CALCIUM ENTRY BLOCKING PROPERTIES OF PIPROFUROL

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Piprofurol (MD710247) is a benzofuran derivative which, when administered in low intravenous doses to anaesthetized dogs, increases coronary blood flow and pO_2 in coronary sinus blood and decreases heart rate and arterial blood pressure (Pourrias et al, 1977). These results led us to postulate that the compound might be a calcium antagonist. We present here additional in vitro data, which appear to support this hypothesis.

Piprofurol and several reference calcium antagonists were studied under various experimental conditions which allow the recognition of calcium antagonistic activity (A. Fleckenstein, 1981). Piprofurol inhibited in a concentration-dependent manner the contractions induced by potassium depolarisation in the proximal and distal portions of the isolated dog coronary artery. The IC's 50 were respectively 20 nM/l (16.2-26) and 27 nM/l (18.4-43.6). Table 1 shows that piprofurol was more potent than the reference calcium antagonists. The inhibitory effect of piprofurol was overcome by raising the calcium concentration of the bath fluid. On rabbit thoracic aorta the apparent pA_2 value was 9.1.

In isolated guinea pig papillary muscle, piprofurol 1.1 µM/l produced an increase (+ 50 %) in action potential duration when measured at 25% decay. The maximum upstroke velocity was decreased (- 52 %) and the contractile force was reduced in a concentration dependent manner (0.2 to 1.1 µM/l). The compound inhibited (IC100= 0.1 µM/l) the contractions evoked in partially depolarized papillary muscle stimulated electrically in the presence of isoprenaline. The inhibition was antagonised by elevation of the concentration of calcium or isoprenaline in the bath fluid. In isolated working heart preparation (Neely et al, 1967), piprofurol 25 nM/l decreased leakage of LDH during coronary ligation and reperfusion, and protected the heart from the effects of coronary ligature and reperfusion on electric cardiac activity (ventricular fibrillation).

These results suggest that piprofurol is a calcium antagonist in both vascular smooth muscle and cardiac muscle. In comparison with the reference calcium antagonists, piprofurol is approximatively equipotent in cardiac muscle but appears to be more specific for coronary vascular smooth muscle and possesses less cardiac depressor activity.

Table 1 Calcium antagonistic effects of piprofurol and the reference calcium antagonists in isolated, potassium depolarized coronary artery of dogs

	IC50 (nM/l)	
	proximal portion	distal portion
Piprofurol	20 (16.2 - 26)	27 (18.4 - 43.6)
Verapamil	320	100
Diltiazem	650	100
Bepridil	550	290
Tiapamil	520	550

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EFFECTS OF COCAINE OR THYROIDECTOMY ON RESPONSES TO PHENYLEPHRINE AND ON BINDING OF ^{45}CALCIUM IN RAT ISOLATED SAPHENOUS ARTERY

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Cocaine selectively increases the sensitivity of adrenergically-innervated smooth muscle to catecholamines by acting presynaptically to block the neuronal uptake mechanism. In some tissues, cocaine acts postsynaptically to increase maximum responses to agonists (Summers & Tillman, 1979). Thyroidectomy also increases maximum responses of smooth muscle to agonists but unlike cocaine, thyroidectomy either does not affect or reduces the sensitivity to agonists (Brown & Pollock, 1980). This study compared the effects of cocaine or thyroidectomy on the sensitivity and maximum response to phenylephrine and on ^{45}calcium ($^{45}Ca^{2+}$) binding in rat isolated saphenous artery.

Thyroidectomised male Wistar rats were obtained commercially. Control and thyroidectomised rats were killed by halothane and isolated saphenous arteries were perfused (2ml/min) with Krebs solution (35^{o}C). Log-dose response curves for phenylephrine were obtained and doses (ED_{50}) that produced 50% of the maximum response were compared with Student's t-test. In $^{45}Ca^{2+}$ binding experiments, arteries were incubated in $^{45}Ca^{2+}$ Krebs solution (2μCi/ml, 37^{o}C, 20 mins), washed with Krebs solution (0^{o}C) in a Millipore filter and digested in KOH (4M, 60^{o}C, 1 hr).

In arteries from thyroidectomised rats, the maximum response to phenylephrine was greater (224±29mmHg (s.e. mean), n=10) than in controls (136±14mmHg, n=13) ($0.01>P>0.001$) but the ED_{50} (4.1±1.5 nmoles, n=13) was not significantly different from controls (2.8±0.6 nmoles, n=14). Cocaine (10^{-5}M) increased the maximum response (178±18mmHg, n=13) ($0.05>P>0.01$) and reduced the ED_{50} value (0.8±0.1 nmoles, n=11) ($0.01>P>0.001$) in arteries from intact rats.

The effect of varying the Ca^{2+} concentration of Krebs solution was also investigated. In control arteries, perfused with normal Krebs solution (2.5mM Ca^{2+}), the standard submaximal response to phenylephrine was 111±9mmHg (n=19). This response was reduced (39±6mmHg, n=11) ($p<0.001$) in Ca^{2+}-free Krebs solution but was unchanged (113±10mmHg, n=11) in Krebs solution containing 5mM Ca^{2+}. In the presence of cocaine (10^{-5}M), the standard submaximal response to phenylephrine was 93±19mm Hg (n=5). This response was reduced (54±12mmHg, n=5) ($0.01>P>0.001$) in Ca^{2+}-free Krebs solution and increased (131±15mmHg, n=5) ($0.01>P>0.001$) in Krebs solution containing 5mM Ca^{2+}. In arteries from thyroidectomised rats, the standard submaximal response to phenylephrine was 94±3mmHg (n=8). This response was reduced (40±8mmHg, n=8) ($p<0.001$) in Ca^{2+}-free Krebs solution and increased (102±10mmHg, n=8) ($0.05>P>0.02$, paired t-test) in Krebs solution containing 5mM Ca^{2+}.

The effect of cocaine and thyroidectomy on $^{45}Ca^{2+}$ binding was also investigated. Cocaine increased (466±87dpm/mg, n=7 ($0.05>P>0.01$) and thyroidectomy increased (556±112dpm/mg, n=7) ($0.01>P>0.001$) the binding of $^{45}Ca^{2+}$ in comparison with control (293±36dpm/mg, n=10). In the presence of phenylephrine, cocaine increased (819±183dpm/mg, n=9) ($0.05>P>0.01$) and thyroidectomy increased (699±59dpm/mg, n=5) ($0.01>P>0.001$) binding of $^{45}Ca^{2+}$ in comparison with control (320±95dpm/mg, n=5).

This study has shown that cocaine and thyroidectomy have similar effects on the maximum response of the isolated saphenous artery to phenylephrine, similar effects on Ca^{2+} metabolism but dissimilar effects on the sensitivity. These changes in Ca^{2+} metabolism and maximum response may be linked.

Acknowledgement - Medical Research Funds of University of Glasgow. J.M.B. is a University of Glasgow Postgraduate Scholar.

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COMPARISON OF THE ACTION OF HYDRALAZINE AND D600 ON RAT PORTAL VEIN

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The results of a recent study (Warburton & Weston, 1982) showed that both hydralazine and D600 inhibited electrically-stimulated tension development in partially depolarised rat papillary muscle, and that this effect could be reversed by increasing the extracellular calcium concentration. In a further comparison of the effects of hydralazine and D600, the action of these drugs on electrical and mechanical activity in the rat hepatic portal vein has been examined.

Hepatic portal veins were removed from male Wistar rats (250-350g) and bathed at 37°C in a MOPS-buffered physiological salt solution (PSS) gassed with 100% O_2. In some experiments electrical and mechanical activities were monitored simultaneously in a perfused capillary bath and quantified using integrators (Jetley & Weston, 1980).

In normal PSS containing 2.5 X 10^{-3}M Ca^{2+} both hydralazine (10^{-5} - 10^{-3}M) and D600 (10^{-7} - 10^{-6}M) produced a concentration dependent reduction in mechanical responses to noradrenaline (NA, 10^{-7} - 10^{-5}M) and to 4 X 10^{-2}M K^+ PSS. Whilst the inhibitory effects of D600 were associated with a parallel reduction of both electrical and mechanical responses to NA, marked electrical discharges were still observed in the presence of mechano-inhibitory concentrations of hydralazine. Both the integrated spontaneous electrical and mechanical activities of portal vein were inhibited by D600 (10^{-7} - 10^{-6}M) whilst hydralazine (10^{-5} - 10^{-3}M) increased the frequency of spontaneous discharges although the integrated electrical and mechanical activities were unchanged.

The dependence of both spontaneous and agonist-induced mechanical activity on extracellular calcium could be demonstrated by the complete inhibition of this activity on removal of calcium from the PSS. In Ca^{2+}-free PSS containing either NA (10^{-6}M) or K^+ (4 X 10^{-2}M) both hydralazine (10^{-5} - 10^{-3}M) and D600 (10^{-9} - 10^{-6}M) inhibited the mechanical responses which occurred on the re-introduction of increasing concentrations of Ca^{2+} to the medium. D600 also inhibited the ability of added Ca^{2+} to restore spontaneous electrical and mechanical activity whilst paradoxically hydralazine potentiated the effect of added Ca^{2+}.

The results of these experiments demonstrate that both hydralazine and D600 inhibit some common Ca-dependent processes. However, the observed differences in their action on portal vein are consistent with earlier results (Warburton & Weston, 1982) which suggest that the two agents do not share the same site or mechanism of action.

The financial support of Ciba Laboratories, the Mason Medical Research Foundation and the Smith Kline & French Foundation is gratefully acknowledged.

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α_1 AND α_2 AGONIST ACTIONS ON CONTRACTION AND Ca FLUXES IN RAT ISOLATED AORTA

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In the pithed rat contractions of vascular smooth muscle by stimulation by α_1- but not α_2-agonists are resistant to the calcium entry blocking drugs verapamil, D600 and nifedipine (Van Meel et al., 1981) suggesting that α_1 stimulated contractions are mostly mediated by the release of intracellularly stored calcium while α_2-stimulated contractions are dependent on extracellular calcium. We have investigated the effects of specific α-adrenoceptor agonists in the isolated rat aorta preparation where it is known that noradrenaline stimulated contractions are partly dependent on extracellular calcium (Ca_o) and partly dependent on the release of intracellular calcium stores (Ca_i) (Godfraind, 1978).

Noradrenaline (1 μM), like the α_1 adrenoceptor selective agonist phenylephrine (1 μM) produces maximal contractions of the isolated rat aorta which consist of an initial rapid, phasic increase in tension independent of Ca_o and a secondary tonic phase during which tension increases more slowly and is dependent on Ca_o. The α_2 adrenoceptor selective agonists, oxymetazoline (1 μM) and clonidine (1 μM) produce maximal contractions which consist only of a tonic increase in tension and amount to 48.6 $\pm$ 5.2 % (n = 14) and 24.1 $\pm$ 2.2 % (n = 47) of the maximal noradrenaline induced contraction respectively and are completely dependent on Ca_o.

The expected order of potency of these α-adrenoceptor agonists if only α_1-adrenoceptors were present is phenylephrine > noradrenaline > oxymetazoline > clonidine (Langer et al., 1981) while the reverse order might be expected if only α_2-adrenoceptors were present. A mixed order of potency was obtained in this artery (noradrenaline > phenylephrine > clonidine > oxymetazoline).

The calcium entry blocking drug cinnarizine (3 μM) which reduces the noradrenaline stimulated influx of ^{45}Ca into the smooth muscle cells of the rat aorta also reduces ^{45}Ca influx stimulated by phenylephrine and abolishes that stimulated by clonidine and oxymetazoline.

These data confirm in vitro that stimulation by α_2-agonists opens calcium channels in the smooth muscle membrane while α_1-agonists stimulation further releases intracellularly stored calcium.

This work was supported by the F.R.S.M. grant n° 3.9001.79).

