevented contracture but active force development declined after c. 10 min and abrupt replacement of the usual [Ca^{2+}] caused more severe contracture than in the absence of an intervening step of zero Ca^{+2} (due to the calcium paradox) and loss of contractile activity. Low Ca^{2+} (0.125 mM) just prior to reoxygenation, with gradual replacement to 2.5 mM over 60 min prevented contracture and allowed recovery of developed force to $97 \pm 7.5\%$ (SE, n=6) of prehypoxic control. Reoxygenation contracture was not influenced by verapamil (10^{-4}M) or lidoflazine (2×10^{-5}M) but was reduced by diltiazem at 10 min ($p < 0.05$) though not at 30 min; none of these agents allowed any recovery of contractile activity. Reoxygenation contracture was likewise reduced ($p < 0.05$) by metabolic acidosis (pH 6.5), 30 mM Mg^{2+} or 8 mM Mn^{2+} at 10 min though not at 30 min by which time all contractile activity was lost. Gradual reoxygenation delayed but did not prevent contracture.

These findings show that reoxygenation contracture and its associated loss of contractile recovery are amenable to therapeutic intervention independently of the preceding hypoxic insult. They further show that the contracture can be influenced by non-specific Ca entry inhibitors and by diltiazem, though it was not influenced in this model by verapamil or lidoflazine.

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333P

AMLODIPINE, A CALCIUM CHANNEL BLOCKER WITH PHARMACOKINETIC PROPERTIES NOVEL TO DIHYDROPYRIDINES

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The use of the established calcium channel blockers in cardiovascular therapy is often limited by the requirement for multiple daily dosing because of rapid clearance by metabolism. For nifedipine, the first representative of the dihydropyridine class of calcium channel blockers, the kinetics have been shown to correlate directly with the onset, intensity and duration of drug effect (Kleinbloesem et al, 1984). Thus pharmacokinetic properties can be a major determinant of the profile of effects of these drugs. Amlodipine, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine, is a new calcium channel blocker (Burges et al, 1985) with a unique pharmacokinetic profile which offers the potential for efficacy on once-daily dosing in man.

A method using h.p.l.c. with fluorescence detection was developed for assaying amlodipine concentrations in plasma and which had a limit of determination of 2ng/ml. Unlike nifedipine, amlodipine does not undergo photosensitive degradation under aqueous conditions.

Plasma concentrations of unchanged drug were determined in dogs following single oral and intravenous doses of amlodipine in a study of crossover design. Following intravenous administration (1mg/kg), plasma concentrations declined in a bi-exponential manner with a terminal elimination half-life of about 30h. The long half-life of amlodipine was associated with properties of moderate plasma clearance (11ml/min/kg) and a high volume of distribution (25 L/kg). Plasma concentrations following oral administration (2mg/kg) attained peak levels in the range 80-100ng/ml from about 3 hours after dosing; the low concentrations in plasma being indicative of the extensive distribution of the drug into the tissues. Comparison of the plasma concentration-time profiles for the oral and intravenous routes demonstrate that oral bioavailability approaches 100%.

Following once-daily oral dosing of conscious normotensive dogs, plasma concentrations of amlodipine gradually increased to a steady state after the fourth dose, at which time concentrations were twice that seen after a single dose. Initial studies in normal human subjects on amlodipine support the findings in dog of a long plasma half-life, a property which is different from other dihydropyridine derivatives. The kinetic profile of amlodipine following once daily dosing is consistent with the gradual onset and long duration of antihypertensive activity observed in dogs (Dodd & Machin, 1985), and potentially is an ideal property for the treatment of cardiovascular disease in man.

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EFFECTS OF KCl, NORADRENALINE AND ADENOSINE TRIPHOSPHATE ON ^{45}Ca INFLUX IN GUINEA-PIG VAS DEFERENS

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Adenosine triphosphate (ATP) depolarizes and contracts the vas deferens and is held responsible for the first phase of twitch response to single electric pulse. Its effects are inhibited by nifedipine but not by verapamil (French & Scott, 1983). Noradrenaline (NA) contracts but does not always depolarize the guinea-pig vas deferens (Sneddon & Burnstock, 1984; Sneddon & Westfall, 1984). Calcium channel blockers inhibit the tonic but not the initial phasic contraction of prostatic portion to NA. In order to find the source of calcium which is utilized for contraction by different stimulants, we studied the effects of KCl, NA and ATP on unidirectional ^{45}Ca influx in guinea-pig vas deferens. The method of Van Breemen was used (Loutzenhiser & Van Breemen, 1981). Studies were carried out on vasa from guinea-pigs pretreated with reserpine (1 mg/kg, i.p., 48 & 24 h before experiment) except when 6-hydroxydopamine (250 µg/ml, for 30 min, followed by 60 min wash) was used to denervate the tissue.

In the epididymal portion of vas deferens, KCl (50 mM) increased ^{45}Ca influx by 56.3±2.7 µmol/kg wet tissue (mean±s.e.mean, n=6). On the other hand, NA (10^{-5}M) and ATP (10^{-3}M), or a mixture of both, did not increase ^{45}Ca influx when compared with normal Krebs. Simultaneous exposure to NA and KCl increased ^{45}Ca influx by only 5.0±1.2 µmol/kg (n=6, $p < 0.001$ when compared with the effect of KCl 50 mM alone).

KCl might increase ^{45}Ca influx through: 1) stimulation of neuronal elements and release of transmitter(s); 2) directly depolarizing the smooth muscle. To find out whether NA acts on prejunctional alpha$_2$-adrenoceptors or directly on the smooth muscle, ^{45}Ca influx was measured in vasa pretreated with 6-hydroxydopamine. In the epididymal portion of these vasa, KCl 50 mM increased ^{45}Ca influx by 14.5±1.4 µmol/kg (n=6, $p < 0.001$ when compared with the reserpinized group). NA did not alter significantly ^{45}Ca influx induced by KCl 50 mM in 6-hydroxydopamine treated tissue.

Similar results were obtained in the prostatic portion of vas deferens.

Based on our results, it is concluded that ATP and NA contract the guinea-pig vas deferens by releasing calcium from intracellular stores. KCl 50 mM contracts this muscle by increasing calcium influx. It seems that the effect of KCl on ^{45}Ca influx is partly exerted through the release of transmitter(s) from neuronal elements. The possibility that 6-hydroxydopamine inhibits ^{45}Ca influx induced by KCl 50 mM by acting directly on the smooth muscle is not excluded by the present results.

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ANTI-HYPERTENSIVE EFFECTS OF AMLODIPINE, A NOVEL DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKER

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Amlodipine is a novel dihydropyridine analogue structurally related to nifedipine possessing the in vitro and haemodynamic properties of a calcium channel blocker (Burges et al, 1985). The following experiments were designed to assess its anti-hypertensive efficacy in conscious hypertensive dogs.

Acute anti-hypertensive effects were assessed using groups of 4-5 male beagles with established hypertension induced by cellophane wrapping of both kidneys. Mean arterial blood pressure (MBP) in these animals was at least 140 mmHg. Systolic, diastolic and mean (diastolic + 1/3 pulse pressure) arterial pressures were obtained from carotid loops, with the animals unsedated and under minimum restraint, using an acoustic manometric technique; heart rate (HR) was calculated from the pulse records. Drugs were administered as solutions, to dogs deprived of food overnight, either by gavage (amlodipine) or in a gelatin capsule (nifedipine).

Single oral doses of amlodipine produced gradually-developing, dose-related reductions in MBP, maximum falls (mean $\pm$ s.e. mean) of 15 $\pm$ 3, 21 $\pm$ 5 and 38 $\pm$ 4 mmHg occurring between 3.5 and 7 h after doses of 0.5, 1.0 and 2.0 mg/kg respectively. No obvious signs of recovery had occurred by the end of a 7 h observation period. In contrast, nifedipine produced rapid falls in MBP, maximum reductions of 11 $\pm$ 4, 16 $\pm$ 4 and 27 $\pm$ 4 mmHg occurring at between 0.5 and 1 h after 0.05, 0.1 and 0.25 mg/kg respectively with complete recovery by 3 h after the lowest and by 6 h after the highest dose. A dose-related tachycardia accompanied the hypotensive effects of both compounds.

In a chronic study, 4 male hypertensive beagles received amlodipine orally as seven daily doses of 0.025 mg/kg followed immediately by 0.05 mg/kg daily for a further seven days. Arterial blood pressure and HR measurements were made as above daily, before and for up to 6 h after each dose. Each daily dose caused negligible acute effects, little alteration from pre-dose MBP being observed during the immediate 6 h post-dose measurement periods. However, a slight fall in daily pre-dose MBP occurred during administration of the lower dose regimen and a similarly progressive, but more substantial, anti-hypertensive effect was achieved with 0.05 mg/kg/day such that before administration of the fourth dose a significant ($P < 0.05$, paired t test) 18 mmHg reduction in pre-dose MBP was observed. This response was essentially maintained for the remainder of the dosing period and was followed by a smooth return to pre-treatment values during a 72 h period after stopping drug administration. HR remained unaltered from pre-treatment values during the whole of the study. These results are consistent with the long plasma half-life of amlodipine in the dog (Beresford et al, 1985).

These results show amlodipine to be an effective anti-hypertensive agent in renal-hypertensive dogs with potentially useful therapeutic advantages such as gradual onset and long duration of action. During chronic administration, at doses ineffective acutely, the compound produced a progressive reduction in blood pressure with negligible effects on heart rate.

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^{45}Ca UPTAKE IN THE RAT VAS DEFERENS: EFFECTS OF DURATION OF DEPOLARIZATION AND CHEMICAL DENERVATION

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There is general agreement that high potassium contracts the rat vas deferens by utilization of extracellular calcium. Contractions to high potassium are inhibited by calcium deprivation and organic calcium entry blockers (see e.g. Hay & Wadsworth, 1982) and ^{45}Ca uptake and efflux are increased by exposure of rat vas deferens to KCl 160 mM (Hay & Wadsworth, 1984). We studied the effect of KCl (50 mM) on ^{45}Ca uptake and unidirectional influx by the method of Van Breemen (Loutzenhiser & Van Breemen, 1981). For measurement of unidirectional ^{45}Ca influx, tissues were exposed to Krebs containing ^{45}Ca for a period of 4 min. ^{45}Ca uptake was measured after incubation of the tissues with medium containing ^{45}Ca for 90 min. Concentration of Ca^{2+} in the medium was 1.5 mM. Extracellular ^{45}Ca was washed during 50 min with 0 ^{o}C Ca^{2+}-free Krebs containing 2 mM EGTA. Studies were carried out on rats pretreated with reserpine (5 mg & 2.5 mg/kg, i.p., 48 & 24 h before experiment, respectively) except when 6-hydroxydopamine was used to denervate the tissues. For chemical denervation, vasa were incubated with Krebs containing 250 µg/ml of 6-hydroxydopamine for 30 min and then washed for 60 min before carrying out the experiments.

In the epididymal portion of vas deferens, KCl 50 mM increased unidirectional ^{45}Ca influx in the first 4 min by 108.6±18.2 µmol/kg wet tissue (mean±s.e.mean, n=6). On the other hand, when the tissues were exposed to KCl 50 mM for 12 min and ^{45}Ca was added in the last 4 min, ^{45}Ca influx was 6.5±1.6 µmol/kg wet tissue (n=6). Vasa pretreated with 6-hydroxydopamine showed no significant increase in ^{45}Ca influx when stimulated with KCl 50 mM for 4 min.