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MECHANISM OF PROTECTION BY CALCIUM CHANNEL BLOCKING AGENTS AGAINST ISCHAEMIA/REPERFUSION DAMAGE IN RAT WORKING HEARTS

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The mechanisms by which calcium slow-channel blocking agents protect cardiac muscle against the effects of ischaemia remain largely a matter for debate. This study therefore sought to examine some possible mechanisms in perfused working rat hearts subjected to acute total ischaemia plus reperfusion. Rat hearts were perfused via the left atrium in a recirculating system with Krebs-bicarbonate containing 5.5mM glucose, 2i.u./l insulin and 1.0mM oleate bound to 0.2mM bovine plasma albumin. After 10min, total global ischaemia was induced by cross-clamping the atrial input and aortic outflow for 30 min. Hearts were then reperfused for 30min and finally freeze-clamped with tongs cooled in liquid nitrogen. Tissue metabolites were measured in neutralised perchloric extracts by automated enzymatic procedures using an ABA-100 Bichromatic Analyser (Higgins, 1981). Intracellular Ca^{2+} was estimated from tissue $^{45}Ca^{2+}$ content after correcting for extracellular space using [^{3}H] inulin. Total cardiac output was measured with a flowprobe on the left atrial input and coronary flow by timed collection of coronary effluent. Treated hearts were perfused throughout in the presence of nifedipine (10^{-7}M), diltiazem (4.10^{-7}M) or verapamil (10^{-7}M).

At these concentrations, none of the above agents altered initial cardiac output or coronary flow. However, all three agents improved recovery of function on reperfusion (from 40.6% to 62.1, 66.7 and 54.0% respectively), decreased release of enzyme (LDH) (from 130 to 56.7, 42.2 and 52.6 i.u./g dry wt.) and reduced intracellular Ca^{2+} accumulation (from 4.7 to 2.6, 2.3 and 2.4 µmol/g) with respect to controls. Despite the increased tissue Ca^{2+} and enzyme leakage in ischaemic hearts, the inulin-impermeable space was not altered, suggesting specific changes in membrane permeability rather than partial sarcolemmal rupture (Bourdillon and Poole-Wilson, 1981).

To determine whether the above cardioprotective actions were due to preservation of high-energy stores, hearts were freeze-clamped after 10 or 30 mins total ischaemia. Intracellular Ca^{2+} did not accumulate during ischaemia alone, but there was a rapid fall in phosphocreatine (75%) and a time-dependent decrease in ATP (18% at 10 and 39% at 30 min) together with massive lactate accumulation (35-fold). None of these parameters were affected by drug treatment.

Thus, under these conditions, the cardioprotective actions of nifedipine, diltiazem and verapamil are not due to depression of cardiac function nor preservation of myocardial energy stores as reported by others (Jolly et al., 1981; Watts et al., 1980), but probably involve antagonism of specific ischaemia-induced alterations in cell membrane permeability.

I am grateful to Mr. Simon Tickner for skilful technical assistance.

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THE EFFECT OF VERAPAMIL ON LETHAL DOSES OF COLLAGEN IN RABBITS

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Verapamil is a coronary vasodilator and displays a strong -ve inotropic effect which is attributed to an inhibition of the transmembrane calcium ion influx into cardiac muscle cells during excitation. Ikeda et al (1981) showed that verapamil inhibited, in vitro, platelet aggregation and secretion acting as an intracellular calcium antagonist.

Arachidonic acid infusion causes sudden death in rabbits (Silver et al, 1974) which may be due to a combination of massive intravascular platelet aggregation, pulmonary and coronary artery constriction, and bronchoconstriction (Cerskus et al, 1978). Thromboxane A_2 (TXA_2) is produced by platelets and has powerful vasoconstricting and platelet aggregating properties.

In the course of studies using the Technicon Autocounter, developed to measure in vivo the effect of both platelet aggregatory and anti-aggregatory agents without the interference of an anticoagulant (Smith, 1981), it was found that infusion of collagen (40 µg/kg) caused death in anaesthetised rabbits. A sharp fall in BP occurred some 30 s prior to the fall in platelet count. The ECG showed death was preceeded by myocardial ischemia. Rats and guinea pigs survived the same dose of collagen and, when given at 15 minute intervals, a reproducible fall in platelet count was achieved. Infusion of verapamil (25 µg/kg/min) in rabbits for one hour protected against the lethal effects of collagen and reproducible falls in platelet count were obtained. When this fall in platelet count was compared with the fall in platelet count caused by collagen which proved lethal in untreated rabbits, no significant difference was found ($P > 0.5$, Student's t-test). Preliminary studies showed that TXB_2 production was not inhibited in verapamil treated rabbits.

Okamatsu et al (1981) suggested that since calcium ion was required for constriction of vascular smooth muscle and platelet aggregation, then verapamil may exert its protective action via calcium channel blockade. Lefer et al (1980) found that CTA_2, a thromboxane A_2 analogue, caused death in rabbits by myocardial ischemia as a result of vasoconstriction in the absence of pulmonary or coronary thrombosis. The results of this study show that verapamil does not inhibit intravascular platelet aggregation, nor does it primarily protect by inhibition of TXA_2 synthesis. Its main protective action may be that of coronary vasodilation opposing the vasoconstrictor actions of TXA_2.

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NALOXONE INHIBITS EARLY POST-INFARCTION ARRHYTHMIAS; POSSIBLE DETRIMENTAL ROLE OF β-ENDORPHIN?

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There is good evidence that β-endorphin is released from the pituitary during stress (Rossier et al., 1977) and this led Holaday and Fadan (1978) to consider the possibility that it is also released in circulatory shock states. We have now examined the possibility that β-endorphin may also be released following the stress of an acute myocardial infarction by evaluating whether naloxone influences the ventricular arrhythmias that result from acute coronary artery ligation in rats. Both conscious and anaesthetised rats were used and the techniques have been previously described in detail (Kane et al., 1980).

In the anaesthetised rat studies naloxone was given as a single i.v. injection (2 mg/kg) 15 min before ligation and then as a continuous infusion ($1\mu g\ kg^{-1}\ min^{-1}$). This treatment reduced the number of ventricular ectopic beats in the initial 30 min post-ligation period from 1486±171 beats in the controls to 454±82 beats in the naloxone group ($P<0.05$). The duration of both ventricular tachycardia (VT) and fibrillation (VF) has also reduced (VT 65±9s to 22±9s; VF 27±7s to 6±2s, $P<0.05$). The incidence of VF was reduced from 50% to 10%.

An even more dramatic effect was seen in conscious rats given either 2 or 4 mg/kg naloxone 15 min pre-ligation. Thus VF was reduced from 89% in the controls to 58% and 18% after 2 or 4 mg/kg respectively ($P<0.001$) and the number of rats surviving coronary artery occlusion was increased from 28% (controls) to 67% and 82% respectively ($P<0.05$) 20 min after occlusion and from 28% to 58% and 73% respectively ($P<0.05$) 16h after occlusion. The appearance of arrhythmias after occlusion was delayed in rats given naloxone and the duration of the arrhythmias much reduced. Infarct size (nitrobluetetrazolium method) was unaffected by naloxone.

These results could be extrapolated as implying that endorphin released in stress exacerbates the serious, life-threatening ventricular arrhythmias that arise following coronary occlusion.

We thank Endo Labs. for the naloxone.

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THE ACTIVITY OF A NOVEL AMINOSTEROID CCI 22277 AGAINST ACONITINE AND CORONARY ARTERY LIGATION-INDUCED DYSRHYTHMIAS IN THE RAT

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A safe, effective drug is required for the active or oral prophylactic treatment of patients with ventricular dysrhythmias or at risk of sudden cardiac death (Julian, 1976). New compounds with a 'class I' mechanism of action (Vaughan Williams, 1980) are being investigated, including the aminosteroid Org 6001. The latter is effective against a variety of ventricular dysrhythmias with low toxicity, and favourably influences the metabolic consequences of myocardial ischaemia (Parratt, 1979; Marshall & Winslow, 1981). We have recently identified a novel aminosteroid CCI 22277 (methyl 2β-ethoxy-3α-hydroxy 11 α-(3-methylbutylamino)-5α-androstane-17 β-carboxylate hydrochloride) with anti-dysrhythmic activity by both i.v. and oral routes.

In the present study, CCI 22277 and reference drugs were compared for their effectiveness in delaying the onset of ventricular tachycardia (VT) produced by aconitine in male Charles River CD rats (250-300g). Dysrhythmias were induced by i.v. infusion of aconitine (5μg min^{-1}) to pentobarbitone-anaesthetised artificially-respired rats using a modification of the method of Vargaftig et al (1975). The ED50 (the dose of test compound that increases by 50% the dose of aconitine required to produce VT) was derived from an assay procedure using Org 6001 as standard. ED50 values obtained when treatments were injected i.v. 3 min before infusion of aconitine were: lignocaine 6.7 mg kg^{-1} (95% fiducial limits 2.6-19); disopyramide 2.0 (0.5-4.5); Org 6001 7.4 (3.5-16) CCI 22277 0.38 (0.24-0.60). ED50 values obtained when treatments were administered orally 1h before aconitine infusion were: disopyramide 150 (105-215); Org 6001 46 (31-68); CCI 22277 1.9 (1.3-2.7).

CCI 22277 and disopyramide were also compared for their effectiveness in reducing the incidence and duration of ventricular fibrillation (VF) in open-chest pentobarbitone-anaesthetised male Charles River CD rats (350-550g) during the 20 min period following ligation of the left coronary artery (Clark et al, 1980). CCI 22277 was as effective but more potent than disopyramide when administered orally 1 h before ligation (Table 1).

Table 1 Antidysrhythmic activity of CCI 22277 and disopyramide in coronary-ligated rats.

Oral Treatment (mg kg^{-1})	Incidence of Death	VF	Duration of VF (s) mean ± s.e. mean
Water (5ml kg^{-1})	7/18	5/11	29 ± 16.1
Disopyramide 100	1/11	2/10	13 ± 8.9
" 200	0/10	2/10	15 ± 14.2
" 400	0/11	1/10	4 ± 4.4
CCI 22277 1	3/13	5/10	14 ± 6.8
" 2	2/12	1/10	3 ± 2.8
" 4	1/11	0/10	0 ± 0.0

We conclude that CCI 22277 is very active against ventricular dysrhythmias produced by aconitine and by regional myocardial ischaemia.

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LONG TERM β-BLOCKADE: EFFECTS ON SIZE OF ZONE AT RISK AND CONTRACTILE FUNCTION DURING ISCHAEMIA IN RAT ISOLATED HEARTS

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We have assessed the effects of long-term treatment with two beta-adrenoceptor blocking agents on the amount of tissue vulnerable to injury during acute regional myocardial ischaemia. We have also assessed whether intrinsic sympathomimetic activity (ISA), an ancillary property of certain beta-blocking agents, can modify any action of these compounds on the zone at risk of infarction and overall contractile function during ischaemia and upon reperfusion of the ischaemic zone. Adult, male rats were treated orally for 14-21 days with equi-potent beta-blocking doses of propranolol and acebutolol (4 mg and 23 mg/kg body wt/day respectively). These compounds differ in the possession by acebutolol of ISA together with a certain degree of cardioselectivity. Hearts were excised at the end of the treatment period and perfused as isolated 'working' heart preparations with Krebs-Henseleit buffer, pH 7.4, containing equi-potent concentrations of propranolol or acebutolol (300 ng/ml or 2000 ng/ml respectively). The group treated with the beta-blocker possessing ISA (acebutolol) exhibited higher spontaneous heart rates than either the propranolol treated group or the untreated control group (324 ± 9 (S.E.) beats/min in the acebutolol treated group compared to 287 ± 9 and 232 ± 7 beats/min in the propranolol treated group and untreated control group respectively). After 20 min aerobic perfusion, an identifiable descending coronary artery was ligated and functional performance measured during a 1h ischaemic period. Heart rate and aortic flow rate were both significantly higher in the acebutolol treated group compared to the control group, whereas treatment with propranolol had no effect. At the end of the ischaemic period, 2 ml of 'cardio-green' dye were infused into the coronary vessels via the root of the aorta and the hearts were immediately frozen in liquid nitrogen and 'freeze-dried' overnight. The dye stains only normal tissue and enabled the ischaemic zone to be visually delineated. The freeze-dried hearts were serially sectioned into approximately 8 1mm thick segments and each segment was divided into normal and ischaemic tissue. The mass of ischaemic tissue was measured and expressed as a percentage of the total ventricular weight. In the control group, the zone at risk of infarction amounted to nearly 40% of the total ventricular mass. Long-term administration of acebutolol reduced this by approximately 25% ($P < 0.005$) and propranolol by 15% ($P < 0.05$). However, the high-energy phosphate content within the ischaemic zone was unaffected by drug treatment (ATP and creatine phosphate levels were 4.9 ± 0.5 and 8.3 ± 0.5 μmol/g dry wt respectively in the control group; 4.6 ± 0.6 and 6.9 ± 0.6 μmol/g dry wt in the acebutolol treated group; and 5.4 ± 0.4 and 7.3 ± 0.7 μmol/g dry wt in the propranolol treated group). In a second series of hearts, reperfused for 30 min after the ischaemic period, only the acebutolol treated group showed increased aortic flow rates compared to the untreated group (37.6 ± 2.5 ml/min compared to 24.9 ± 2.6 ml/min, $P < 0.01$).

Therefore these studies indicate that long-term treatment with either acebutolol or propranolol may reduce the size of the ischaemic zone, but neither compound was capable of reducing the degree of tissue injury within that zone. However, only acebutolol (possessing ISA) was able to support pump function during ischaemia and reperfusion. Therefore it is suggested that the possession of ISA by a beta-blocking compound may be advantageous in maintaining pump function during periods of myocardial ischaemia.

EFFECTS OF SELECTIVE AND NON-SELECTIVE β-BLOCKADE ON REPERFUSION-INDUCED VENTRICULAR FIBRILLATION IN RAT ISOLATED PERFUSED HEARTS

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Beta-blockade is known to reduce arrhythmias caused by myocardial ischaemia (Khan et al. 1972; Pearse et al. 1978). However, propranolol has been found to be ineffective against reperfusion-induced rhythm disturbances (Sheridan et al. 1980). Therefore it was the aim of the present investigation to study firstly if beta-blockade per se is ineffective against reperfusion-induced ventricular fibrillation and secondly whether cardioselectivity associated with some beta-blockers has any beneficial effect. Isoprenaline challenge studies were first carried out to determine equi-potent concentrations of all beta-blockers used. Oxprenolol (300 ng/ml) and timolol (50 ng/ml) were used as examples of non-selective beta-blocking agents and metoprolol (300 ng/ml) and acebutolol (2000 ng/ml) as examples of cardio-selective beta-blockers. Adult, male rats were lightly anaesthetized with diethyl ether and the hearts excised and perfused with Krebs-Henseleit buffer, pH 7.4 at 37°C, as 'working' heart preparations. The buffer contained 11.1 mM glucose and 5 x 10^{-6} M adrenaline bi-tartrate. Adrenaline was added in order to replace some of the exogenous sympathetic support absent in isolated heart preparations. Hearts were perfused aerobically for 20 min and a bi-polar epicardial ECG was recorded. After 20 min an identifiable coronary artery was ligated for 15 min and then the ligation was cut thereby reperfusing the ischaemic tissue. The incidence of fibrillation upon reperfusion was divided into hearts that exhibited irreversible fibrillation and hearts that exhibited fibrillation but returned to normal sinus rhythm either spontaneously or upon electrical defibrillation. Figure 1 shows that over 90% of control hearts exhibited spontaneous and irreversible ventricular fibrillation 60-90 s after the onset of reperfusion. Both of the cardioselective drugs greatly reduced the overall incidence of ventricular fibrillation such that 50% or less exhibited fibrillation and in only a small proportion was this designated irreversible. In contrast neither non-selective drug altered the overall incidence of fibrillation and only partially reduced the incidence of irreversible fibrillation.

In conclusion, it appears that beta blockade with metoprolol and acetutolol is much more effective than non selective beta blockade in reducing the incidence of reperfusion induced ventricular fibrillation

Figure 1 Effect of beta-blockade on the incidence of reperfusion-induced ventricular fibrillation in the isolated rat heart (n) indicates number of determinations.

Group	Incidence of Ventricular Fibrillation	
	Spontaneous	Irreversible
Control (34)	93%	65%
Cardioselective beta-blockade		
Metoprolol (11)	27%	18%
Acebutolol (12)	50%	0%
Non-selective beta-blockade		
Oxprenolol (12)	93%	58%
Timolol (13)	93%	46%

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LACK OF ADRENERGIC INFLUENCES ON VENTRICULAR ARRHYTHMIAS IN CORONARY ARTERY LIGATED RAT ISOLATED HEARTS

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It has been suggested that the arrhythmias which occur during the early phase of coronary artery occlusion are initiated by adrenergic mechanisms in the myocardium (Corr et al, 1978). This investigation studied the influence of adrenergic mechanisms on ischaemia-induced ventricular arrhythmias in the isolated rat heart, using a variety of pharmacological tools. The preparation has been described previously (Daugherty & Woodward, 1981).

l-Propranolol reduced the incidence of arrhythmias, although the equipotency of the d-isomer implies that the antiarrhythmic effect is not due to beta-adrenoceptor antagonism. Atenolol did not modify the incidence of arrhythmias. The adrenergic neurone blockers, guanethidine and bretylium, also failed to modify the incidence of arrhythmias.

Both phentolamine and prazosin reduced the incidence of arrhythmias. However, both drugs were equally effective despite prazosin being a more potent alpha-adrenoceptor antagonist (Doxey et al, 1977). Therefore, the effects of these drugs may be independent of alpha-adrenoceptor antagonism.

Table 1. Effects of drugs on premature ventricular contractions (PVC's), ventricular tachycardia (VT) and ventricular fibrillation (VF).

Drug treatment		n	PVC's	%VT	VT duration (sec)	%VF	VF duration (sec)
Control		14	607 ± 93	86	54 ± 11	64	563 ± 134
l-Propranolol	(1uM)	10	213 ± 39*	90	16 ± 4*	20	13
d-Propranolol	(1uM)	9	193 ± 44*	67	14 ± 3*	22	52
Atenolol	(1uM)	10	636 ± 110	100	52 ± 9	80	541 ± 168
Phentolamine	(1uM)	10	747 ± 127	100	65 ± 10	60	186 ± 45
	(10uM)	12	247 ± 50*	33	8 ± 3	0*	
Prazosin	(1uM)	10	525 ± 144	100	51 ± 14	50	243 ± 150
	(10uM)	9	90 ± 23*	67	6 ± 2	11	5
Prazosin solvent		10	697 ± 79	100	53 ± 9	70	333 ± 146
Guanethidine	(1uM)	11	484 ± 72	100	59 ± 11	82	379 ± 146
Bretylium	(1uM)	11	460 ± 54	100	53 ± 8	72	478 ± 163

* $P < 0.01$ compared to controls

A.Daugherty is a British Heart Foundation Research Fellow. Gifts of atenolol, propranolol (ICI) and prazosin (Pfizer) are gratefully acknowledged.

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DIETARY LIPIDS AS MODULATORS OF CARDIAC RESPONSES TO INOTROPIC DRUGS

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Several recent studies have demonstrated that the fatty acid content of cardiac cell membranes can be changed by manipulation of the saturated:polyunsaturated fatty acid content of the diet (Gudbjarnason & Oskadottir, 1977; Innes & Clandinin, 1981). Changes in left ventricular work capacity have also been reported in similarly fed animals (De Deckere & Ten Hoor, 1979, 1980). We have examined the effects of dietary lipids on the response of atria and papillary muscles from rats fed a fat supplemented diet and compared the fatty acid composition of cardiac membrane phospholipids resulting from this dietary regime.

Two groups of male Hooded Wistar rats weighing approximately 400 g were fed, for a total of nine weeks, a diet comprising conventional rat pellets soaked in either sunflower seed oil or sheep kidney fat. The polyunsaturated fatty acid content of the lipids was 80.3% and 39% respectively, and the lipids constituted a total of 13.1% and 17.5% respectively of total diet by weight. The rats were killed by decapitation and the tissues suspended in Krebs-Henseleit solution at 37°C in a conventional tissue bath, and stimulated at 1 Hz. The length-tension relationship of the cardiac tissues was not different between the two dietary groups. The resting state twitch tension of the papillary muscles from the kidney fat fed group was significantly greater than that from the seed oil fed group (4.56 ± 0.34 mg/mg tissue vs. 2.77 ± 0.29 mg/mg tissue, $P < 0.01$).

Concentration effect curves were obtained to calcium, isoprenaline and verapamil. While the ED_{50} values were the same for tissues from the two dietary groups, the magnitude of the response of the papillary muscles from the kidney fat fed group was much greater than that from the seed oil fed group ($P < 0.01$). On the other hand the left atria from the seed oil fed group manifested a greater response to increases in the bathing solution calcium concentration or to isoprenaline ($P < 0.05$). The response to verapamil was not different in either atria or papillaries. Analysis of total membrane phospholipid fatty acids confirmed that diet had induced changes in the proportion of unsaturated fatty acids, but not saturated fatty acids, present. Animals fed kidney fat had lower levels of 18:2, but substantially greater percentages of 22:6 than animals fed seed oil ($P < 0.001$). In addition ventricles had greater percentages ($P < 0.001$) of both of these polyunsaturated acids than the corresponding atria in each dietary group.

These results may indicate a role of membranes in modulating the magnitudes of physiological and pharmacological responses, and that this effect is sensitive to the quality of dietary lipid in omnivores. The mechanism whereby the fatty acid composition of membranes affects the responses requires elucidation, but at present, biophysical properties, calcium binding and prostaglandin synthesis are all contenders.

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EFFECTS OF AMRINONE AND PHOSPHODIESTERASE INHIBITORS ON TENSION RESPONSES AND CYCLIC NUCLEOTIDE LEVELS IN RABBIT PAPILLARY MUSCLE

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We have previously reported (Rodger & Shahid, 1981) that cyclic AMP in rabbit myocardium is a determinant of isoprenaline-induced positive inotropism. However, the ratio of cyclic 3',5'-adenosine monophosphate to cyclic 3',5'-guanosine monophosphate ([cyclic AMP]/[cyclic GMP]) does not correlate well with changes in mammalian cardiac muscle contractility. We have now extended our study to include the effects of amrinone and the phosphodiesterase inhibitors UK31-557-10 and 3-isobutyl-1-methylxanthine (IBMX) on cyclic nucleotide levels and developed tension in the rabbit myocardium.

Papillary muscles from the right ventricle of male white rabbits were suspended in Krebs-Henseliet solution at 32°C and their electrically evoked contractions recorded by conventional methods. Cyclic AMP and cyclic GMP levels were measured using standard assay kits (Radiochemical Centre, Amersham).

The results are summarised in Table 1.