Uptake measurements showed that, ^{45}Ca content of epididymal portion increased by 282.6±42.2 µmol/kg wet tissue (n=11) when the tissue was stimulated for 4 min, but only by 79.8±12.7 µmol/kg (n=16) when the tissue was stimulated for 10 min.

Similar results were obtained in the prostatic portion.

The results suggest that, in the rat vas deferens, exposure to KCl 50 mM increases ^{45}Ca influx and uptake in the first 4 min. However, calcium channels seem to be mostly inactivated after 8 min. Release of transmitter(s) from neuronal elements probably plays a major role in calcium influx and uptake induced by KCl 50 mM.

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337P

EFFECT OF INDOMETHACIN ON THE ANTIHYPERTENSIVE AND SYMPATHETIC INHIBITORY ACTIVITIES OF ACE INHIBITORS IN THE RAT

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Clinical experience has shown that the antihypertensive effect of captopril in patients with essential hypertension was attenuated by cyclo-oxygenase inhibition with indomethacin or aspirin (Moore et al, 1981; Silberbauer et al, 1982). In the high-renin 2-kidney renal hypertensive (2KRH) rat the hypotensive response to captopril was unaffected by infusion of indomethacin (Omata et al, 1981), and in the spontaneously hypertensive (SH) rat, which has a smaller renin component contributing to its high blood pressure (Koletsky et al, 1972), indomethacin was without effect on the hypotensive response to captopril (Antonaccio et al, 1979; DiNicolantonio et al, 1981). The lowering of blood pressure by inhibitors of angiotensin-I converting enzyme (ACE) may be related to their effect in reducing the vasopressor response to sympathetic outflow stimulation in the pithed rat (Antonaccio and Kerwin, 1980). We have therefore examined the effect of treating SH rats and 2KRH rats with indomethacin, 10 $mg.kg^{-1}$ po (a dose which prevents the vasodepressor response to arachidonic acid) on the hypotensive response to ACE inhibitors, and on the inhibitory effect of ACE inhibitors on the vasopressor responses to sympathetic outflow stimulation in the pithed SH rat (Gillespie and Muir, 1967). Pretreatment of 2KRH and SH rats with indomethacin did not affect their systolic arterial pressure (recorded by a non-invasive tail-cuff method (Gerold and Tschirky, 1968)) throughout 24 hr. This treatment did not affect the hypotensive responses to orally administered enalapril (3 $mg.kg^{-1}$) and cilazapril (10 $mg.kg^{-1}$) in the 2KRH rat, but significantly reduced those to captopril (30 $mg.kg^{-1}$), enalapril (30 $mg.kg^{-1}$) and cilazapril (10 $mg.kg^{-1}$) in the SH rat. This is in contrast to the observations for captopril by Antonaccio et al (1979) and DiNicolantonio et al (1981). The vasopressor responses to sympathetic outflow stimulation were significantly reduced by the three ACE inhibitors in the pithed SH rat; this reduction was unaffected by indomethacin, 2 $mg.kg^{-1}$ po at -48, -24 and -1 hr, a regimen which also significantly reduces the antihypertensive effect of the ACE inhibitors in the SH rat. We therefore suggest that (i) ACE inhibitors lower blood pressure in high renin hypertension by preventing the formation of causative angiotensin II; (ii) ACE inhibitors lower blood blood pressure in normal renin hypertension by releasing dilatory products of cyclo-oxygenase; (iii) inhibition of the effects of sympathetic stimulation is not relevant to the indomethacin-sensitive antihypertensive effect in the SH rat.

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PRE- AND POSTJUNCTIONAL EFFECTS OF PINACIDIL ON SYMPATHETIC NEUROEFFECTOR TRANSMISSION IN RABBIT BLOOD VESSELS

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Pinacidil is a potent vasodilator (Arrigoni-Martelli et al., 1980) which is effective in the treatment of mild to moderate hypertension (Ward, 1984). The aim of the present study was to examine the effect of pinacidil on sympathetic neuroeffector transmission and the vasoconstriction evoked by various agonists and potassium. The rabbit isolated pulmonary artery and aorta preparation were used. The methods described in detail (Nedergaard, 1980) were used.

Pinacidil (10^{-6} - $3x10^{-4}$M) inhibited the contractions of pulmonary artery evoked by selective electrical-field stimulation (150 monophasic pulses; 0.5 msec; 3 Hz; 200 mA) of postganglionic sympathetic neurone terminals in the absence and presence of cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M). Pinacidil (10^{-4}M) caused a submaximal steady state block of stimulation-evoked contractions of pulmonary artery within 30 min. The inhibition was reversible.

Pinacidil (10^{-5} - $3x10^{-4}$M) caused a marked increase (up to 220% of control) of the ^{3}H-overflow evoked by field stimulation (300 pulses; 3 Hz; 200 mA) of pulmonary artery preloaded with (-)-^{3}H-noradrenaline (^{3}H-NA). The enhancement was also seen in the presence of cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M). Neither rauwolscine (10^{-6}M) nor propranolol ($3x10^{-7}$M) prevented the pinacidil-induced enhancement. The relationship between stimulation frequency (1-30 Hz) and ^{3}H-overflow was examined. At 1-10 Hz, but not at 30 Hz, pinacidil (10^{-4}M) caused a significant increase in ^{3}H-overflow.

Pinacidil (10^{-5} - $3x10^{-4}$M) in a non-competitive manner antagonized the contractions of aorta evoked by noradrenaline ($3x10^{-9}$ - $3x10^{-5}$M), phenylephrine ($2x10^{-8}$ - $3x10^{-4}$M), adrenaline (10^{-9} - $3x10^{-5}$M), histamine (10^{-6} - $6x10^{-4}$M), serotonin (10^{-8} - 10^{-4}M), and potassium (16-55 mM).

Pinacidil ($3x10^{-5}$ - $3x10^{-4}$M) reduced the accumulation of ^{3}H by aorta preloaded with ^{3}H-NA (10^{-8}M). The aorta was treated with pargyline (10^{-4}M) and U-0521 (3',4'-dihydroxy-2-methylpropiophenone) to inhibit monoamine oxidase and catechol-O-methyltransferase, respectively.

Smooth muscle relaxation of blood vessels by acetylcholine and several other drugs is dependent on the release from the intact endothelium of a relaxant factor (EDRF; Furchgott, 1984). Pinacidil (10^{-6} - $3x10^{-4}$M) and methacholine ($3x10^{-8}$ - 10^{-6}M) relaxed aorta preconstricted with noradrenaline (10^{-7}M). The relaxation caused by pinacidil was not dependent on the presence of endothelial cells, while that seen with methacholine was.

These results suggest that pinacidil is a direct acting vasodilator and is not dependent on the release of EDRF. Furthermore, pinacidil facilitates depolarization-evoked sympathetic transmitter release which does not involve prejunctional adrenoceptors of either the α_2- or β-subtype.

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COMPARISON OF DILATOR ACTIVITY IN RAT HEPATIC PORTAL VEIN AND RAT MESENTERIC ARTERIES

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The true resistance vessels are relatively inaccessible and vascular reactivity is often investigated in larger vessels or vascular beds. Among the most frequently used models of resistance vessels are rat mesentery and hepatic portal vein. The latter shows more resemblance in innervation, myogenic activity and calcium-dependence to the true resistance vessels than do large arterial preparations (Pegram, 1980). The present work reveals considerable differences between these two preparations.

Rat hepatic portal veins were suspended under 0.5g tension in oxygenated Krebs-bicarbonate solution containing 0.1mM ascorbic acid at 37°C. Isolated rat mesenteries were perfused with the same solution at 4 $ml.min^{-1}$ at 37°C (McGregor, 1965). Each preparation was maximally constricted by noradrenaline (NA) (5 x 10^{-6}M for portal vein and 10^{-5}M for mesentery) or KCl (0.1M) before applying each dose of dilator. One constrictor and one dilator agent were tested in each preparation (N = 5-6). The highest dose of verapamil and papaverine produced approximately 90% reduction in constrictor response so that passive tone was unaffected. The highest dose of nitroprusside and Dantrolene produced maximal dilatation. The IC_{50} values were calculated from the concentration-dilatation curves. Dilatation is also expressed (Table 1) as the ratio:- % reduction in NA/% reduction in KCl induced constriction (NA/KCl ratio), a modification of the method of Kent et al (1982). This index summarises differential actions of dilators on receptor-mediated and non-receptor-mediated constriction.

Table 1. Comparison of dilator activity in rat portal vein and mesentery

Portal Vein	Max reduction in vasoconstriction (%)		IC_{50}		Ratio of
Vasodilator	NA	KCl	NA	KCl	NA/KCl
Nitroprusside	56.2 ± 4.8	5.7 ± 1.4*	2 x 10^{-6}M	-	9.8 ± 1.0
Dantrolene	44.2 ± 3.1	25.6 ± 3.5*	-	-	1.7 ± 0.2
Verapamil	86.8 ± 14.3	86.1 ± 8.2	4 x 10^{-8}M	2 x 10^{-7}M	1.0 ± 0.2
Papaverine	91.1 ± 8.5	80.0 ± 8.8*	8 x 10^{-6}M	7 x 10^{-6}M	1.1 ± 0.1
Mesentery					
Nitroprusside	79.0 ± 12.4	36.0 ± 1.0*	7 x 10^{-8}M	-	2.2 ± 0.4
Dantrolene	47.2 ± 4.2	9.9 ± 3.5*	-	-	4.8 ± 2.0
Verapamil	64.3 ± 6.5	96.5 ± 6.8*	5 x 10^{-7}M	2 x 10^{-8}M	0.7 ± 0.1
Papaverine	88.6 ± 4.7	70.0 ± 6.8*	2 x 10^{-6}M	8 x 10^{-6}M	1.3 ± 0.1

*Different from reduction in NA constriction ($P<0.01$, Mann-Whitney Test)

The results (Table 1) indicate that the effects of dilators depend on the agent used to elevate tone and vary with the vascular tissue used. Only papaverine caused comparable effects on the two tissues as indicated by similar IC_{50} and Na/KCl ratios. The results in both tissues differ from those reported for rat aorta by Kent et al (1982) and Keraki et al (1984).

Dantrolene was a generous gift from Norwich-Eaton Pharmaceuticals.

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ADENOSINE RETARDATION OF ADRENOCEPTOR RESENSITISATION IN THE PROSTATIC RAT VAS DEFERENS

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Purines occur endogenously in the prostatic rat vas deferens and are known to exert potent modulatory effects pre and post synaptically on many transmitter systems including the noradrenergic (Stone, 1981). Additionally, there is a considerable body of evidence suggesting that ATP may act as a co-transmitter in this tissue (see for instance French and Scott, 1983).

We have recently identified a further action of purines on noradrenergic contractility, as adenosine has a significant effect on the rate of resensitisation of adrenoceptors in rat vas deferens,

The vasa of Wistar rats were removed, bisected and prostatic portions set up isometrically in 10ml organ baths, bathed in oxygenated Krebs-Henseleit solution. After 30 mins equilibration, control responses to 6μM noradrenaline (NA) were obtained. The vasa were then desensitised by exposing them to 1000μM NA for 1 min periods at 2 min intervals for 20 mins. The rate of resensitisation was assessed by testing the contractile response to 6μM at 5 min intervals immediately after the last desensitising dose of NA had been washed out. One vas of each pair was used to follow the spontaneous rate of resensitisation, the other vas being used to examine the effect of various purines on resensitisation. Purines, where used were added 1 min before the amine.