Table 1 Effects of Amrinone, IBMX and UK31-557-10 on tension responses and cyclic nucleotide levels in rabbit papillary muscles. ± S.E. mean (n = 3-5)

Drug	Concentration (μg/ml)	Tension (mg)	c-AMP (pmol/mg)	c-GMP (pmol/mg)	c-AMP/c-GMP
Amrinone	solvent control	303 ± 60	0.74 ± 0.06	0.039 ± 0.009	19
	100	314 ± 90	0.77 ± 0.08	0.036 ± 0.007	21.4
	500	882 ± 206	1.22 ± 0.09	0.079 ± 0.006	15.4
	1000	1250 ± 109	1.47 ± 0.14	0.102 ± 0.010	14.4
		(r = 0.99)	(r = 0.98)	(r = 0.98)	
IBMX	solvent control	317 ± 56	0.63 ± 0.09	0.055 ± 0.008	11.5
	10	517 ± 103	1.11 ± 0.10	0.082 ± 0.017	13.5
	50	779 ± 208	1.42 ± 0.17	0.117 ± 0.018	12.1
	100	1067 ± 152	2.36 ± 0.20	0.135 ± 0.021	17.5
		(r = 0.98)	(r = 0.97)	(r = 0.95)	
UK31-557-10	100	565 ± 62	1.42 ± 0.20	0.049 ± 0.009	28.9
	500	954 ± 141	2.03 ± 0.29	0.067 ± 0.010	30.3
	1000	1200 ± 110	2.47 ± 0.31	0.073 ± 0.008	33.8
		(r = 0.98)	(r = 0.98)	(r = 0.94)	

All three drugs induced concentration-related increases in developed tension and in the levels of both cyclic AMP and cyclic GMP. In each case the ratio of cyclic AMP to cyclic GMP shows no clear trend. The levels of both cyclic AMP and cyclic GMP in the tissues, and the associated developed tensions, correlated well with the concentrations of drugs used. The correlation coefficients for each drug are given at the bottom of the appropriate columns in Table 1.

The results show that in the rabbit ventricular papillary muscles the mechanism underlying amrinone-induced positive inotropism may involve phosphodiesterase inhibition with consequent elevation of cyclic nucleotides. These data, however, are in contrast to the findings of Alousi et al (1979) in cat atria. The results using IBMX and UK31-557-10 are consistent with their known effects on phosphodiesterase. Furthermore, these data support our previous claim that the cyclic AMP/cyclic GMP ratio is of little significance in determining positive inotropism in rabbit cardiac muscle.

Our thanks to Sterling-Winthrop and Pfizer for gifts of Amrinone and UK31-557-10.

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HEMICHOLINIUM-3 BLOCKS CURRENTS THROUGH SINGLE ACETYLCHOLINE-RECEPTOR CHANNELS

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Analysis of the action of hemicholinium-3 (HC-3) on endplate currents recorded from frog cutaneous pectoris muscles suggest that HC-3 blocks the acetylcholine (ACh) activated ionic channel (Henderson et al, 1981). By use of the patch clamp technique (Neher and Steinbach 1978) we have now examined the effects of HC-3 on single ACh-activated channels recorded from chick cultured myotubes. Myotubes were prepared by the method of Fischbach (1972) and used 6-14 days after plating. All recordings were obtained from myotubes at resting membrane potential (-45mV to -60 mV) and at room temperature (21-23°C).

In the presence of HC-3 (25-50 μM) single ACh channel events occurred in bursts, similar to those observed for the local anaesthetic QX-314 (Neher and Steinbach 1978). The measured mean channel open time $\bar{t}_o$ was decreased in a concentration dependent manner by HC-3 from a control value of 6.6 ± 1.49 ms (mean ± S.D., n = 13 cells) to 3.01 ± 0.43 ms (n = 7 cells) in the presence of 25 μM HC-3 and to 1.7 ± 0.15 ms (n = 7 cells) in the presence of 50 μM HC-3. The relationship between $1/\bar{t}_o$ and HC-3 concentration was found to be linear suggesting that the blocking of the ACh channel by HC-3 is a bimolecular reaction.

The data were further analysed using a sequential channel blocking model (Neher and Steinbach 1978). From this model the forward rate constant (G) for HC-3 binding to the ACh channel was 0.86×10^7 s^{-1} M^{-1}, the backward rate constant (F) for HC-3 unbinding from the ACh channel was 20 s^{-1} and the equilibrium dissociation constant (F/G) was 2.3×10^{-6}M. In comparison with the effects of the local anaesthetic QX-222 on the ACh channel (Neher and Steinbach 1978) HC-3 had a similar blocking rate but a much slower unblocking rate suggesting that HC-3 is a more effective blocker of the ACh channel than QX-222.

Preliminary experiments with HC-3 congeners suggest that chemical modification of the HC-3 molecule will allow a better understanding of the kinetics of ACh-activated ionic channels.

This work was supported by a Wellcome Research Travel Grant to J.J.L. and an M.R.C. Training Fellowship to M.L.J.A.

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TETRAETHYLAMMONIUM-METHOHEXITONE INTERACTIONS AT THE CHICK MUSCLE: SUCROSE-GAP AND MOVING FLUID ELECTRODE TECHNIQUE

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Using the sucrose-gap apparatus, to record changes in resting membrane potential induced by drug action, in the chick biventer cervicis nerve-muscle preparation, dose-response curves and ED50s for acetylcholine (ACh) were determined. Mean ED50 for ACh depolarization in the control krebs solution was 0.89 ± 0.59 mM(n=6). Maximum depolarization of4.9±0.18mV(n=6) was produced by 5.5 mM ACh. In the presence of methohexitone(88 µM) the ED50 was 24±0.09mM. Ach(5.5 mM) produced a mean depolarization of 1.3±0.08mV(n=6).

Tetraethylammonium (TEA) produced small depolarizations of the resting membrane potential in the chick muscle. The mean ED50 was 5.8±0.09 mM (n=6). Maximum depolarization of 0.63±0.03mV(n=6) was produced by 24 mM TEA in the control krebs solution. In the presence of methohexitone(88 µM) the mean ED50 was 1.78±0.15mM (n=6). Maximum depolarization was 0.76±0.03mV (n=6)with 9.5mM TEA.

The difference between the ED50s for ACh depolarizations in the control kerbs and in methohexitone was significant at the 1% level, as were the corresponding values for TEA.Tubocurarine(12.7 µM)reduced the ACh and the TEA-induced depolarizations in the control krebs solution, while eserine (0.77 µM)greatly potentiated ACh-induced depolarizations and only slightly potentiated the TEA-induced depolarizations.After treatment with prolonged repetitive indirect stimulation in the continued presence of 26µM hemicholinium,to deplete ACh stores,TEA(24.0mM) still produced small depolarizations(mean of 0.48±0.03mV,n=4).

The present results confirm those previously obtained by Bell & Wali(1981) with moving fluid electrode technique(Fatt,1950).Using the latter technique,the depolarizations induced by TEA(24mM)were greatly reduced in the presence of the local anaesthetic lidocaine(xylocaine)(0.93mM).Mean ED50s for TEA depolarizations in the control krebs and in lidocaine were: 1.1±0.08mM and 16.0±0.19mM, n=6,respectively.On the other hand, the contracture responses induced by TEA (24.0mM)were greatly potentiated in lidocaine (0.93mM).Mean ED25s for TEA contractures in the control krebs solution and in lidocaine were: 8.2±0.32mM and 0.88±0.14mM,n=6,respectively.The difference between the ED25s for TEA contractures in the control krebs and in liodcaine was significant at the 1% level. Similar results were obtained by Elliott(1981)in the chick biventer cervicis muscle.

TEA may act presynaptically by releasing ACh(Collier & Exley,1963),and postsynaptically either by combining with nicotinic receptor sites or by blocking potassium ion channels(Kirkpatrick,1975).TEA may act by releasing Ca^{2+} ions from the sarcoplasmic reticulum(Beaulieu & Frank,1967), which may explain why TEA produced a proportionately larger contracture tension than ACh for the same level of depolarization(Bell & Wali,1981).

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THE EFFECTS OF TRIMETAPHAN ON TETANIC FADE AND ON ENDPLATE ION CHANNELS AT THE RAT NEUROMUSCULAR JUNCTION

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The ganglion-blocking agent trimetaphan has been shown to produce a neuromuscular block that is similar in characteristics to that produced by non-depolarizing muscle relaxants (Dale & Schroeder, 1976; Nakamura et al, 1980). It has been suggested that tetanic fade produced by non-depolarizing relaxants may be due to an interaction of the drugs with prejunctional ganglion-like nicotinic receptors (Bowman, 1980), but it is also possible that postjunctional endplate ion channel block may contribute to the fade. The effects of trimetaphan have now been studied on the rat isolated phrenic nerve-hemidiaphragm preparation in an attempt to assess the possible contribution of ion channel blockade to the tetanic fade produced.

Tension studies showed that trimetaphan (2×10^{-4}M) produced complete tetanic fade (50 Hz for 1.9 s) but no reduction in single twitch tension (0.1 Hz). The concentrations producing 50% tetanic fade and 50% twitch block were $1.66 \pm 0.08 \times 10^{-4}$M and $4.09 \pm 0.04 \times 10^{-4}$M respectively (mean ± s.e., n = 4). Electrophysiological recording techniques showed that trimetaphan ($0.25 - 2 \times 10^{-4}$M) produced a concentration dependent reduction in the time constant of decay (τ) of extracellularly recorded miniature endplate potentials in intact hemidiaphragm preparations. Voltage clamp studies showed that these low concentrations of trimetaphan produced a concentration and voltage dependent reduction of τ of both endplate currents (epcs) recorded from cut muscle fibres and miniature endplate currents (mepcs) recorded in tetrodotoxin treated fibres. Trimetaphan (5×10^{-5}M), reduced τ mepc at -60 mV from 1.45 ± 0.13 ms to 1.01 ± 0.15 ms and τ mepc at -100 mV from 2.46 ± 0.3 ms to 1.1 ± 0.24 ms (n = 3). Thus trimetaphan produced a greater shortening of τ at hyperpolarized endplates. The voltage-dependence of τ mepc conformed to the relationship $\tau(V_m) = \tau(0) \exp(-V_m/H)$ where H is the change in membrane potential required to produce an e-fold change in τ. In trimetaphan (5×10^{-5}M), H increased from 95.7 ± 16.9 mV to 295 ± 133 mV (n = 3), indicating a reduction in the voltage sensitivity of τ. These results are consistent with trimetaphan blocking acetylcholine-activated ion channels. In the present study trimetaphan (10^{-4}M) also produced a voltage-dependent rundown of trains of epcs (50 Hz for 0.4 s). We conclude that trimetaphan possesses an endplate ion channel blocking action which contributes to its neuromuscular blocking action and ability to cause tetanic fade.

This work was supported by grants from the Medical Research Council and the Smith Kline & French Foundation. AJG holds a Science Research Council CASE award.

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BILE-INDUCED COLONIC MOTILITY INCREASE MAY BE MEDIATED BY ACTIVATION OF A KALLIKREIN-LIKE ENZYME

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The presence of bile salts in the colonic lumen stimulates a marked motor response (Kirwan et al, 1973). We have previously reported that bile salts in the rat duodenum release and activate kallikrein-like activity (KLA) forming spasmogens (Frankish and Zeitlin, 1977). KLA is also present in the rat colon (Frankish and Zeitlin, 1980). We have therefore examined the role of KLA in the action of bile on rat colonic motility.

Glandular KLA was assayed by incubation with the glandular kallikrein-selective chromogenic substrate (Claeson et al, 1978) H.D. Val-Leu-Arg. pNA (pH 8.2, 37°C), in the presence of excess soyabean trypsin inhibitor (250$\mu g.ml^{-1}$) to inhibit trypsin, plasmin and plasma kallikrein.

Fasted male wistar rats (200-350g) were anaesthetised with urethane (1.5$g.kg^{-1}$). In one group the large bowel was occluded, a cannula was inserted just distal to the caecum and a drainage cannula inserted into the rectum. Intraluminal perfusion of the colon with Kreb's solution containing sodium chenodeoxycholate (3.86mM) produced a 275 (SEM = 40)% increase in mean perfusate concentration of glandular KLA compared with a bile-free perfusion (U-test $P < 0.01$, $n = 8$). The release occurred concomitant with a 53% reduction in the tissue concentration of glandular KLA (U-test, $P < 0.01$, $n \geq 6$). The glandular KLA was totally inhibited by aprotinin (Trasylol (R), Bayer A.G., 2000 $KIU.ml^{-1}$).

In a second group of animals a small balloon (3.5mm diameter) was inserted per rectum 6cm into the proximally occluded colon and attached to a Statham pressure transducer. Colonic motility was monitored for a 30 min control period using a Grass polygraph. Saline (2.5ml), alone or containing drugs, was then instilled into the colon via a fine cannula also inserted per rectum and normally kept closed, and motility was monitored for 30 min. Percentage motility (PM) (Flynn et al, 1979) was calculated as percentage of time during which pressure waves were present during the 15 min of maximum activity pre-and post-saline instillation. Mean basal PM was 0% and was not increased by instillation of saline alone ($n = 5$). On instillation of saline containing sodium chenodeoxycholate (30mM), PM increased in every case to a mean of 50.9 (SEM = 8.2)%.

A further 7 animals received a bolus injection of aprotinin (100,000 $KIU.kg^{-1}$) 5 min prior to intraluminal instillation of saline containing chenodeoxycholate (30mM) and aprotinin (40,000 $KIU.ml^{-1}$), in the presence of an intra-arterial infusion of aprotinin (100,000 $KIU.kg^{-1}.h^{-1}$). In these animals the mean PM rose only to 4.0 (SEM = 1.6)%. Use of intravascular aprotinin alone did not consistently suppress the motility response.

These animals were killed 1.5h after the instillation of saline and it was noteworthy that after bile instillation there was a bloody discharge and on macroscopic examination the colon looked oedematous and congested. After aprotinin treatment there was no bloody discharge after bile instillation and on macroscopic examination the colon was not congested nor oedematous.

H.A.R. Al-Dhahir was supported by a scholarship from Baghdad University. We are grateful to Professor G.L. Haberland of Bayer AG for a kind gift of Trasylol (R).

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SUBCELLULAR LOCALISATION OF SMOOTH MUSCLE INHIBITORY FACTOR ISOLATED FROM THE BOVINE RETRACTOR PENIS MUSCLE

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The retractor penis and anococcygeus muscles possess a powerful inhibitory innervation whose neurotransmitter is unknown. Following on the work of Ambache et al (1975) we have isolated, semipurified and determined some of the properties of a novel smooth muscle inhibitory material isolated from the bovine retractor penis (BRP) (Gillespie & Martin, 1980; Gillespie et al, 1981). If this inhibitory factor is the neurotransmitter then it is likely to be localised within membrane-bound vesicles which, after homogenisation, should sediment with ultracentrifugation. This paper reports the results of such an investigation into the subcellular distribution of the inhibitory factor in the BRP.

Smooth muscle is a difficult tissue to homogenise because of its high collagen content. We used two methods of homogenisation: (1) Passage of frozen tissue four times through an LKB X-Press under a pressure of 2,000 kg/cm^2; (2) Frozen tissue sectioned at 50 μ then incubated in Krebs saline at 37°C containing collagenase 2 mg/ml for 75 min followed by homogenisation in a Tri-R homogeniser. The homogenate, whichever method was used, was then centrifuged at 4°C, first at 1,000 *g* for 10 min to give a P1 pellet, the S1 supernatant centrifuged at 15,000 *g* for 20 min to give a P2 pellet and the S2 supernatant finally centrifuged at 100,000 *g* for one hour to give a P3 pellet and a final supernatant, S3. The weight of each pellet and the volume of the final supernatant were measured. The pellets were extracted with methanol and these methanol extracts and the S3 fraction in phosphate buffer applied to separate anion exchange columns, washed with distilled water and eluted with 250 mM NaCl solution. The inhibitory activity of each was then assayed on the BRP and compared with the activity extracted directly by methanol from a separate control sample of muscle from the same animal.

The results were similar whatever the method of homogenisation. The total inhibitory activity recovered from the subcellular fractions averaged 81% of the control. The distribution of this inhibitory activity showed little in the large P1 pellet, more in P2 but most in P3. When inhibitory activity was expressed per mg of pellet, P3 was approximately seven times more concentrated than either the original tissue or the P2 fraction and fifty times more than P1.

These results suggest at least part of the inhibitory factor is present in a particulate fraction which, like other transmitter vesicles, sediments with the P3 fraction and supports the suggestion that it may be the inhibitory transmitter.

J.C.H. is an MRC Scholar. Our thanks are due to the Medical Research Funds of Glasgow University.

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THE ELECTRICAL AND MECHANICAL RESPONSES OF THE RABBIT AND RAT ANOCOCCYGEUS MUSCLES TO FIELD STIMULATION AND TO AN INHIBITORY EXTRACT

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An extract with smooth muscle inhibitory activity resembling that of non-adrenergic non-cholinergic (NANC) nerve stimulation was isolated recently (Bowman et al, 1979). The electrical basis of the inhibition of smooth muscle tone produced by stimulation of NANC nerves varies from a hyperpolarization in the rabbit anococcygeus (Creed & Gillespie, 1976) to either an inhibition of the oscillations or no consistent change in membrane potential in the rat anococcygeus (Creed et al, 1975). To determine whether the inhibitory material also differentiated similarly between these two muscles - a prerequisite for its having a transmitter role - its electrical properties have been compared with those of nerve stimulation in the anococcygeus of the rat and rabbit using mechanical and intracellular electrical techniques (Blakeley et al, 1979). The extract was cleaned of adenine nucleotides and activated (Gillespie et al, 1981); cleaned unactivated extract served as control. The heat labile extract at 0-5°C was added directly to the bath.

In the rat, in approximately 25% cells, both field stimulation (1-10Hz) and extract inhibited the frequency and amplitude of oscillations in membrane potential and relaxed tone induced by guanethidine ($1\text{-}3\times10^{-5}$M). In the majority of cells however, no apparent electrical change accompanied the decrease in guanethidine-induced tone produced by either field stimulation or extract.

In the rabbit field stimulation (1-10Hz) and extract invariably reduced guanethidine-induced tone. The electrical response varied. In one group of cells the electrical response to both field stimulation and extract was either an inhibition of oscillations or no significant change in membrane potential. In the case of extract, inhibition of oscillations was accompanied by a hyperpolarization of the membrane potential. In another group of cells in which guanethidine produced no oscillations, field stimulation and less frequently extract hyperpolarized the membrane potential. Apamine (100nM) inhibited the electrical and mechanical response to extract but not to field stimulation. After some 10 min the mechanical and electrical inhibitory responses were partially restored in the presence of apamine.

These results show that in both species the extract and field stimulation produce apparently similar effects - a reduction in tone accompanied either by no electrical change or by an inhibition of oscillations and/or a hyperpolarization of the membrane potential. The results with apamine suggest however that the inhibitory effect of field stimulation and extract may be produced by different mechanisms.

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THE MECHANISM OF ACTION OF AZATHIOPRINE ON THE MIXED LYMPHOCYTE REACTION (MLR)

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The inhibition of the MLR by azathioprine was previously reported (Vas & Lowenstein 1965; Skopiniska et al, 1968; Bach & Bach, 1972). The mechanism of the immunosuppressive effect of azathioprine is still unclear. We utilized the human unidirectional MLR to study the problem. Blood samples were obtained from normal healthy volunteers. Lymphocytes were separated (Boyum, 1976) and cultured in microtitre plates (Parker & Lukes, 1971). Our results were in agreement with Bach & Bach (1972) in that azathioprine almost completely inhibited the MLR at 36 μM. Low concentrations (0.036-3.6 μM) caused inconsistent effects. However, Bach & Bach (1972) reported that azathioprine had no effect when added at or after 48h, whilst our results showed a significant ($p<0.005$) decrease of 27.9% when azathioprine addition was delayed 48h. The azathioprine metabolite 6-MP inhibited the MLR significantly at and above 36 μM. However, at an equimolar concentration (36 μM), azathioprine was more effective than 6-MP by 33.7% ($p<0.02$). Addition of erythrocytes or glutathione to split azathioprine to 6-MP could not abolish the difference in activity between both drugs. Reversal of the inhibitory effect of azathioprine or 6-MP was achieved by washing the cells with culture medium (Table 1).

Table 1 Effect of washing on the inhibitory effect of azathioprine or 6-MP in the MLR at different intervals

Time (hrs)	n	% OF REVERSAL OF INHIBITION ± SEM AZT (36 μM)	6-MP (36 μM)
2	6	111.7 ± 14.5 ($p<0.001$)	138.1 ± 26.4 ($p<0.005$)
6	6	87.7 ± 18.6 ($p<0.005$)	101.1 ± 8.0 ($p<0.001$)
12	9	47.2 ± 6.4 ($p<0.001$)	104.3 ± 19.0 ($p<0.001$)
24	7	42.7 ± 5.9 ($p<0.001$)	77.5 ± 20.0 ($p<0.005$)

Restimulation of azathioprine (36 μM) treated MLR culture after washing at 24h, whether by the same or different antigenic stimulating cells, resulted in no significant difference in the reversal of inhibition. Washing the azathioprine (36 μM) treated responding or stimulating cells, cultured separately prior to the MLR, after 24h resulted in a significant ($p<0.001$) reversal of the inhibitory effect by 55.3 and 60.5% respectively. Addition of 5-amino-4-imidazolecarboxamide (AIC) as a de novo purine synthesis precursor did not influence the inhibitory effect of azathioprine (36 μM) but reversed the inhibitory effect of 6-MP (100 μM) by 15.4, 41.7, 39.7 and 46.5% at 100, 300, 500 and 1,000 μM of AIC respectively. Addition of erythrocytes and AIC (500 μM) reversed the inhibitory effect of azathioprine by 5.7% ($p<0.05$). The main conclusions that can be drawn from our study are:

1. Effect of azathioprine is greater than that of an equimolar concentration of 6-MP.
2. Effects of azathioprine and 6-MP on the MLR are reversible showing that the "clonal deletion" hypothesis may not be true.
3. Azathioprine affects stimulating and responding cells to the same extent.
4. The reversal and irreversal of 6-MP and azathioprine inhibitory effect respectively by AIC indicates a difference in the mechanism of action between azathioprine and 6-MP on de novo purine synthesis.

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THE EFFECT OF BETAMETHASONE ON ALTERED RESPONSIVENESS OF ISOLATED INTESTINE FROM RATS INFECTED WITH *Nippostrongylus brasiliensis*

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Immune expulsion of the nematode *Nippostrongylus brasiliensis* from rat intestine changes responsiveness to agonists of isolated gut (Farmer,1981). There occurs a specific subsensitivity to 5-hydroxytryptamine (5-HT), possibly due to elevated intestinal levels of this substance during infection (Murray et al. 1971), and a non-specific increase in maximum responses to 5-HT and acetylcholine (ACh). The rate of worm expulsion (Murray et al.1971) and the increased maximum responses of the gut are maximal at day 14 of infection but it is unknown whether the two processes are linked. Treatment of infected rats with betamethasone inhibits worm expulsion (Ogilvie,1965), and the present study set out to examine whether this drug also prevents changes in intestinal responsiveness.

Male hooded rats (200-250g) were infected with *N. brasiliensis* larvae (5000/rat, injected s.c.) and on days 6, 8, 10 and 12 post-infection injected with betamethasone (1.5mg/kg s.c.). A group of uninfected rats also received betamethasone on corresponding days. Rats were killed on day 14 of the experiment and segments of small intestine suspended in oxygenated Krebs' solution (37°C), and responses recorded isometrically. Dose response curves to ACh and 5-HT were obtained, and mean pD_2 values and maximum responses for both groups were compared with control and infected means using Students' t-test.

Betamethasone did not affect pD_2 values or maximum responses of gut from uninfected rats (Table 1). Neither did it prevent the fall in pD_2 to 5-HT during infection, suggesting that this is not involved in worm expulsion. However, betamethasone markedly reduced the increase in maximum responses (Table 1), which suggests that this phenomenon may play a part in expulsion. It is likely that betamethasone inhibits worm expulsion by virtue of its immunosuppressive activity (Parrillo & Fauchi, 1979). As betamethasone also inhibited the increase in maximum responses of the gut, it is conceivable that this phenomenon is a consequence of the immune response to, rather than a direct effect of, the parasites.