Our results show that adenosine significantly retarded the rate of resensitisation of adrenoceptors in this tissue. The time to recovery to 50% control responses (R_{50}) was used as the index to the rate of resensitisation and was measured by graphical interpolation. Spontaneous resensitisation was complete within 25-30 mins and had an R_{50} of 19.1±0.6 mins (s.e.m.) (n=60); in the presence of 30μM adenosine this value was 25.0±2.5 mins (n=6), ($p<0.001$; Students paired t-test). This effect was prevented by the specific P_1-purinoceptor antagonist 8-phenyltheophylline (10μM) and by yohimbine (10nM). Atropine (2μM) did not prevent this effect. Neither 8-phenyltheophylline, yohimbine or atropine by themselves had any effect on the rate of resensitisation. ATP (30μM) and βγ methylene ATP (30μM) were also investigated and shown not to alter the rate of resensitisation. The effect of adenosine did not occur when the responsiveness of the tissue was tested with phenylephrine. The effect of adenosine thus appears to be specific to that purine and mediated via an α_2 adrenoceptor.

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ENDOTHELIUM-DEPENDENT RELAXATION IS INHIBITED BY A HIGH MOLECULAR WEIGHT PROTEIN FRACTION OF WHOLE HUMAN PLASMA

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Endothelium dependent relaxation (Furchgott 1983) has been shown to be mediated by both basal and stimulated release of an unstable endogenous vasodilator (Griffith et al 1984a). To study the effects of plasma constituents on this phenomenon, 2mm wide strips of descending rabbit aorta were mounted isometrically in a 3 ml organ bath containing oxygenated Holman solution, as previously described (Griffith et al 1984b). In preparations preconstricted by 5-hydroxytryptamine (10^{-5}M) developed tension was 1325±55 mg, the addition of the calcium ionophore A23187 (10^{-7}M) resulted in endothelium dependent relaxation of 61±3.5% (n=21). Blood (collected so as to avoid haemolysis) was obtained from healthy male volunteers into lithium heparin tubes and whole plasma obtained by centrifugation at 3,500 rpm for 20 min. 0.3 ml of this whole plasma added to the 3 ml organ bath inhibited the endothelium-dependent relaxation by 106±13%; 0.03 ml by 40±4% and 0.003 ml by 17±7% (n=8-10). Inhibitory activity was abolished by boiling for 5 minutes. Similar effects were demonstrated against endothelium dependent relaxation induced by acetylcholine (10^{-6}M). Whole rabbit plasma also exhibited similar inhibitory properties.

Whole human plasma was fractionated by gel filtration on G200 Sephadex and eluted in 0.05M phosphate buffer, pH 7.0, containing 0.3M NaCl. An inhibitory peak was eluted with proteins M.Wt. ca. 200,000 (i.e. with immunoglobulins and haptoglobin). Whole human plasma was also fractionated on DEAE Sepharose with 0.02 M phosphate buffer pH 8.0, and developed with a gradient to 0.35 M NaCl. The inhibitory activity could be eluted in buffer with a conductivity of between 8.4 - 11.6 mS and preceded the major albumin peak. Inhibitory activity was lost over a period of two days. It has not yet been possible to combine the two fractionation steps.

One plasma protein common to both active fractions is haptoglobin. Haemoglobin ($> 10^{-7}$M) has marked inhibitory action against endothelium dependent relaxation (Furchgott 1984). It is therefore possible that the inhibitory action of plasma is due to the haptoglobin-haemoglobin complex.

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ENDOTHELIUM AND CALCIUM FLUX IN RABBIT AORTIC PREPARATIONS

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Endothelium-derived relaxant factor (EDRF) is an unstable humoral agent whose release is stimulated by acetylcholine (ACh) (Furchgott and Zawadski 1980; Griffith et al 1984). Its smooth muscle relaxant effect has been attributed to an increase in arterial cyclic guanosine monophosphate (cGMP) levels (Rapoport et al 1983).

We have investigated the effects of EDRF on calcium flux in rabbit aorta. 3-5 mm-wide ring segments from the thoracic aorta were prepared. Endothelium was removed from half the rings by gentle abrasion. Rings were immersed in Hepes buffer at 37°C, pH 7.3 gassed with 100% O_2 and equilibrated for 90 min. For influx studies rings were placed in buffer containing ^{45}Ca (c. 1.5 μCi/ml) for 1.5 or 3 min before being transferred into ice cold buffer containing 2 mM EGTA for 45 min, then gently blotted, weighed and placed in hypotonic EDTA solution (5 mM) overnight. The supernatant radioactivity was measured. For efflux studies, rings were equilibrated for 3 hrs in buffer containing ^{45}Ca, then placed in ice cold buffer containing 5 mM EGTA and 6.5 mM $CaCl_2$ for 45 min. Rings were transferred through a series of 3 ml aliquots of buffer at 5 min intervals for measurement of efflux rates over 60 min, blotted, weighed and residual ^{45}Ca content measured. Efflux is expressed as fractional ^{45}Ca loss/min. Results are given as mean ± SEM and compared using students t test for unpaired data.

Ca influx in resting preparations was greater in the presence of endothelium (45±1.4 μM/kg) than in its absence (37±1.4 μM/kg) (n=21, $p<0.001$). In neither case was it affected by verapamil (10^{-5}M) nor by agents which inactivate EDRF - dithiothreitol, potassium borohydride, phenylhydrazine (5 x 10^{-4}M) or phenidone (10^{-5}M), nor by ACh (10^{-6}M) which stimulates EDRF release. The endothelium-dependent increment was however reduced by flurbiprofen (10^{-5}M).

Noradrenaline (10^{-5}M) increased Ca influx in the presence (75±4 μM/kg) or absence (67±2 μM/kg) (n=6, $p<0.001$ cf respective controls) of endothelium. ACh (10^{-6}M) reduced Ca influx to 56±1.4 μM/kg (n=6, $p<0.001$) but only when endothelium was present. This effect of ACh was blocked by dithiothreitol, potassium borohydride or phenylhydrazine (all at 5 x 10^{-4}M) but not by flurbiprofen (10^{-5}M).

Noradrenaline (10^{-6}M) increased the rate constant for efflux in both endothelialised (0.57±0.004/min) and deendothelialised (0.0695±0.006/min) rings. ACh (10^{-6}M) reduced the ^{45}Ca efflux only in those rings with an intact endothelium to (0.04 ± 0.003/min) (n=6, $p < 0.001$). This effect of ACh was reversed by dithiothreitol (5 x 10^{-4}M) but not affected by flurbiprofen.

Thus in the basal state there is a small endothelium-dependent cyclo-oxygenase product-mediated increase in Ca influx which does not occur through voltage-dependent slow channels. When the arteries were stimulated by noradrenaline, however, EDRF reduced net ^{45}Ca influx and efflux. These results imply that EDRF-mediated relaxation of noradrenaline stimulated arteries is associated with a reduction of Ca influx and that this may contribute to the mechanism of its relaxant effect.

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STIMULATION AND INHIBITION OF ENDOTHELIAL-DEPENDENT RELAXATIONS OF RAT AORTIC STRIPS

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Furchgott & Zawadski (1980) first reported that acetlycholine (ACh) required an intact endothelium in order to relax pre-contracted strips of rabbit aorta. Further experiments have revealed that many other dilators act indirectly via an endothelial-derived relaxant factor (EDRF). Using rat aorta our studies have shown that vasoactive intestinal polypeptide (VIP) is an endothelial-dependent relaxant (Davies & Williams, 1984). We have now extended the range of vasodilators studied and also report the effectiveness of a variety of agents to inhibit such responses.

Circular muscle strips of rat thoracic aorta were prepared and mounted in an organ bath as described (Davies & Williams, 1984). Each strip was contracted to approximately 80% of maximal tension with noradrenaline (NA) or phenylephrine (PE) and relaxants added cumulatively. Papaverine (PAP) or isoprenaline (ISO) were used as negative controls. Endothelium was removed by rubbing the intimal surface of each strips with a probe. Drugs tested for inhibitory activity were first equilibrated with aortic strips for 15 minutes. Using ACh and histamine as positive controls, experiments showed that ATP (7×10^{-7}M), ADP (9×10^{-7}M), the calcium ionophore A23187 (3×10^{-8}M) and lysophosphatidylcholine (5×10^{-7}M) (n=4) all exerted relaxant actions which were abolished after de-endothelialisation. The relaxant effects of hydralazine, theophylline, sodium nitroprusside, magnesium sulphate and verapamil were not altered by endothelial cell removal.

ACh-induced relaxations were inhibited by the dual lipoxygenage/cyclo-oxygenase inhibitors 5,8,11,14 eicosatetraynoic acid (ETYA) and BW755C. ETYA and BW755C (5×10^{-7}M - 2.5×10^{-6}M) caused parallel shifts to the right in the ACh dose-response curves (n=5). At higher concentrations the maximal response to ACh was depressed. Indomethacin did not affect relaxations induced by any endothelial-dependent relaxant. Similarly other reported lipoxygenase inhibitors e.g. benoxaprofen, esculetin and nafazatrom were ineffective. Mepacrine (2×10^{-5}M) inhibited the effects of endothelial-dependent relaxants but prolonged incubation (3h) with dexamethasone or corticosterone (2.5×10^{-5}M) was ineffective. Several anti-oxidants inhibited relaxant responses: hydroquinone (10^{-5}M), L- and D- cysteine (8.25×10^{-3}M), retinol (3.5×10^{-5}M) and potassium borohydride (1.9×10^{-3}M). However, ascorbic acid, dithiothreitol and reduced glutathione were inactive. Thus anti-oxidant activity per se is not sufficient to inhibit EDRF generation. The effectiveness of ETYA, suggests that an oxygenated product of the lipoxygenase, such as an evanescent hypdroperoxide or epoxide, may be an EDRF in rat aortic strips.

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PRODUCTION OF ENDOTHELIUM DERIVED RELAXANT FACTOR IS BOTH ATP AND CALCIUM DEPENDENT

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EDRF is an unstable endogenous vasodilator whose production occurs continuously in the basal state but may be stimulated by many pharmacological agents (Griffith et al, 1984; Furchgott 1983). Mechanisms underlying its production were studied in isometrically mounted aortic strip preparations of the rabbit and a bioassay in which an intact endothelialised aorta was perfused in series with a preconstricted deendothelialised coronary artery preparation. Oxygenated Holman's buffer (NaCl 120 mM; KCl 5.4 mM; $CaCl_2$ 2.5 mM; Na_2HPO_4 1.3 mM; sucrose 10 mM; glucose 11 mM) at 37°C was used in all experiments.