Table 1: The effect of betamethasone treatment on *N. brasiliensis* infection-induced changes in responsiveness of isolated rat intestine

Group	Treatment	ACh pD_2	ACh Maximum (g)	5-HT pD_2	5-HT Maximum (g)
I	None	6.03 ± 0.05	1.80 ± 0.17	7.28 ± 0.10	1.81 ± 0.14
II	Day 14 of infection	6.14 ± 0.04	10.23 ± 1.16***	6.81 ± 0.05**	5.26 ± 0.64***
III	Betamethasone	6.14 ± 0.07	1.52 ± 0.25†††	7.09 ± 0.09	1.66 ± 0.26†††
IV	Day 14 of infection + betamethasone	6.50 ± 0.07***†††	2.98 ± 0.34***†††	6.84 ± 0.04**	3.56 ± 0.34†***

Each value is expressed as mean ± s.e. mean, of at least 6 observations.
Groups II, III & IV compared to group I: **, $P < 0.01$; ***, $P < 0.001$
Groups III & IV compared to group II: †, $P < 0.05$; ††, $P < 0.01$; †††, $P < 0.001$.

The author gratefully acknowledges financial support from The Wellcome Trust.

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CHARACTERISATION OF THE SPECIFIC GLUCOCORTICOID RECEPTOR PROTEIN IN RAT SKIN

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Glucocorticoids are used in the treatment of skin diseases. They initially interact with a specific cytosolic receptor protein, hormone action is then mediated by the receptor-glucocorticoid complex (Munck & Leung, 1977). The present work investigated the binding of glucocorticoids to a specific cytosolic receptor protein in rat skin.

Dorsal skin from prepubertal male Wistar rats (50-70g) was pulverised in a mortar cooled in liquid nitrogen and homogenised with a Polytron. The homogenate was centrifuged at 100,000 x g for 1h to yield a cytosol, or high speed supernatant, fraction. Cytosol was immediately incubated with 10-15nM [^{3}H]-triamcinolone acetonide in the presence or absence of 1μM non-radioactive triamcinolone acetonide (to assess non-specific binding). The binding of glucocorticoid to the receptor was quantified by charcoal-dextran adsorption and Sephadex G-25 chromatography. The binding of [^{3}H]-triamcinolone acetonide to the 'free' (not bound to endogenous corticosterone) and 'occupied' (bound to endogenous corticosterone) forms of the cytosolic receptor was complete after incubation for 20h at 4°C. High affinity (K_d=0.65 ± 0.11nM s.e. mean n=7) low capacity (400-600fmol/mg protein) binding of [^{3}H]-triamcinolone acetonide was detected. Fluorinated corticosteroids bound to the receptor with a greater affinity than did natural corticosteroids. Progesterone also bound to the receptor, but androgenic and oestrogenic steroids did not bind at all. Heating of the cytosol at 40°C, incubation with proteolytic enzymes and omission of dithiothreitol and molybdate from the homogenisation buffer all resulted in receptor degradation. Adrenalectomising the rats increased the assayable receptor concentration from 286.8 ± 7.7fmol/mg protein n=19 in intact animals to 336.8 ± 32.3fmol/mg protein n=21 in animals one day after adrenalectomy. Maximum levels of receptors, 516.1 ± 18.4fmol/mg protein n=23, were found five days after adrenalectomy. The receptor concentration thereafter declined to the level of intact rats. When adrenalectomised rats were injected i.p. with corticosterone and killed 30 min later there was a dose-dependent (2.5-1280μg) decrease in the assayable cytosolic receptor concentration. A similar decrease was observed after injecting triamcinolone acetonide, however, progesterone, oestradiol 17β and testosterone were without effect at all doses tested. These results are consistent with in vivo depletion of the cytosol receptor following corticosteroid injection and further experiments are now in progress to measure the glucocorticoid receptor in rat skin nuclei to determine whether the in vivo depletion of cytosol receptor is accompanied by an increase in the levels of the nuclear receptor.

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AMINE OXIDASES IN HUMAN AMNIOTIC FLUID AND HUMAN LUNG

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Amine oxidase activities have been found distributed widely in human tissues (Lewinsohn et al, 1980) and amniotic fluid (Lewinsohn & Sandler, 1982). In this study an attempt has been made to identify and trace the source of these enzymes, particularly the clorgyline-resistant activity towards benzylamine (Bz), benzylamine oxidase (BzAO), in amniotic fluid (AF) and to differentiate it from histaminase (Tufvesson, 1978) and polyamine oxidase (Illei & Morgan, 1980).

Enzymic activity was measured by a radiochemical method (Lewinsohn et al, 1980). Final concentrations of ^{14}C-Bz were from 10µM to 5mM in the kinetic studies, and 250µM to 1mM in all others; and of ^{3}H-5-hydroxytryptamine (5-HT), from 25µM to 1.6mM and 1mM, respectively. Bz assays were carried out at pH 9.0, all others at pH 7.8, in the presence of oxygen.

A dialysable inhibitor was found in AF and in foetal but not adult lung. The effect of dilution on the specific activity of Bz deamination in AF was proportional up to 75% dilution; dialysis caused an increase of 75% in specific activity. In foetal lung, dialysis produced a 30% increase in specific activity of Bz oxidation; sensitivity towards clorgyline, phenelzine or NH_4Cl was not affected. Neither dialysis nor dilution caused any change in specific activity of foetal liver, child's aorta, or adult lung.

Dialysed AF gave biphasic Lineweaver-Burk plots for Bz oxidation without inhibitor yielding approximate K_m values of 32 (± 8.5) and 198 (± 78) µM (means and s.e. of 5 individual assays). 10^{-3}M clorgyline, which inhibits MAO B, reduced the number of K_m components to one, 23 (± 5) µM (mean and s.e., 3 individual assays). One dialysed AF in the presence of clorgyline, at pH 9.0, gave K_m = 17µM; at pH 7.8, in the presence and absence of clorgyline, the K_m was 770 and 630 µM, respectively. Where K_m plots showed more than one component, none of the following compounds had any effect: deprenyl 10^{-7}, phenelzine 10^{-8}, NH_4Cl 10^{-4}, spermine 4 x 10^{-4}, spermidine 2 x 10^{-3}, putrescine 2 x 10^{-4} or 3 x 10^{-5}M. Values from foetal, child's and adult lungs gave single-component plots for Bz oxidation without inhibitor, with K_m values ranging from 26 to 47µM; mean = 37.7 (± 2.7) µM (n = 10). 5-HT oxidation in human lung (n = 6) gave a K_m of 201.1 (± 24) µM. No age-related or other differences were found. No activity of AF was found against 5-HT, β-PEA or tyramine. Six out of 16 AF samples were sensitive towards 10^{-3}M clorgyline or 10^{-7}M deprenyl, confirming previous studies (Lewinsohn & Sandler, 1982). Except for 3 immature specimens, all other human lungs (n = 14) were sensitive to 10^{-3}M clorgyline and gave monophasic plots showing clorgyline resistance of 10-20%. Semicarbazide (10^{-3}M) totally inhibited Bz oxidation in AF, while in lung about 9 to 16% of control activity remained. 10^{-3}M KCN reduced Bz oxidation to 28% of control in AF, and 69% of control in lung. High concentrations of histamine, spermine and putrescine had an inhibitory effect on Bz deamination in AF, but not in lung.

These results suggest that BzAO in human AF and lung differs from histaminase and polyamine oxidase. It is possible that the foetal lung is the source of BzAO and MAO B in AF; further studies to test this hypothesis are under way.

This work was supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brasilia, Brazil, and The Wellcome Trust, London, U.K.

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AH22216, A NEW LONG ACTING HISTAMINE H_2-RECEPTOR ANTAGONIST

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AH22216 (1-methyl-N^5-[3-[3-(1-piperidinyl methyl)phenoxy]propyl]-1H-1,2,4-triazole-3,5-diamine) is a new H_2-receptor antagonist which differs in structure and pharmacological profile from cimetidine and ranitidine (Daly et al, 1980; 1981), the two H_2-blocking drugs now available clinically.

Although AH22216 (0.03 - 0.10 μg/ml) was a potent inhibitor of the positive chronotropic action of histamine on the guinea-pig isolated atrium, it did not fulfil the criteria for competitive antagonism on this preparation. Using a 45 min contact time, the histamine concentration-response curves were displaced in a dose-related but non-parallel fashion and their maxima were depressed. Furthermore, the slope of the Schild plot was significantly different from unity, being 3.08 (with 95% confidence limits of 1.03 - 5.07). The histamine antagonism shown by AH22216 was specific since responses of the guinea-pig isolated ileum to histamine (H_1-receptors) or to acetylcholine (muscarinic receptors) were not antagonised by AH22216 except at extremely high concentrations (30 - 100 μg/ml).

AH22216 inhibited histamine-induced gastric acid secretion in the anaesthetised rat perfused stomach preparation and in the conscious Heidenhain pouch dog. In the rat, AH22216 had a prolonged duration of action and was approximately 8 x more potent than ranitidine, intravenous antisecretory ED_{50} values being 0.017 (0.012 - 0.025) mg/kg and 0.13 (0.08 - 0.22) mg/kg respectively. AH22216 was also more potent than ranitidine in the dog (n=5), oral ED_{50} values being 0.029 (0.025 - 0.033) mg/kg and 0.23 (0.17 - 0.29) mg/kg respectively. In these experiments, in which AH22216 was given during a plateau response to histamine, the antisecretory effect of AH22216 was prolonged, with little or no recovery of secretion over a 5 hour period. The time course of action of AH22216 was studied in 4 dogs by measuring the secretory response to a 2 hour infusion of a standard, submaximal dose of histamine (0.3 or 0.5 μg/kg/min) at different times after dosing with ranitidine or AH22216. Representative results are given in Table 1.

Table 1 Inhibition of secretory response to histamine by ranitidine or AH22216

Drug	Oral dose mg/kg	Mean ± s.e. % inhibition of secretion at 2†h	4h	8h	18h	24h
Ranitidine	1.0	95 ± 2	62 ± 8	23 ± 9		
AH22216	0.03	4 ± 8	67 ± 9	55 ± 11	33 ± 10	16 ± 20
	0.10	67 ± 9	95 ± 3	89 ± 2	54 ± 8	32 ± 13

† Results calculated from plateau experiment 2h after drug

Whereas inhibition of secretion following ranitidine at 1 mg/kg lasted approximately 8 hours, the antisecretory effect of AH22216, 0.03 - 0.10 mg/kg persisted for 18 - 24 hours.

Thus AH22216 differs from ranitidine in its interaction with H_2-receptors *in vitro* in being more potent, and in having a very persistent antisecretory action. This profile may have specific benefit for certain acid peptic disease states e.g. Zollinger-Ellison syndrome.

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GENETIC VARIABILITY IN THE MURINE VAS DEFERENS HISTAMINE H_2-RECEPTOR

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Classically, drug response is considered to be a function of two factors, namely, the availability of free drug at its site of action and the characteristics of the drug-receptor interaction. Both the pre-receptor dispositional and receptor events may, in principle, display genetically determined variation. A number of metabolic variables for example, have been characterized as displaying genetic polymorphism in both laboratory animals and man (Wood & Taylor 1979; Idle and Smith, 1980). By comparison, little attention has been directed towards identifying genetically determined variation in receptor mediated responses.

This communication describes an investigation of the possible influence of genetic factors on receptor sensitivity, assessed by the dose-response characteristics of isolated tissues taken from different inbred strains of mice. The ability of histamine to inhibit the electrically-induced twitch response of the mouse vas deferens via a histamine H2-receptor (Marshall, 1978) has been characterized in nine strains, both in terms of the histamine concentration required to elicit half-maximal twitch inhibition (150), and also the apparent dissociation constant of the histamine H2-receptor antagonist cimetidine (K_B), as judged by the dose ratio method (Arunlakshana & Schild, 1959). Stripped vasa deferentia from mice of nine inbred strains (see Table 1) were suspended in oxygenated magnesium-free Krebs solution at 37°C between parallel platinum electrodes. Responses to stimulation (0.2 Hz, 2ms, 64V) were recorded isometrically. Cumulative dose-response curves to histamine (0.1- 300 μM) were obtained in the absence and presence of cimetidine (10, 30 and 100 μM). Three strains (SWR, A2G and C57BL/10/ScSn)

Table 1 Inter-strain variability in the murine vas deferens histamine H2-receptor dose-response characteristics. (Mean ± S.D.; n = no. of animals).

STRAIN	n	HISTAMINE 150 (μM)	CIMETIDINE K_B (μM)
SWR	12	1.4 ± 0.2	6.0 ± 1.7
A2G	4	1.6 ± 0.3	6.0 ± 0.7
C57BL/10/ScSn	13	2.4 ± 1.3	6.9 ± 0.9
C3H	15	6.3 ± 1.6	32.1 ± 12.1
A	9	4.9 ± 1.0	21.4 ± 5.4
C57BL/6	7	5.9 ± 0.9	35.1 ± 4.6
DBA/2	6	10.0 ± 0.8	42.5 ± 5.0
Balb/c	4	7.4 ± 0.5	28.6 ± 3.8
129/Sv	4	8.5 ± 0.6	33.0 ± 5.0

yielded values for both parameters similar to those previously reported (Marshall, 1978) whilst the remaining six strains were relatively insensitive to both histamine and cimetidine (see Table 1). In all strains the antagonism of histamine by cimetidine was competitive (slope of Schild plot not significantly different from unity), and the response to histamine was unaffected by the presence of mepyramine (100 nM), atropine (100 nM), methysergide (1 μM), haloperidol (1 μM) or yohimbine (100 nM). In conclusion, studies of nine inbred mouse strains have revealed six-fold differences in the characteristics of the response of the mouse vas deferens to histamine. This variability appears to be due to differences in the properties of the histamine H2-receptor and is presumably genetic in origin.

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THE EFFECTS OF BARIUM AND METHOXAMINE ON CALCIUM UPTAKE IN THE RAT BISECTED VAS DEFERENS

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Methoxamine and barium each produce rhythmic contractions in the rat vas deferens. These contractions are dependent on extracellular calcium but are blocked by organic calcium antagonists only in high concentrations (Hay & Wadsworth, 1980). Apart from their effect on Ca^{2+} channels, verapamil and methoxyverapamil have other effects: methoxyverapamil has affinity for α-receptors and muscarinic receptors (Fairhurst et al, 1980) and verapamil blocks Na^{+} channels (Singh & Vaughan Williams, 1972). We have investigated whether the inhibitory effect of verapamil and nifedipine on the vas deferens can be explained by their action on Ca movements.

Cellular ^{45}Ca uptake was measured in bisected vasa deferentia from Wistar rats. Tissues were incubated for 50 min at 37°C in Krebs-Henseleit solution then for 5 min at 37°C in a tris buffered solution (both containing ^{45}Ca 0.5 μ Ci ml^{-1}) and finally for 60 min at 0.5°C in a tris buffered solution containing $LaCl_3$ 50 mM. Residual ^{45}Ca was extracted with EDTA 5 mM and measured by liquid scintillation counting.

$BaCl_2$ 1mM increased ^{45}Ca uptake in both the prostatic and epididymal halves. Ba-stimulated uptake was abolished by nifedipine 14μM or verapamil 61μM. Methoxamine 8.1μM had no effect on ^{45}Ca uptake in either half. Basal ^{45}Ca uptake was not affected by verapamil or nifedipine (Figure 1).

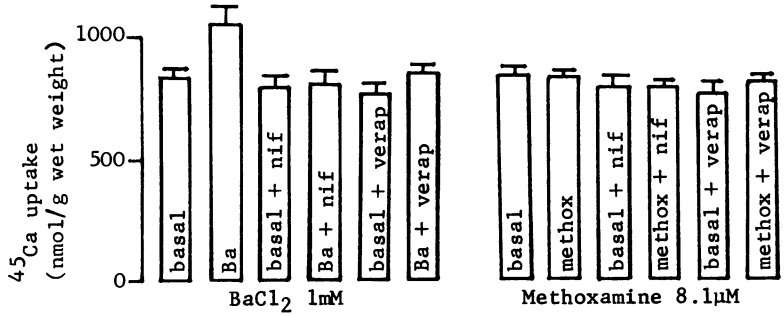

Figure 1 Effect of nifedipine 14μM (Nif) or verapamil 61μM (Verap) on ^{45}Ca uptake stimulated by $BaCl_2$ or methoxamine in epididymal halves of rat vasa deferentia.

The increase in ^{45}Ca uptake in the presence of Ba shows that Ba opens ion channels in the membrane which allow entry of Ca^{2+}, leading to contraction. We conclude that inhibition of these contractions by verapamil 61μM or nifedipine 14μM is due to block of these channels which have lower affinity for the calcium antagonists than those opened by K^{+} (Hay & Wadsworth, 1981). Methoxamine-induced rhythmic contractions, although similar in form to those produced by Ba, and also dependent on $[Ca^{2+}]_o$ are not associated with influx of ^{45}Ca. Methoxamine-induced contractions may involve a small amount of trigger calcium (which would escape detection in uptake experiments) that enters through Ca channels and releases more calcium from intracellular stores.

D.W.P.H. was supported by an MRC studentship. We thank Bayer and Knoll for drugs.

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SODIUM DEPENDENT BINDING OF (^{125}I)-ANGIOTENSIN II TO KIDNEY CORTEX MEMBRANES

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Angiotensin II stimulates sodium transport in the kidney by direct effects on epithelial cells, possibly by alterations in blood flow and by stimulating aldosterone release. Angiotensin II binding sites have been identified in glomeruli (Sraer et al, 1977; Foidart et al, 1980) but not in transporting cells. This study reports the presence of such binding sites in membranes prepared from epithelial cells of rat renal cortex.

A crude basolateral/brush border membrane fraction was prepared from rat renal cortex as described by Heidrich et al (1972). Freshly prepared lysed membranes (15-20 µg protein) were incubated with 1 nM (^{125}I)-angiotensin II (S.A. 1880 Ci per mmol) in 20 mM Tris HCl pH 7.4 buffer containing 120 mM NaCl, 5 mM Na_2 EDTA, 0.1 mM PMSF and 0.2% Bovine Serum Albumin. Non-specific binding was assessed by the addition of 1 µM angiotensin II to half of the tubes. Free and bound ligand were separated after 5 min incubation at 22 °C by filtration as fmol bound per mg protein.

Specific binding of angiotensin to this membrane fraction was multicomponent (ligand concentration range 0.1-5.0 nM), a high affinity binding site (Kd 0.5 nM Bmax 200 fmol per mg) with an affinity constant in the range of circulating hormone was chosen for further study. A similar pattern of multicomponent binding was found in renal glomeruli (Sraer et al, 1977). The antagonists [Sar^1,Ileu^8]AII and [Sar^1,Leu^8]AII were the most potent displacers of specific angiotensin binding (Ki 3.2 nM and 8.0 nM respectively) followed by angiotensin II (17.5 nM), [desAsp^1 Ileu^8]AII (27.0) and [Sar^1,Ala^8]AII (42.7 nM). Angiotensin I (154.2 nM) and angiotensin III (240.5 nM) were much less active. Bradykinin, substance P and converting enzyme inhibitor were 10,000 less active than angiotensin II in displacing specific binding while the N terminal dipeptide asp-arg was inactive.

Specific (^{125}I)-angiotensin binding was maximal in the presence of 120 mM NaCl in contrast to non-specific binding which was unaffected by NaCl. The specific binding was reduced to 24% of control values in the absence of sodium and was sodium concentration-dependent, 50% binding occurring with 60 mM NaCl. Potassium, lithium and rubidium, but not choline, were totally effective in replacing sodium, and alterations in anions had no effect.

The results suggest the presence of sodium dependent binding sites for angiotensin II on renal epithelial membranes which may have physiological significance.

The authors wish to thank the Smith Kline Foundation for financial support.

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NALOXONE-REVERSIBLE HYPERALGESIA IN FEMALE RATS FOLLOWING COPULATORY EXPERIENCE

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Recently, it has been suggested that mating is a biological stimulus for endorphin release. In support of this suggestion, it has been found that copulation results in analgesia in male rats, that prolonged mating stimulates opioid activity in mid-brain and that naloxone significantly extends the post-ejaculatory refractory period (Szechtman et al, 1981), the present study investigated the possible role of endorphins in female sexual behaviour.

30 adult virgin female hooded lister rats (200-270g) from Bradford University colony were used. Animals were individually housed, under reversed lighting, for 28 days prior to experimentation. On test days, base-line pain latencies were determined for all animals using the tail-flick assay (radiant heat method). Following base-line estimates, each animal was subjected to saline lavage, to determine stage of oestrous. Animals found to be in late proestrous or oestrous were then administered with either saline, 1mg/kg naloxone or 5mg/kg naloxone (i.p. 1ml/kg). Ten minutes following injection, a sexually experienced male conspecific was introduced into the cage of each female. After a 15 minute encounter, females were removed and immediately retested for pain latency. Analysis of variance revealed no overall significant effects of test condition ($F(2,29) = 0.231$) or any condition x time interaction ($F(2,27) = 2.23$), however, a significant effect was found for time ($F(1,27) = 15.521$, $p<0.001$). Further investigation indicated that this effect was due to significant decreases in tail-flick latencies in both saline control group ($t(df=27) = 5.03$, $p<0.0005$) and the 1mg/kg naloxone group ($t(df=27) = 3.78$, $p<0.0005$). However, animals treated with 5mg/kg naloxone failed to show a significant reduction in pain latencies between base-line and post-encounter tests ($t(df=27) = 0.89$, n.s.). These data clearly demonstrate that female copulatory experience results in hyperalgesia and that this effect is naloxone-reversible.

Whilst the mechanisms involved in post-copulatory hyperalgesia and its reversal by naloxone cannot currently be defined, our data nevertheless suggests the strong possibility of marked sex-differences in the role of endogenous opioids following copulatory experience. In particular, the adaptive significance of post-copulatory analgesia in male rats (Szechtman et al, 1981) but post-copulatory hyperalgesia in female rats remains to be determined.

The authors wish to thank Dr M.J. Ferster (Endo laboratories, Brussels) for the kind gift of naloxone hydrochloride. C.A. Hendrie is supported by an M.R.C. research studentship (G 77/7274).

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THE CARDIOVASCULAR EFFECTS OF TOLMESOXIDE IN THE ANAESTHETIZED DOG

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Tolmesoxide (4,5-dimethoxy-o-tolyl methyl sulphoxide) lowers blood pressure by a direct relaxing effect on vascular smooth muscle (Doxey, 1978). The present study determines the effect of tolmesoxide on the haemodynamics of the anaesthetized greyhound. Nine greyhounds in the weight range 19 - 25 Kg were anaesthetized with chloralose (80 mg/kg i.v.), given pancuronium (3 mg i.v.) and mechanically ventilated with oxygen. Cannulae were inserted into the femoral vein and artery for the infusion of tolmesoxide (1 mg/kg/min for 20 min) and measurement of systemic blood presure (SBP). A cannula in the left carotid artery was passed into the left ventricle from which left ventricular pressure (LVP) and its first derivative, left ventricular dP/dt was computed. A Swan-Ganz catheter inserted via the jugular vein enabled the measurement of pulmonary artery pressure (PAP) and pulmonary artery wedge pressure (PAWP). Cardiac output was monitored using the thermodilution method (IL 601). Electromagnetic flow probes (Statham) were used to monitor the blood flow through the renal, femoral, carotid and mesenteric blood vessels.