Inhibitors of mitochondrial electron transport (antimycin A, 10^{-6}M, or rotenone, 10^{-6}M), uncouplers of oxidative phosphorylation (carbonyl cyanide m-chlorophenylhydrazone (CCCP), 10^{-5}M or valinomycin, 10^{-4}M) and an inhibitor of the F_1 ATPase (oligomycin 10^{-4}M) rapidly (within 5 min) abolished the endothelium dependent relaxation induced by acetylcholine (10^{-6}M) or A23187 (10^{-7}M) in aortic strip preparations preconstricted by 5HT (10^{-5}M). Inhibition was complete and irreversible except with CCCP when the endothelium response returned after 60-90 min of repeated washing. The constrictor response to 5HT was unaltered by any of these five mitochondrial inhibitors. Aortic strip preparations were also incubated with 25 mM 2-deoxyglucose in the absence of glucose to inhibit glycolysis. Endothelium dependent relaxation to acetylcholine (10^{-6}M) declined from 70±3% (control without glucose) to 45±5% after 30 min and to 29±6% after 60 min incubation with 2 deoxyglucose (n=10). The constrictor response to 5HT remained unaltered.

In bioassay experiments intact aortae were first stimulated to produce EDRF by acetylcholine (10^{-5}M) and the response of the preconstricted coronary measured. The aortae were then incubated for 20 minutes with the mitochondrial inhibitors (all at 10^{-6}M, n=4 in each case). Their subsequent ability to produce EDRF on stimulation with acetylcholine was completely lost, irreversibly except in the case of CCCP where partial restoration of the response occurred after 60 min of washing. Incubation of aortae in glucose-free buffer containing 25 mM 2-deoxyglucose resulted in progressive loss of EDRF-induced dilatation to ca. 30% of control after 60 min, though it was still demonstrable at 150 min (n=3).

Endothelium-dependent relaxation has previously been shown to depend on extracellular calcium (Singer and Peach, 1982). By altering the infusion site of calcium into the bioassay circuit (such that the concentration of calcium remained 2.5 mM at the coronary artery) EDRF production was shown to be rapidly (within 5 sec) responsive to the presence or absence of extracellular calcium.

Thus EDRF production is dependent (within seconds) on Ca^{++} and (within minutes) on ATP ,with mitochondria rather than glycolysis being the major source of ATP. Three possible mechanisms may be involved : i) release by Ca^{++} and ATP-dependent exocytosis ii) Ca^{++} and ATP-dependent biosynthesis iii) Ca^{++} and ATP-dependent protein phosphorylation.

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SUPERNATANTS FROM STIMULATED PLATELETS INDUCE HISTAMINE RELEASE FROM HUMAN BASOPHILS

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We have previously demonstrated that supernatants from thrombin-stimulated human platelets will enhance anti-IgE induced release of histamine from human basophils (Knauer et al, 1984). This platelet-derived substance is non-dialyzable and stable to boiling. The experiments reported here demonstrate that a platelet derived supernatant (PDS) will also induce direct release from basophils collected from specific atopic subjects.

Mixed leukocytes were separated from blood by dextran sedimentation, washed twice and suspended in PIPES buffer, pH 7.4, containing 1 mM $CaCl_2$ (PCG) (Lichtenstein & Osler, 1964). Platelets were isolated from blood collected from normal donors, washed twice and suspended in PCG to a count of $6x10^8$/ml (Knauer et al, 1984). Following stimulation at 37°C with thrombin for 5 min, PDS was obtained by centrifugation (12,300 g for 20 min) and concentrated 5 fold (Amicon YM2). The PDS was incubated with the mixed leukocytes, the cells pelleted (1000 g for 2 min) and the resulting supernatants assayed for histamine by the method of Siraganian (1975). Histamine release was expressed as a percentage of the histamine released by perchloric acid (2%) treated cells.

Table 1. Net basophil histamine release (% total) from 5 subjects ($\bar{x} \pm$ s.e.mean)

Buffer	PDS (10^8 equivalents/ml)				Anti-IgE (µg/ml)
	2.5	5	10	20	0.02
5 ±0.3	2 ±1.7	9 ±3.6	19 ±4.1	32 ±2.3	31 ±7.8

Incubation of mixed leukocytes with PDS over the range of $2.5x10^8$ to $2x10^9$ platelet equivalents/ml resulted in a dose dependent release of histamine (Table 1). As with anti-IgE, D_2O enhanced histamine release induced by PDS and incubation of the leukocytes at 0°C, 46°C or with 5 mM EDTA reduced release to 1.6%, 19.6% and 0%, respectively, of that at 37°C and 1 mM $CaCl_2$. The time course of histamine release was faster than that seen with a maximal concentration of anti-IgE. Further study revealed that release of activity from platelets was dependent on the thrombin concentration with a maximum at 2 U/ml. Over the range of 0.2 to 5 U/ml thrombin alone did not induce histamine release from human leukocytes. Collagen (5 µg/ml) also generated histamine releasing activity from platelets. Initial purification has revealed that the histamine releasing activity from platelets does not bind to heparin and is retained by a membrane with a 30,000 Mwr cut-off, suggesting that this activity is not due to platelet factor 4 (Brindley et al, 1983).

Thus, it is apparent that platelets, once activated, can initiate basophil mediated reactions and therefore can participate directly in allergic and inflammatory events.

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EFFECT OF CIMETIDINE, TIMOPRAZOLE AND ATROPINE ON PENTAGASTRIN-INDUCED GASTRIC ACID SECRETION IN THE ANAESTHETISED FERRET

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A number of workers (Pfeiffer & Peters, 1969; MacKay & Andrews, 1983) have shown that there are many similarities between the gastric physiology and anatomy of the ferret and man, and we have therefore investigated the potential of the ferret for testing gastric anti-secretory drugs.

Ferrets of both sexes weighing 0·6-1·8 kg were starved for 20-24h but allowed water ad libitum. Anaesthesia was induced with 40-50mg kg^{-1} pentobarbitone i.p. and maintained with an i.v. infusion of 6 mg ml^{-1} pentobarbitone as required. When necessary the animals were artificially respired.

The pylorus was ligated through a midline abdominal incision. Gastric contents were aspirated every 15 min using a No 21 Salem double lumen gastric tube passed into the stomach via the oesophagus. The volume of secretion was measured and an aliquot titrated to pH7 using 0·1M NaOH. 5 ml distilled water was instilled into the stomach at the start of each 15 min period. After a basal period gastric secretion was stimulated with a near maximal i.v. infusion of 4-8 µg $kg^{-1}h^{-1}$ pentagastrin. Once this response had plateaued drugs were administered by i.v. bolus, and the effects calculated as a % of the last two pre-dose responses in each animal. Only one dose of compound was tested in each ferret.

Cimetidine and timoprazole both inhibited acid output in a linear dose-related manner, with ED_{50}'s of 5·5 and 6·5 µmol kg^{-1}, respectively (Figure 1). Secretion was inhibited by 84 and 89% at 30 µmol kg^{-1} of each compound, respectively, and was due mainly to a reduction in acid concentration although the volume of gastric juice was also reduced. Timoprazole, however, was slower acting than cimetidine, its peak effect occurring 1-2h after administration compared to 0·5-1h after cimetidine. The effect of these two drugs appeared to be long lasting, with few signs of recovery of the secretory response within 2-3h of dosing.

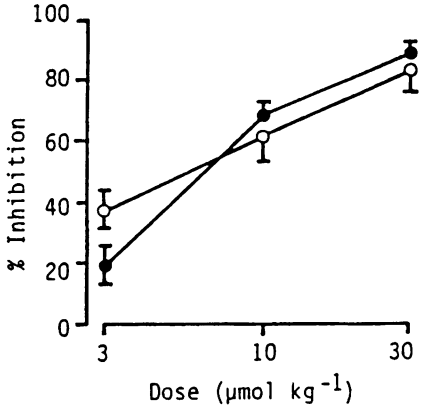

Figure 1 Effect of cimetidine (○) and timoprazole (●) on acid output in anaesthetised ferrets (mean ± s.e. mean, n = 4-8)

Atropine sulphate had inconsistent activity at 1-30 µmol kg^{-1}, and overall had little effect. This was not due to the use of pentobarbitone as the anaesthetic since atropine was inactive in chloralose anaesthetised ferrets. The reason may be increased sympathetic activity due to the acute surgical nature of the preparation (Schofield et al, 1975).

The ferret model described will detect H_2 antagonists and H^+/K^+ - ATPase inhibitors, and therefore offers an alternative carnivorous species to cats and dogs for gastric anti-secretory testing.

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SOLUBILISATION OF DOPAMINE D_2 RECEPTORS FROM HUMAN PUTAMEN USING THE ZWITTERIONIC DETERGENT CHAPS

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The rat striatal dopamine D-2 receptor identified by ^{3}H-spiperone can be solubilised in an active form using the zwitterionic detergent CHAPS (3-[3-cholamidopropyl]-dimethylammonio-1-propanesulphonate) (Lew et al.,1981; Jenner et al., 1984). We now report the effects of CHAPS treatment on D-2 receptors present in human putamen, and the properties of the solubilised preparation.

Normal human putamen was obtained from Dr.G.Reynolds (Brain Tissue Bank, Cambridge). Membrane preparations were prepared in 50 mM Tris HCl buffer (pH 7.4) at a final dilution of 270 volumes. Soluble preparations were prepared in 50 mM Tris HCl buffer containing 1 mM EDTA (pH 7.4) by exposure to 5 mM CHAPS for 1 h at a final dilution of 10 volumes. The specific binding of ^{3}H-spiperone (0.1-8.0 nM; defined by the incorporation of 3×10^{-5} M (±)-sulpiride) to soluble and membrane preparations was determined as previously described (Jenner et al., 1984). In both membrane and soluble preparations the specific binding of ^{3}H-spiperone was saturable and of high affinity. Scatchard plots of specific binding were linear suggesting a single component (membrane preparations Bmax 10.5 pmoles/g tissue; K_D 0.14 nM; soluble preparations Bmax 1.7 pmoles/g; K_D 1.6 nM).Compared to membrane preparations a 16% yield of specific ^{3}H-spiperone binding sites and 21% of original protein was obtained following CHAPS solubilisation. Pre-incubation of putamen membranes at 50°C for 10 min resulted in a minimal loss of specific ^{3}H-spiperone binding but a complete loss of binding sites in soluble preparations. The specific binding of ^{3}H-spiperone to soluble preparations was not retained on Millipore HAWP 02400 filters. The specific binding of ^{3}H-spiperone to both membrane and soluble preparations was stereoselectively displaced by the isomers of butaclamol and by haloperidol and sulpiride (Table 1). Dopamine and 6,7-ADTN also displaced ^{3}H-spiperone. In all cases drugs exhibited lowered affinities for the sites labelled by ^{3}H-spiperone in the soluble preparation. Gel filtration of the solubilised putamen preparation on a calibrated Sepharose CL-4B column revealed 2 components of specific ^{3}H-spiperone binding. The major component eluted between the peaks obtained for the soluble proteins thyroglobulin and ferritin.

Table 1. IC_{50} (nM) values for displacement of ^{3}H-spiperone binding

Drug	Membrane preparation	Soluble preparation
(+)-Butaclamol	1.1	8.1
(-)-Butaclamol	3,800	>10,000
(±)-Sulpiride	32	570
Haloperidol	3.6	35
6,7-ADTN	1,000	1,200
Dopamine	36,000	81,000

In conclusion, the dopamine D-2 receptor identified by specific ^{3}H-spiperone binding can be solubilised in an active form, but at a reduced affinity,using the zwitterionic detergent CHAPS.

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NEUROTOXIC ACTIONS OF MPTP IN THE MOUSE: MODIFICATION BY MONOAMINE OXIDASE INHIBITORS

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1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) causes degeneration of nigrostriatal dopamine neurones which can be antagonised by treatments with inhibitors of monoamine oxidase (MAOIs) (Heikkila et al, 1984). In the present study we extend the neurotoxic actions of MPTP to the limbic system and investigate the ability of MAOIs to modify the effects of MPTP in the mouse.

MPTP was given daily to male albino mice, 30mg/kg i.p. on days 1 and 2, 40mg/kg i.p. on days 3 and 4 and 50mg/kg i.p. on days 5 and 6, this being the maximum dose regime tolerated. Other mice received daily injections of vehicle, clorgyline 5mg/kg i.p., deprenyl 1mg/kg i.p. or clorglyline plus MPTP and deprenyl plus MPTP. Locomotor activity was measured in individual photocell cages 1h and 12h after each MPTP treatment. Animals were killed 92h after the last treatment and the striatum and limbic tissue (tuberculum olfactorium and nucleus accumbens) dissected for determination of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels using HPLC with EC detection.

Mice exhibited a prostration associated with signs of general toxicity 1h following MPTP administration alone or with the MAOIs. At this time a non-specific reduction in locomotion could be recorded, but all animals showed normal locomotion after 12h. The major biochemical consequences of the MPTP treatments were marked reductions in striatal DA (-90%), DOPAC (-82%), HVA (-65%) and limbic DA (-66%), DOPAC (-72%) and HVA (-61%) which could be antagonised by deprenyl but not by clorgyline which, in its own right, reduced the levels of DA, DOPAC and HVA (Table 1).

Table 1. MPTP and MAOI effects on limbic and striatal DA function.

Treatment	DA ng/mg	DOPAC pg/mg	HVA pg/mg	DA ng/mg	DOPAC pg/mg	HVA pg/mg
		STRIATUM			LIMBIC	
Vehicle	8.56±.90	644±65	926±112	3.149±.22	466±44	348±22
MPTP	0.81±.16***	116±16***	322±40**	1.049±.43***	132±13***	134±6***
Clorgyline	7.92±.68	192±38***	339±15***	2.874±.14	118±10***	130±10***
Deprenyl	8.03±.7	501±44	718±57	2.844±.18	349±30	286±28
MPTP+Clorg.	1.47±.22***	71±20***	187±20***	1.180±.36**	60±18***	62±20***
MPTP+Depr.	7.48±1.48^{++}	427±107^{+}	683±101^{+}	2.055±.35^{+}	249±61*	225±34*$^{+}$

n = 5. *P<0.05, **P<0.01, ***P<0.001 (reductions, comparison to vehicle), $^{+}$P<0.05, $^{++}$P0.01 (antagonism of the MPTP-induced reductions)

It is concluded, firstly, that MPTP given peripherally to the mouse can disrupt both the striatal and limbic DA systems. This occurs in the absence of any persistent behavioural change. Secondly, the antagonistic actions of deprenyl confirm that oxidase enzyme inhibition, at least of monoamine oxidase-B, may confer some protection against the neurotoxic actions of MPTP. Whilst inhibition of monoamine oxidase-A by clorgyline failed to protect against MPTP neurotoxicity, the role played by monoamine oxidase-A requires further study in view of the long-lasting changes caused by clorgyline in its own right.

Heikkila, R.E. et al (1984) Nature 311, 467-469

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EFFECTS OF ONE MONTH'S ADMINISTRATION OF AMINE DEPLETING DRUGS AND NEUROLEPTICS TO RATS ON STRIATAL DOPAMINE RECEPTOR FUNCTION

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Tetrabenazine and oxypertine deplete brain monoamines but may also interact directly with brain dopamine receptors (Hong et al.,1984; Reches et al.,1983; Nakahara et al.,1980). We compare the effects of repeated administration of amine depleting drugs with that of neuroleptics on striatal dopamine function in rats.

Male Wistar rats (180 ± 3 g at the start of the experiment) received either chlorpromazine hydrochloride (34-42 mg/kg/day), trifluoperazine dihydrochloride (3.0-5.0 mg/kg/day), oxypertine (7.0-12.5 mg/kg/day, tetrabenazine (5.5-7.5 mg/kg/day) or reserpine (0.25-0.35 mg/kg/day) dissolved in distilled water for 28 days.

Spontaneous locomotor activity was reduced by the administration of chlorpromazine, trifluoperazine or tetrabenazine for 28 days, but the other drugs were without effect. Following drug withdrawal, locomotor activity in drug-treated groups was not different from that of control animals. Stereotyped behaviour induced by apomorphine (0.5 or 1.0 mg/kg sc, 15 min previously) was inhibited by the administration of trifluoperazine for 28 days; other drugs were without effect. Following drug withdrawal, no alterations in stereotypy were observed. Striatal dopamine concentrations were decreased by treatment with chlorpromazine, trifluoperazine and tetrabenazine for 28 days, but not by oxypertine or reserpine; these returned to control levels following cessation of treatment. Bmax for striatal specific ^{3}H-spiperone (0.02-1.0 nM; defined using 10^{-5}M (±)-sulpiride) binding sites was increased by 28 days treatment with chlorpromazine or oxypertine; trifluoperazine, tetrabenazine and reserpine administration had no effect. Chlorpromazine and trifluoperazine treatment increased the dissociation constant K_D for specific ^{3}H-spiperone binding. No changes in Bmax and K_D for ^{3}H-spiperone binding were found on drug withdrawal.

Table 1. The behavioural and biochemical effects of 1 month's drug administration

Drug treatment	Locomotor activity (counts/ 60 min)	Striatal DA (µg/g tissue)	^{3}H-Spiperone binding Bmax (pmol/g tissue)	K_D (nM)
Control	1304 ± 81	14.8 ± 1.6	13.4 ± 0.2	0.04 ± 0.01
Chlorpromazine	370 ± 53*	9.6 ± 1.0*	20.0 ± 1.5*	0.22 ± 0.07*
Trifluoperazine	530 ± 72*	9.4 ± 1.0*	12.5 ± 0.5	0.09 ± 0.01*
Oxypertine	1111 ± 101	12.4 ± 1.3	16.2 ± 0.7*	0.06 ± 0.01
Tetrabenazine	881 ± 108*	7.3 ± 1.0*	14.5 ± 0.6	0.05 ± 0.01
Reserpine	1026 ± 124	10.4 ± 1.7	13.3 ± 0.7	0.08 ± 0.03

Results expressed as mean ± SEM; n = 3-10 animals. * $p < 0.05$ compared to controls

With the exception of reserpine all drugs showed evidence of affecting striatal dopamine function during the period of drug administration. However, such short term oral administration with amine depleting drugs or neuroleptics produced no persistent effects following drug withdrawal.

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THE NEUROTOXIC ACTION OF 1-METHYL-4-PHENYLPYRIDINE (MPP^+) ON THE NIGROSTRIATAL DOPAMINE SYSTEM OF THE RAT

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MPP^+ has been shown to occur in the brain as an oxidation product of the neurotoxic agent 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Markey et al, 1984). The present study investigates whether MPP^+ has neurotoxic action on the nigrostriatal dopamine system, and makes comparisons with the effects of MPTP.

Male Sprague-Dawley rats were subject to standard stereotaxic techniques for the implantation of guide cannulae to allow subsequent drug infusion into the substantia nigra (SN, Ant. 2.2, Vert. -2.0, Lat. ±2.0). MPTP, MPP^+ (10µg/24h) or vehicle were bilaterally infused into the SN via injection units coupled to Alzet osmotic minipumps located subcutaneously in the back neck region. During infusion the locomotor activity of rats was assessed in photocell cages, and difficulties in forelimb movement and rigidity in the limbs and trunk were noted. At a time of maximum behavioural change (4 days) animals were killed and areas of the striatum (CP) removed for determination of the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC with EC detection.

Table 1. Biochemical changes in CP following infusions of MPTP and MPP^+into SN

	Anterior CP			Medial CP		
Infusion	DA ng/mg	DOPAC pg/mg	HVA pg/mg	DA ng/mg	DOPAC pg/mg	HVA pg/mg
Vehicle	8.98±.14	674±28	533±15	3.40±.85	364±58	272±35
MPTP	8.38±.40	723±18	404±22*	3.60±.88	410±81	200±28
MPP^+	5.42±.48**	354±22**	326±34**	0.83±.26*	111±28*	100±19**

n = 6. Reductions significant to *P<0.05, **P<0.01

The rat is less susceptible than higher species to the neurotoxic actions of MPTP (Chiueh et al, 1984). When infused directly into the SN the neurotoxicity is limited to this area and does not extend to the striatum (Table 1, also Bradbury et al, unpublished). This contrasts with the neurotoxicity of MPP^+ which extends to the striatum where DA, DOPAC and HVA are reduced by 75, 70 and 63% (medial CP) and by 40, 49 and 39% (anterior CP) respectively. The MPTP infusion does, however, lead to motor deficits with locomotor activity levels reduced by 50-60%, but the effects of MPP^+ infusion into the SN are more marked (80-90% reduction in locomotion, associated with deficits in front and hind limbs and with rigidity of limbs and trunk).

It is concluded that a) MPP^+ can disrupt the nigrostriatal dopamine system as assessed both behaviourally and biochemically where the degree of change is dependent on the striatal area analysed, b) the pyridinium metabolite of MPTP may contribute to its neurotoxic actions, and c) MPP^+ may be an important investigative tool if its actions can be shown to be specific for the nigrostriatal dopamine system.

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EFFECT OF SERUM FACTORS ON SODIUM-PUMP ACTIVITY AND DNA SYNTHESIS IN CULTURED CEREBELLAR NEURAL CELLS

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Chronic exposure of neural cell cultures from rat brain to foetal calf serum (FCS) markedly enhances the in vitro development of their Na^+, K^+-ATPase activity when compared with cells grown in the absence of serum (Atterwill et al, 1985). Furthermore, thyroid hormone may be one important serum component for the development of the Na^+, K^+-ATPase of certain neural cell types in vitro (Atterwill et al, 1984). It has also been shown that certain serum factors acutely stimulate the Na^+ pump of quiescent cultured fibroblasts and neuroblastoma cells deprived of serum. These cells rapidly respond to serum addition with an influx of Na^+ ions, an increase in Na^+-pump activity and subsequent initiation of DNA synthesis (Smith & Rozengurt, 1978; Moolenaar et al, 1983). In this study, therefore, the effect of acute serum addition to cultured cerebellar (CBL) neural cells grown under serum-free conditions was investigated.

Rat CBL granule neurones or astrocytes were grown in a serum-free medium (CDM) as previously described (Atterwill et al, 1985) and Na^+ pump activity measured in situ by monitoring ouabain-sensitive ^{86}Rb uptake (Atterwill et al, 1984). [^{3}H] Thymidine incorporation into cellular DNA was also measured.

The CBL granule neurone-enriched cultures (4-8DIV) maintained in CDM behaved similarly to other cell types in that 10% FCS addition elicited a rapid increase (20-80% above control; 15 min. max response) in ouabain-sensitive, Na^+ pump activity. This effect was inhibited by amiloride and mimicked by the Na^+ ionophore, monensin. It seemed likely that this effect on ^{86}Rb uptake was occurring primarily in the neurones, since besides the very low numbers of astrocytes present in these cultures, ^{86}Rb uptake was unaffected by serum addition in CBL cultured astrocytes. Acute thyroid hormone (T_3) addition was found to have no effect on Na^+-pump activity in either cell type. Interestingly, cultured granule neurones (4DIV) and astrocytes (14DIV) in CDM also responded to the addition of serum with a large increase in the incorporation of [^{3}H] thymidine into DNA. However, autoradiographic studies showed that astrocytes and other contaminating 'flat cells' were the principal cells accumulating [^{3}H] thymidine in the 4 DIV 'neurone- enriched' cultures.

Taken together, these results suggest that in cultured neural cells derived from the early postnatal CNS an enhancement of Na^+-pump activity induced by serum addition or raising intracellular [Na^+] is not linked to an initiation of DNA synthesis. This also contrasts with findings obtained for cultured neurones from chick embryonic brain where raising intracellular [Na^+] was reported to initiate neuronal DNA synthesis (Cone & Cone, 1976)

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BLOCKADE OF THE ANTINOCICEPTIVE EFFECT OF INTRATHECAL DOPAMINE AGONISTS IN NAIVE AND SUPERSENSITIVE RATS

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Recent experiments have suggested that the dopamine (D-2) agonists apomorphine and LY 171555 produce spinal antinociception in barbiturate anaesthetised rats (Barasi et al., 1985). In contrast, the D-I agonist SKF 38393 was ineffective in this model. However, in DA-supersensitive rats the D-I agonist SKF 38393 displayed marked antinociception whilst the effects of the D-2 agonists were equivocal. The present study sets out to further characterise these responses by using two DA antagonists in naive and DA-supersensitive rats.

Experiments were performed on male Wistar rats (WSP stock) lightly anaesthetised with sodium pentobarbitone (Barasi et al., 1984). Nociceptive sensitivity was determined by measuring the tail flick latency (TFL) in response to noxious radiant heat. DA-supersensitivity was induced after withdrawal from 28-day haloperidol administration in drinking water (50 mg/l).

In naive rats the antinociceptive effects of intrathecally administered (i.t.) apomorphine (75 µg/kg) and LY 171555 (75 µg/kg) were significantly blocked by sulpiride (10 mg/kg i.v.). Although SKF 38393 (up to 150 µg/kg i.t.) was devoid of antinociceptive activity in naive rats, when administered to DA-supersensitive animals at a dose of 75 µg/kg i.t., SKF 38393 produced a significant increase in TFL. In subsequent experiments this effect of SKF 38393 in sensitised rats was significantly antagonised by both sulpiride (10 mg/kg i.v.) and the D-1 antagonist SCH 23390 (Iorio et al., 1983) at 250 µg/kg (i.v.).

The present results provide further evidence for the existence of a D-2 mediated antinociceptive effect of DA agonists in the spinal cord of the naive rat. However, since the antinociceptive activity of SKF 38393 observed in supersensitive animals is susceptible to blockade by both types of DA receptor antagonist this may suggest a lack of DA receptor selectivity in the mechanism of this antinociceptive response.

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353P

DOPAMINE D_1 AND D_2 RECEPTOR AGONISTS REDUCE GASTRIC SECRETION IN THE RAT

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Apomorphine reduces gastric secretory volume and acid concentration in the rat via agonist action on β_2 type adrenoceptors and dopamine (neuroleptic-sensitive) receptors respectively (Costall et al, 1984, also unpublished data). The present study investigates the nature of the dopamine receptors mediating the inhibition of gastric acid concentration.

Male Sprague-Dawley rats were implanted with chronically indwelling stainless steel gastric cannulae exteriorised from the fundus of the stomach. Before each experiment the rats were fasted for 18h but allowed water ad libitum. Stomachs were rinsed and the sample of gastric secretion collected in the first 15 min discarded. Samples were then collected at hourly intervals for 4h to determine the volume and titratable acid concentration.

The dopamine D-1 receptor agonist SK&F38393 (10-20 mg/kg s.c., 10 min pretreatment), the dopamine D-2 receptor agonist LY141865 (1.25-5 mg/kg s.c., 10 min pretreatment) and the mixed D-1/D-2 agonist apomorphine (0.25-0.5 mg/kg, 5 min pretreatment) reduced both the volume (control values 1.56±0.1 ml/h) and acid concentration (control values 84.16±1.76 µM/ml) of gastric secretion (by approximately 60% at the higher doses, n = 5-10, $P<.001$ for all agents). The inhibitory effects of apomorphine and SK&F38393 were present for 1 to 2h whilst LY141865 could reduce both the volume and acid concentration for at least 4h. The selective D-2 receptor antagonist metoclopramide (0.1-10 mg/kg i.p. 30 min pretreatment) and the mixed D-1/D-2 receptor antagonist cis-flupentixol (0.25-1 mg/kg i.p. 30 min pretreatment) when administered alone failed to modify either the volume or acid concentration. However, metoclopramide (10 mg/kg, 30 min pretreatment) completely antagonised the actions of apomorphine (0.5 mg/kg s.c.), SK&F38393 (20 mg/kg s.c.) and LY141865 (2.5 mg/kg s.c.) to reduce acid concentration ($P<.001$ for all agents). Cis-flupentixol (0.25 mg/kg, 30 min pretreatment) also antagonised the effects of apomorphine and LY141865 ($P<.001$) with only a trend to reduce the inhibitory effects of SK&F38393 ($P>.05$). In contrast to the above antagonism afforded by the neuroleptic agents, the dopamine receptor antagonists failed to antagonise the dopamine agonist induced reduction in gastric secretory volume.

It is concuded a) that both dopamine D-1 and D-2 receptor agonists can reduce both the volume and acid concentration of gastric secretion in the rat, b) that the reduction in acid concentration is achieved via an action on dopamine receptors which can be antagonised by neuroleptics having D-1 and/or D-2 antagonist potential and c) that no evidence was obtained to support a selective action of the agonists or antagonists on D-1 or D-2 receptors in the control of gastric acid secretion.

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ANTAGONISM BY LITHIUM OF CYCLIC CHANGES IN LOCOMOTOR ACTIVITY INDUCED BY DOPAMINE INFUSED INTO RAT NUCLEUS ACCUMBENS

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Lithium is the drug of choice for the treatment of manic-depressive illness in man. However, the precise site and mechanism of its action have remained uncertain, mainly because experimental analysis has been limited by the lack of a suitable animal model of phasic behavioural change. Recently we have described a phasic hyperactivity response to dopamine infusion into the mesolimbic nucleus accumbens of rat (Costall et al, 1982), and in the present studies we assess the ability of lithium and antidepressant drugs to stabilise such phasic changes.

Female Sprague-Dawley rats weighing 280±25g were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae to allow the bilateral infusion of dopamine into the nucleus accumbens (Ant. 9.4, Vert. 0.0, Lat ±1.6, De Groot, 1959). This was achieved via injection units coupled to Alzet osmotic minipumps located subcutaneously in the back neck region (see Costall et al, 1982 for experimental details). The locomotor activity of rats receiving dopamine (25µg/day, 0.48µl/h) or vehicle infusion was measured daily during the 13 day infusion period using individual cages equipped with photocell units. Lithium chloride and antidepressant drugs were administered during infusion by the intraperitoneal route, 3 doses daily.

The infusion of dopamine into the nucleus accumbens caused biphasic increases in locomotor activity with peaks of hyperactivity occurring on days 3-4 and 9-11 (254±25.9, 286±30.2 counts/60min, $P<0.001$ compared with control counts from vehicle infused animals, 87±7.3-125±15.2 counts/60min, n=6). The administration of lithium (3 x 2.5 mg/kg/day) during the period of dopamine infusion prevented the peaks of hyperactivity, and levels of locomotor activity remained indistinguishable from those of vehicle-treated rats. It is emphasised that the lithium treatment did not depress the level of activity below the control values. Higher doses of lithium (15 mg/kg/day) were toxic. Imipramine (3 x 5 mg/kg), amitriptyline (3 x 2.5 mg/kg) and nomifensine (3 x 1.25 mg/kg) administered daily enhanced the induced hyperactivity to obscure the two peaks. Higher doses of imipramine (3 x 20 mg/kg), amitriptyline (3 x 5 mg/kg) and nomifensine (3 x 5 mg/kg) could be shown to reduce the peaks of hyperactivity but only when toxic effects were also apparent. However, bupropion given daily (3 x 5, 10 or 20 mg/kg) during the dopamine infusion prevented the peaks of hyperactivity responding and animals remained healthy with levels of locomotor activity returning to control values.

It is concluded that the administration of lithium to the rat can antagonise the phasic changes in hyperactivity caused by the infusion of dopamine into a limbic brain area without reducing locomotor activity below normal levels. This property was not observed using antidepressants with the exception of bupropion, which is suggested in a preliminary report to be effective in bipolar affective illness (Shopsin, 1983). The model of phasic locomotor activity caused by dopamine infusions into the mesolimbic nucleus accumbens may thus present a means of studying the actions of mood stabilising agents.

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355P

A NOVEL ISOLATED IN VITRO PREPARATION OF THE RETRACTOR PENIS FROM THE GIANT AFRICAN SNAIL (ARCHACHATINA MARGINATA)

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The West African giant land snail ((GLS) belongs to the pulmonates group amongst gastropod molluscs.) The molluscs constitute the second largest invertebrate phyllum. The GLS is hermaphroditic, oviparous, and largely terrestrial. The nervous system comprises distinct or partially united cerebral, pedal, pleural, visceral, abdominal and buccal ganglia with their commissures and connectives and nerves supplying all parts of the body. The kidney is usually single. The GLS consists of a large visceral mass covered by a stout calcareous shell which protects against desiccation and osmotic changes. The nervous system is highly concentrated and the nerve cords are not twisted. The GLS and the British garden snail, Helix pomatia belong to the same group of gastropod molluscs. Archachatina is active during the rainy season and becomes minimal in the activity during the dry season, when it undergoes aestivation. GLS can be maintained on decomposing plant matter, fruit, bark and leaves (Segun, 1975).

The GLS (Archachatina marginata) was collected from various places especially in the Eastern and Western parts of Nigeria. About 150 snails have been employed for this preliminary investigation. For the dissection, a mid-line incision is made across the outer calcareous shell and the snail is completely drained of all haemolymph. The inner shell is excised with forceps and the entire snail transferred to a dissecting board. The mantle is pulled up and incision made through it to expose the intestine. A parallel incision exposes the genitalia which comprises an upper penial and a lower retractor penis running adjascent to the vas deferens. The whole retractor penis is then cut from the fusion of the penial base and by another lateral incision at the upper part. The excised snail retractor penis (SRP) is quickly transferred to a 5 ml organ-bath containing Meng's solution gassed with a mixture of 95% oxygen and 5% carbon dioxide at the room temperature of 26 $\pm$ 1^{o}C. The SRP usually measures on the average 30-40mm in length and 4-5mm in width and comprises a whitish strip of longitudinal muscle fibres. (Adebanjo and Ononiwu, 1985).

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FAILURE OF N-2-CHLOROETHYL-N-ETHYL-2-BROMOBENZYLAMINE (DSP4) TO AFFECT POSITIVELY REINFORCED OPERANT BEHAVIOUR

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It has been proposed that the noradrenergic innervation of the forebrain plays an important role in the acquisition and maintainance of operant behaviour by positive reinforcement (e.g., Crow, 1973). Systemic administration of DSP4 produces a selective and irreversible depletion of noradrenaline in the neocortex, hippocampus and cerebellum (Jonsson et al, 1981). In the present experiment we examined the effect of DSP4 on operant behaviour maintained under variable-interval (VI) schedules of food reinforcement. Our method, which is based on Herrnstein's (1970) quantitative model of performance in these schedules, allows separate evaluation of the effects of pharmacological interventions upon reinforcer effectiveness, and the capacity to emit operant responses (Morley et al, 1984).

The rate of responding (R) in VI schedules is a hyperbolic function of reinforcement frequency (r):

$$R = R_{max} \cdot r / (K_H + r) \quad (1)$$

where R_{max} and K_H are constants expressing the theoretical maximum response rate and the reinforcement frequency needed to maintain the half-maximum response rate (Herrnstein, 1970). The value of R_{max} is sensitive to variables which impair the capacity to respond, whereas K_H is sensitive to variables which devalue reinforcers (see Morley et al, 1984). Equation 1 implies that a treatment which elevates K_H (i.e., which reduces reinforcer effectiveness) will have a more profound suppressant effect on performance maintained by low reinforcement frequencies than on performance maintained by high reinforcement frequencies. This would be reflected in the ratios of response rates maintained by a low and a high reinforcement frequency, the ratio being decreased by treatments which elevate K_H. We have examined whether treatment with DSP4 would produce such an effect.

Ten female Wistar rats maintained at 80% of their free-feeding body weights were trained to press levers in operant conditioning chambers, using 0.05 ml of 0.6 M sucrose as the reinforcer. After performance had been established under VI 28-s, one group (N=5) received intraperitoneal injections of DSP4 (50 mg/kg), and the other group (N=5) injections of distilled water. Training under VI 28-s was continued for a further 21 sessions, and then the schedule was changed to VI 300-s. After 48 sessions of training under this schedule, the rats were sacrificed and their brains assayed for concentrations of catecholamines using high-performance liquid chromatography with electrochemical detection.

The concentrations of noradrenaline in the parietal cortex, hippocampus and cerebellum of the DSP4-treated group were less than 10% of those of the control group (t-test, $P < 0.001$). The mean ratios ($\pm$ s.e.mean) of the steady-state response rates maintained under the two schedules were 0.14 ± 0.01 (DSP4-treated) and 0.20 ± 0.04 (control group) (t-test: $P > 0.1$). Analysis of the transitional performance following the change in schedule indicated that the two groups did not differ significantly in the rate at which performance adjusted to the new schedule ($F(1,8) = 1.86$, $P > 0.1$).

These results do not provide any support for the proposed role of forebrain noradrenaline in the maintainance of operant behaviour by positive reinforcement.

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DIFFERENTIAL EFFECT OF CHRONIC ANTIDEPRESSANT ADMINISTRATION ON ENTRAINED CIRCADIAN WHEEL-RUNNING BEHAVIOUR IN THE MOUSE

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The suprachiasmatic nuclei (SCN) of the hypothalamus have been identified as a central circadian pacemaker regulating physiological and behavioural processes (Rusak & Zucker 1979). The SCN receive a dense serotonergic (5-HT) projection from the raphe nuclei (Rusak & Zucker 1979). Following chronic administration of imipramine SCN neurones become supersensitive to ionophoresed 5-HT (Mason & Meijer 1982). In this communication we report the effect of chronic administration of imipramine, iprindole and the selective 5-HT uptake inhibitors fluoxetine and zimelidine on entrained circadian locomotor behaviour in mice.

Male mice (C57 black, 20-25g) were housed individually in cages containing running wheels, on a 12h light : 12h dark lighting regimen. Food and water were provided ad libitum. Circadian locomotor activity was monitored continuously via micro-switches actuated by the running wheels. A delay (-) or advancement (+) in the entrainment of circadian locomotor activity to the light-dark cycle was determined from measurement of the time of onset of wheel running activity with respect to the onset of the dark phase (19.00h) of the lighting cycle. Imipramine, fluoxetine, iprindole and zimelidine were administered daily (10 $mg.kg^{-1}$, i.p. in 0.9% saline at 16.00h) for 12-28 days. Mice were administered saline, i.p., for 7-14 days prior to drug administration.

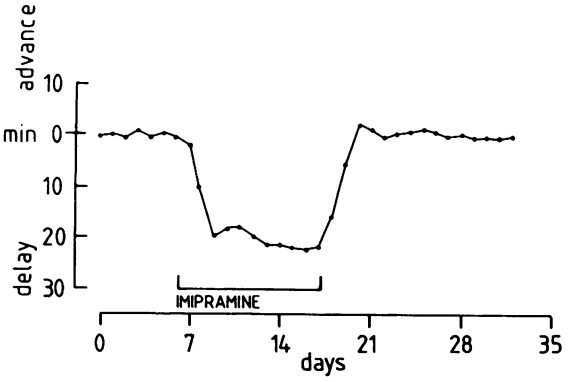

Figure 1 Imipramine-induced delay in the onset of entrained wheel-running activity of a single mouse with respect to the start of the dark period (0 min).

Chronic administration of imipramine induced a delay in the onset of wheel-running activity (Figure 1) ranging from (-) 18 to (-) 31 min (mean = 23.5 min, n = 8). A delay in the onset of wheel-running activity was also seen with the atypical anti-depressant iprindole (mean = 18.3 min, n = 4). With both drugs the delay appeared within 2-5 days of the start of drug administration. In contrast, fluoxetine and zimelidine did not produce detectable delay or advancement in the onset of locomotor activity. It seems unlikely from these results that the delay in the onset of wheel-running is due to blockade of 5-HT uptake in the SCN.

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THE USE OF p-CHLOROAMPHETAMINE-INDUCED BEHAVIOUR IN RODENTS AN AN IN VIVO SCREEN FOR 5-HT UPTAKE INHIBITORS

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The 5HT releasing property of para-chloroamphetamine (pCA) is dependent on prior active transport by the 5-HT uptake system (Meek et al., 1971). Therefore, the ability of drugs to inhibit the behavioural (and 5-HT depleting) actions of pCA can be used as an index of in vivo 5-HT uptake inhibition. In the present study we have examined the effects of 5-HT uptake inhibitors, and other drugs, on the behavioural changes produced by pCA in mice and rats.

pCA was administered i.p to either female Tuck (T/O strain) mice (21-28g) or male Sprague-Dawley rats (250-350g). In the mouse pCA induced an amphetamine-like hyperactivity and hyper-reactivity whereas in the rat it evoked a characteristic 5-HT - mediated stereotypy (the '5HT syndrome'). In subsequent experiments the effects of drugs on these behaviours were examined as follows:-

(i) mice: drug or vehicle was administered p.o. to pairs of mice 60 min before pCA (10 $mg.kg^{-1}$ i.p.) The activity of the paired mice (each member of a pair received the same treatment) was then recorded automatically during the period 10-30 min after pCA.

(ii) rats: drug or vehicle was administered p.o. to rats 90 min before pCA (10 $mg.kg^{-1}$ i.p.). Thirty minutes later the intensity of the '5-HT syndrome' was assessed blind (hind-limb abduction, fore-paw treading and head-weaving were each scored 0,1,2 or 3 (according to severity); tremor was scored as absent (0) or present (1) i.e. maximum score per rat = 10).

In the rat the 5-HT uptake inhibitors zimelidine, panuramine and fluvoxamine were found to inhibit the pCA-induced '5-HT syndrome' in a dose-dependent manner (ED_{50} values: 5.7, 16.2 and 17.2 $mg.kg^{-1}$ p.o. respectively). Zimelidine and panuramine also antagonised pCA-induced hyperactivity in mice (ED_{50} values: 12.2 and 40.4 $mg.kg^{-1}$ p.o. respectively) whereas fluvoxamine maleate was inactive over the dose-range 5-80 $mg.kg^{-1}$.

The dopamine antagonists haloperidol and pimozide were found to be potent inhibitors of pCA-induced hyperactivity in mice (ED_{50} values: 0.6 and 2.6 $mg.kg^{-1}$ p.o. respectively). In the rat, neither haloperidol (0.2-1.8 $mg.kg^{-1}$) nor pimozide (1.0-9.0 $mg.kg^{-1}$) significantly affected the intensity of the '5-HT syndrome' evoked by PCA.

Using dosing regimens shown to induce profound and selective depletion of 5-HT or catecholamines, monoamine synthesis inhibitors had differential effects on pCA-induced behaviour in the two species. Para-chlorophenylalanine (pCPA; 200 $mg.kg^{-1}$ p.o. administered at 54 and 30h before testing) inhibited pCA-evoked stereotypy in the rat by 85% whereas alpha-methyl-para-tyrosine (α MPT; 200 $mg.kg^{-1}$ i.p. 4h before testing) had no significant effect. In the mouse,either pCPA (500 $mg.kg^{-1}$ p.o. 54 and 30h before testing) or α MPT (400 $mg.kg^{-1}$ i.p. at 4h and 100 $mg.kg^{-1}$ at 1h before testing) pretreatment significantly inhibited pCA-induced hyperactivity (by 40% and 52% respectively).

We conclude that pCA-induced hyperactivity in mice involves catecholaminergic mechanisms in addition to those utilising 5-HT. In contrast, pCA-evoked stereotypy in the rat is predominantly a result of 5-HT release, rendering it more suitable as a rapid, in vivo preliminary screen for 5-HT uptake inhibitors.

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A COMPARISON OF TOLERANCE TO THE ANTICONVULSANT EFFECTS OF CLOBAZAM AND ITS N-DESMETHYL METABOLITE IN MICE

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Tolerance to the anticonvulsant effects of clobazam, a 1,5-benzodiazepine, has been demonstrated in both clinical (Gastaut & Low, 1979) and animal studies (Gent et al, 1984). N-desmethylclobazam (NDMC), its principal metabolite also has anticonvulsant actions in mice (Fielding and Hoffman, 1979) and plays a major role in the anti-pentylenetetrazole (PTZ) effect of clobazam (Haigh et al, 1984). We have therefore studied the anti-PTZ effects of repeated administration of clobazam and NDMC with concomitant measurement of plasma levels of these active compounds.

Adult male mice (Tuck No. 1), 25-35g in weight, were dosed orally, twice daily for 10 days, with either clobazam (10 mg/kg) or NDMC (40 mg/kg) suspended in 1% methylcellulose. In both experiments, control mice received equal volumes (5 ml/kg) of methylcellulose alone on the same schedule. On the first day and subsequently every 3 days, groups of 5 experimental and 5 control animals were randomly selected and 2 h after their morning dose were given an i.v. infusion of PTZ until a clonic convulsion was elicited. Protection was calculated as the difference between the mean minimal convulsant doses for experimental and control groups. Immediately after the test, blood samples were taken from each experimental mouse and plasma levels of clobazam and/or NDMC analysed by GC and HPLC respectively. Details of these procedures have been described previously (Gent et al, 1984). Results were analysed by single classification analysis of variance.

In the clobazam study, protection was significantly reduced ($P < 0.005$) from 43.4 $\pm$ 2.4 mg/kg PTZ (mean $\pm$ s.e. mean) on day 1 to 26.5 $\pm$ 2.7 mg/kg PTZ on day 4 and did not change significantly thereafter. Plasma concentrations of clobazam were very low (< 113 ng/ml) compared with those of NDMC (2300 - 3920 ng/ml). Levels of both compounds were significantly reduced on day 4 ($P < 0.005$); although clobazam levels remained low, concentrations of NDMC rose again so that on days 7 and 10 they were not significantly different from the initial levels ($P > 0.05$). The initial protection afforded by NDMC (35.4 $\pm$ 5.1 mg/kg PTZ) fell to 27.2 $\pm$ 2.6 mg/kg PTZ by day 4, but this change was not significant ($P > 0.1$); the protection remained at this level for the duration of the study. Despite fluctuations in the plasma levels of NDMC (3580 - 1910 ng/ml) no consistent change was seen over the 10 days.

From these results we can say that the tolerance developed in response to repeated administration of NDMC was markedly less than that to clobazam. Any change in protection which may have occurred with NDMC was clearly not the result of a change in metabolism. Nor was the tolerance to clobazam a result of changes in NDMC concentration, but it might have been attributable to some change in metabolism as illustrated by the significant reduction in plasma levels of clobazam. However, we have previously shown that NDMC is principally responsible for the protection afforded 2 h after clobazam administration in mice (Haigh et al, 1984). As tolerance development is the major drawback for long-term use of clobazam, the lesser degree of tolerance seen here with NDMC might well have clinical relevance.

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EXCITATORY EFFECTS OF CATECHOL ON SYNAPTIC TRANSMISSION IN THE RAT OLFACTORY CORTEX SLICE

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Parenteral administration of catechol (1,2-dihydroxybenzene) to rats induces a variety of motor activities (spontaneous convulsions, sensory evoked myoclonic jerks and tremor) some of which have been attributed to an increase in acetylcholine release (Angel & Dewhurst, 1978; Dewhurst, 1984). The present study investigates the possible effects of catechol on other excitatory transmitter systems by monitoring its actions on the evoked field potentials recorded from rat OC slices, a brain region where aspartate and glutamate are neurotransmitter candidates (Collins, 1979; Collins et al, 1981).

Rat OC slices (500μm thick) were preincubated and perfused at room temperature (Pickles & Simmonds, 1976) in a solution containing 25μM picrotoxin to abolish GABA-mediation inhibition (Collins et al, 1982). The surface fields evoked on lateral olfactory tract (LOT) stimulation (50μsec pulse width; supramaximal voltage, 0.0033Hz) were recorded using chlorided silver ball electrodes.

The dropwise application of catechol significantly ($P < 0.01$) increased the amplitudes of all fields in a concentration dependent (10μM to 1mM) manner; for example 1mM increased the peak N'a' amplitude from 2.92 ± 0.57 to 3.81 ± 0.76, that of the N'b' from 0.87 ± 0.43 to 1.81 ± 0.44 and the P-wave from 0.51 ± 0.18 to 0.83 ± 0.21 (mean amplitudes in mV ± s.d. mean, n = 6 to 11). Partial recovery was observed within 30-60 min of perfusion with drug-free solution.

The recorded fields are thought to reflect excitatory transmission at synapses of the LOT-superficial pyramidal cells (N'a'), superficial and deep pyramidals (N'b') and at sites deeper within the slice (P) (Collins et al, 1982). The present results suggest that catechol possesses excitatory effects on transmission which, of course, might be mediated at either pre- or postsynaptic sites. In experiments where changes in the d.c. potential between pial and cut surfaces of OC slices were recorded (Brown & Galvan 1979), 1mM catechol failed to affect responses to aspartate, glutamate or GABA implying that changes in neuronal excitability cannot provide the basis of an explanation for the present results.

As catechol increases the amplitude of all three fields it might facilitate transmission at all three sites but the present findings could equally be the result of a primary action at the LOT-superficial pyramidal cell synapse, a site where aspartate but not glutamate is a transmitter candidate (Collins et al, 1981). Minchin & Pearson (1981) reported that catechol increases D-[^{3}H] aspartate release from thalamic slices : preliminary experiments indicate that catechol selectively increases the K^+-evoked release of endogenous aspartate from OC slices, an effect consistent with a presynaptic action at the LOT-superficial pyramidal cell synapse.

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361P

ARE THERE TWO FUNCTIONALLY DIFFERENT NMDLA RECEPTORS IN RAT CORTEX?

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The effects of topically applied N-methyl-D,L-aspartic acid (NMDLA) on the somatosensory evoked potential (SEP) indicate some form of desensitisation of the NMDLA receptors (Addae & Stone, 1985). Since iontophoretic application of excitatory amino acids does not exhibit any apparent desensitisation (Curtis et al., 1960) we have conducted experiments to monitor single cell responses during topical applications of NMDLA, DL-homocysteic acid (DLH) and carbachol.

Male Wistar rats were anaesthetised with urethane and the area of cortex representing the contralateral forepaw exposed. Solutions of compounds were made in 0.9% NaCl and used to form a static pool on the cortex at room temperature. The cortex was washed with saline between drug applications. Seven-barrelled micropipettes with a separate single recording electrode were used for iontophoresis (Stone, 1985) with the tip at 1.2-1.6 mm below the cortical surface. Experiments were performed on pyramidal cells identified by their sensitivity to acetylcholine and the SEP from forepaw stimulation at 0.2 Hz was recorded simultaneously.

Topical application of 0.5 mM NMDLA caused within one minute an increase, followed by complete abolition of cell firing, with a decrease in responses to iontophoretic NMDLA, DLH, L-glutamate and acetylcholine. Leaving the NMDLA on the cortex for a longer time resulted in a return of cell firing and iontophoretic responses to control levels. A second application of NMDLA had no effect on the SEP or iontophoretic responses if applied within 5 minutes but was effective if applied 60 minutes after the first application. Topically applied DLH (10 mM) gave similar results whilst 1 mM carbachol usually caused only a partial decrease of cell activity.

Tetrodotoxin (TTX) 10 μM, applied topically for 30 mins caused a parallel decrease in the SEP and cell activity. Topical application of 0.5 mM NMDLA 10 minutes after removal of the TTX led to the partial (2 cases) or total (8 cases) prevention of the effects of iontophoretic NMDLA. In 3 experiments NMDLA responses were unchanged after TTX.

The lack of an effect of a second topical NMDLA when applied within 5 minutes of the first, whilst single cell activity and responses to iontophoretic NMDLA were normal, suggests the possible existence of two functionally distinct types of NMDLA receptor, one of which (presumably the superficially located variety) exhibits apparent desensitisation, whilst the other type (possibly located closer to the neuronal somata) does not. The results from the TTX experiments suggest that interneurones might be mediating the effects of topically applied NMDLA.

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THE EFFECTS OF FORSKOLIN AND PAPAVERINE ON NERVE CONDUCTION IN THE PRESENCE OF TETRODOTOXIN

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It has been described that adenosine, stable adenosine analogues, dibutyryl cyclic AMP and methylxanthines enhance the inhibitory effect of tetrodotoxin (TTX) on nerve conduction (Ribeiro & Sebastião, 1984). The present work was undertaken to investigate the effects of forskolin, an activator of adenylate cyclase in axons (Kilmer & Carlsen, 1984) and papaverine, a non-xanthine phosphodiesterase inhibitor (Kukovetz & Poch, 1970) on the axonal blockage induced by TTX.

The experiments were carried out at room temperature (22 - 25^{o} C) on the partially desheated sciatic nerve trunk of the frog. The preparations were arranged so that the solutions containing the drugs could be applied to the desheated part of the trunk. The nerve was stimulated supramaximally with rectangular pulses of 10 μs duration applied once every 5 s. Throughout the experiments compound action potentials were recorded in the conventional way and photographed. The bathing solution contained (mM): NaCl 117, KCl 2.5, NaH_2PO_4 1, Na_2HPO_4 1, $CaCl_2$ 1.8, $MgCl_2$ 1.2 (pH 7.0).

Forskolin (0.5 - 10 μM) decreased in a concentration - dependent way the amplitude of compound action potentials when nerve conduction was partially blocked by TTX. The full effect of forskolin was usually seen within 5 - 15 min after its application to the nerves and was washed out in about 60 min. Fifty percent decrease in the action potential amplitude recorded in the presence of TTX (45 - 80 nM) was obtained with about 2.5 μM forskolin. Papaverine (1 - 25 μM) also decreased the amplitude of compound action potentials partially inhibited by TTX, with a full effect usually observed within 5 - 15 min of its application to the preparations. Fifty percent decrease in the action potential amplitude recorded in the presence of TTX (30 - 80 nM) was obtained with about 10 μM papaverine. Both substances were devoid of effect on nerve conduction when applied to the nerves in the absence of TTX.

Since a cyclic AMP - dependent protein kinase phosphorylates the TTX - sensitive subunit of the sodium channel (Costa et al., 1982), the enhancement of the TTX - induced axonal block caused by forskolin and papaverine can result from their ability to increase the intra-axonal cyclic AMP content and by this mechanism to decrease the entry of sodium through the voltage - sensitive sodium channels.

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FLUNITRAZEPAM AND MUSCIMOL BINDING TO POST-MORTEM BRAIN TISSUE IN EPILEPSY

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It has often been proposed that epilepsy is caused by a reduction in GABA mediated inhibition in the CNS. The benzodiazepines have marked anti-convulsant activity which is utilized clinically for the treatment of certain epileptic disorders. These drugs facilitate GABA mediated transmission (1,2) at the $GABA_A$ receptor and the physical structure on which the binding site for the benzodiazepines is located also exhibits binding sites for compounds active at the $GABA_A$ receptor (3). Studies in vitro have shown that GABA increases the affinity of the benzodiazepine receptor for agonist ligands (4) and this may represent the neurochemical counterpart of the observed physiological phenomena.

The following study was carried out to discover if the number of binding sites for GABA and for the benzodiazepines or the facilitation effect of GABA on benzodiazepine binding is altered in epilepsy. Samples from 4 brain areas collected post-mortem from 6 epileptic patients and from 6 age-matched controls were used in binding studies with ^3H flunitrazepam (Fnz) and with ^3H muscimol.

Single point binding with 0.5 nM Fnz and with 2 nM muscimol (Table) demonstrated regional variation in the binding of both ligands but these were unaltered in the epileptic brain samples. Saturation analysis with Fnz was carried out in samples from the hippocampus and frontal cortex and yielded similar K_D and B_{max} values in all samples. 10^{-4} M GABA facilitated the binding of 0.5 nM Fnz to a similar extent in all samples (Table). In conclusion therefore, this study did not demonstrate any change in GABA or benzodiazepine binding induced by epilepsy.

Binding of 0.5 nM Fnz and 2 nM muscimol (fmol/mg protein)

means ± s.e.mean	0.5 nM Fnz n=5	GABA* Facilitation	Fnz Saturation Study n=3		2 nM Muscimol n=6
			K_D	B_{max}	
Caudate					
control	115 ±12	1.58±0.085			101±17
epileptic	98.1±20	1.81±0.09			111±17
Hippocampus					
control	159 ±20	1.80±0.09	1.76±0.29	952±242	89±17
epileptic	126 ±12	1.90±0.09	3.39±0.96	711±106	78±11
Frontal Cortex					
control	243 ±35	1.83±0.09	3.77±0.92	1337±290	152±21
epileptic	226 ±59	1.75±0.12	2.50±0.31	1253± 81	127±16
Temporal Cortex					
control	205 ±12	1.80±0.11			117±14
epileptic	172 ±36	1.70±0.09			152±14

* Ratio of binding of 0.5 nM Fnz with 10^{-4} M GABA/Binding 0.5 nM Fnz without GABA.

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