Tolmesoxide produced a progressive fall in mean blood pressure (130±7 to 86±11 mmHg) associated with a marked increase in heart rate (168±18 to 256±11 beats/min) and dP/dt (3280±360 to 7390±590 mmHg/s) however a parallel reduction in stroke volume (19±3 to 12±2 ml/beat) resulted in no net change in cardiac output. Although there was a reduction of peripheral resistance from 47.3±2.4 to 35.6±2.8 units due to the vasodilator action of tolmesoxide, there were no consistent changes in regional blood flow of the carotid, renal, femoral or mesenteric circulation. In contrast to the significant reduction in systemic resistance the pulmonary artery resistance rose from 2.4±0.4 to 4.3±0.4 units and maintained PAP at control levels. PAWP was reduced but not significantly.

PaO2 was unaltered by tolmesoxide indicating the maintenance of a consistent level of respiratory shunting (according to the method of Hyde, 1970) however PaCO2 was elevated (34.6±2.3 to 50.8±5.7 mmHg) as was base excess (-1.9±1.0 to -6.0±1.6 mEq/L) and lactic acid production (3.4±0.6 to 6.3±1.7 mg/100ml plasma) indicative of enhanced aerobic and anaerobic metabolism. The hypotensive effect of tolmesoxide induced an increase in plasma renin (1.9±0.2 to 4.5±0.9 ng/ml/hr plasma). Cardiac P-R, QRS, QT intervals and QRS amplitude were not significantly altered by tolmesoxide and no dysrhythmias were induced.

Thus tolmesoxide decreases left-heart afterload by decreasing systemic peripheral resistance without altering right heart haemodynamics enabling a reduction in systemic blood pressure without impairing right heart circulation.

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THE EFFECT OF ACTIVE IMMUNISATION ON DIGOXIN HANDLING AND RESPONSE

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Previous studies have indicated that passive immunisation with digoxin-specific antibodies may alter both digoxin disposition and effect (Griffiths et al, 1981, Lloyd and Smith, 1978, Butler et al, 1977) to the extent that this provides a possible approach to the treatment of digoxin toxicity (Smith et al, 1976). While less attention has been paid to date to the effects of active immunisation on digoxin handling and response, it has been demonstrated in actively-immunised rabbits given digoxin intravenously that serum levels were increased (Schmidt et al, 1974) and digoxin cardiotoxicity decreased (Schmidt and Butler, 1971). In an attempt to provide further information on the effects of active immunisation, we have carried out two studies, firstly on digoxin tissue distribution and lethality in guinea-pigs and secondly on the serum elimination of digoxin in the rabbit.

Digoxin immunogen (non-radioactive or ^{3}H-labelled, 13.6 μCi μg^{-1}) was prepared by coupling digoxin to human serum albumin by the periodate oxidation method and animals were actively immunised by intradermal injection of the conjugate (800 μg) in Freund's adjuvant at several intradermal sites (Smith et al, 1970).

In the study in guinea-pigs, digoxin cardiotoxicity and tissue digoxin levels were determined 9 weeks after active immunisation. Groups of 5 control and treated animals were anaesthetised with urethane and digoxin infused (250 μg ml^{-1}, 3.8 ml h^{-1}, i.v.). Control animals showed some abnormalities in ECG pattern within 15 min, with a time to death of 36 $\pm$ 3.3 min (mean $\pm$ SEM), and a digoxin dose of 0.64 $\pm$ 0.014 mg kg^{-1}. Three of the 5 immunised animals had normal ECG patterns throughout, with all animals surviving 80 min infusion (equivalent to a dose of 1.36 $\pm$ 0.06 mg kg^{-1}). Serum digoxin concentrations measured at the end of the infusion in the treated animals were some 10 times the values at death in the control animals, whereas digoxin concentration in bile was approximately one eighth of the control values. Immunisation had no significant effect on digoxin concentration in the tissues studied i.e. liver, kidney, spleen, heart and lymph nodes.

In the second study, the serum elimination of ^{3}H digoxin following intradermal administration in Freund's adjuvant was determined in 4 rabbits 4 weeks prior to active immunisation ($t\frac{1}{2}$, 2.0 $\pm$0.49 days, mean $\pm$ SEM) and redetermined 6 weeks ($t\frac{1}{2}$, 11.3 $\pm$ 3.03 days) and 44 weeks ($t\frac{1}{2}$, 25.5 $\pm$ 1.7 days) after immunisation. Serum digoxin $t\frac{1}{2}$ values correlated positively with digoxin antibody titre (estimated from the extent of ^{3}H-digoxin binding to serum in equilibrium dialysis) but neither correlated with the $t\frac{1}{2}$ of the ^{3}H-digoxin conjugate ($t\frac{1}{2}$ = 2.86 $\pm$ 0.37 days).

These studies indicate that, as with passive immunisation, active immunisation produces a cardioprotective effect, raises serum total digoxin concentration and markedly prolongs the serum elimination half life.

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HUMAN PLATELET MEMBRANE POTENTIAL ASSESSED WITH A FLUORESCENT PROBE

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Fluorescent cationic dyes partition across the membrane of intact cells according to their membrane potential (Hoffman & Larris 1974, Rink, Montecucco et al 1980). In this study the fluorescent dye 3,3'-diethylthiocarbacyanine iodide ($diSC_2(5)$) has been used to estimate the potential difference across the surface membrane of human washed platelets. In some experiments the antibiotic valinomycin, which is a potassium ionophore, was used to raise the potassium permeability of the platelet.

Blood from the antecubital vein of donors A or B was collected in trisodium citrate 3.8%(1:9v/v), centrifuged at 200 g for 10 min and the separated platelet rich plasma was centrifuged at 1000 g for 15 min to obtain a platelet pellet. The pellet was washed twice with a medium (pH 7.4) containing HEPES 2 mM, glucose 5.0 mM, KCl 1 mM and NaCl 150 mM, divided into 8 aliquots and these were finally resuspended in a medium containing HEPES 2 mM, glucose 5.0 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1 mM, KCl ranging from 1-200 mM and NaCl from 28.5-138.0 mM (in order to maintain 300 mosmoles osmolarity). The final platelet count of all aliquots was adjusted to 50,000/mm^3. Five µl of $diSC_2(5)$ in DMSO (0.5 µg/ml) were added to 2 ml of platelet suspension and the intensity of fluorescence recorded (with excitation and emission wavelengths at 634 nm and 666 nm and slit widths at 2.5 and 10 nm) on a PERKIN-ELMER fluorimeter. Valinomycin in DMSO (5×10^{-3} M final concentration) was added when the signal had reached a plateau (at 12 min) and it was then followed for a further 5 min.

The uptake of $diSC_2(5)$ by the platelets showed an initial rapid component followed by a slower component. Uptake was inversely proportional to the external potassium concentration (K_o), the relationship between (K_o) and change in fluorescence being non-linear. At low (K_o) valinomycin caused a small increase in dye uptake suggesting a hyperpolarization of the platelet membrane whereas at high (K_o) the antibiotic caused a decrease in dye uptake (depolarization). These results suggest that the membrane of the platelet is permeable to potassium and one or more other ions. Studies currently in progress are designed to determine the contributions of these other ions so that the membrane potential can be calculated.

I wish to thank Professor P.N.R.Usherwood, Department of Zoology and Professor J. Crossland, Department of Pharmacy, for their assistance with these studies.

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PERIPHERAL α_1 - AND α_2 -ADRENOCEPTOR EFFECTS AND CENTRAL CARDIOVASCULAR ACTIONS OF CLONIDINE-LIKE DRUGS IN THE RAT

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Clonidine-like drugs are hypotensive agents that exert their effect by an action on central α- adrenoceptors (Kobinger 1978). We have attempted to elucidate the α- adrenoceptor subtype these agents act on by comparing the peripheral α_1- and α_2- adrenoceptor stimulating properties of these agents to their central hypotensive and bradycardic effects.

Clonidine, UK14,304,guanfacine, lofexidine (clonidine-like agents) and also phenylephrine were given intracerebroventricularly (i.c.v.) to female Sprague-Dawley CD rats (250-350 g) anaesthetised with urethane (1.25 g/kg). Phenylephrine (0.5-40 µg i.c.v.) caused hypotension and bradycardia. The rank order of potency for these effects was UK14,304 > clonidine > lofexidine > guanfacine.

The pressor action of the agonists was tested in pithed rats anaesthetised with urethane in the absence or presence of prazosin (0.1 mg/kg i.v.) or rauwolscine (1 mg/kg i.v.). All clonidine-like agents had a higher selectivity for α_2-adrenoceptors than phenylephrine, (Table I).

α_2- stimulating effects were also assessed by inhibition of the twitch response of the prostatic portions of rat vasa to single pulsefield stimulation. (0.3 msec, supramaximal voltage) and α_1- adrenoceptor stimulating effects by assessing the contractile action of these agents on the epididymal portions of vasa (Brown et al 1979, Michel & Whiting 1981). All clonidine-like agents (4-32 ng/ml) inhibited the twitch response whereas phenylephrine augmented it. The rank order of potency was UK14,304 > clonidine > guanfacine > lofexidine. All agents contracted the epididymal portions but UK14,304 was the least active.

The results show UK14,304 to be a selective agent for α_2-adrenoceptors. A reasonable correlation existed between peripheral α_2-adrenoceptor activity and central hypotensive and bradycardic activity of clonidine-like agents in the rat.

Table I - Vascular α_1- and α_2- activity of α- adrenoceptor agonists in the pithed rat.

	$ED_{50}C$	$ED_{50}P$	$ED_{50}R$	$ED_{50}P/ED_{50}R$
UK14,304	13.5 ± 3.0	24.3 ± 7.8	65.1 ± 21.1	0.37
Clonidine	5.6 ± 2.0	18.7 ± 3.3	39.5 ± 5.1	0.47
Lofexidine	3.7 ± 1.9	22.3 ± 8.2	27.0 ± 6.8	0.83
Guanfacine	80.8 ± 25.2	653.3 ± 89.2	361.7 ± 59.7	1.8
Phenylephrine	35.8 ± 8.8	482.9 ± 52.9	38.5 ± 7.4	12.5

$ED_{50}C$, $ED_{50}P$, $ED_{50}R$ - dose required (µg/kg) to raise the diastolic B.P. of rats to 50% maximum in the absence or presence of prazosin (0.1 mg/kg) or rauwolscine (1 mg/kg) respectively. n = 5-7 for all experiments. Mean ± S.E. mean are shown.

P.J.B. is supported by a S.R.C. CASE award with Pfizer Ltd.

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IN VITRO ACTIONS OF HYDRALAZINE ON VASCULAR AND NON-VASCULAR SMOOTH MUSCLE: PRE AND POST-JUNCTIONAL EFFECTS

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It has previously been demonstrated (Worcel, 1978 ; Chevillard et al., 1981) that the in vitro post-junctional anti-spasmogenic action of hydralazine (Hyd) on the rat tail artery is modulated by substances, probably purines, released from sympathetic nerve terminals. Hyd has also been shown to exert a pre-junctional action in the same preparation, in inhibiting field stimulation-induced ^3H release from (^3H) noradrenaline (^3H-NA) loaded arteries (Chevillard et al., 1980). Since all the previous work was performed using the same arterial smooth muscle preparation it seemed important to investigate the actions of Hyd on other vascular and non-vascular smooth muscles.

The tissues and organs used were rat vas deferens (epididymal portion), anococcygeus muscle and perfused kidney and rabbit ear artery and perfused kidney. Hyd inhibited phenylephrine (Phe)-induced increases in perfusion pressure in the innervated rat kidney (IC_{50} 74±10nM Hyd ; plateau inhibition of 70.5±4.5% at 0.3μM Hyd, n=7). However, higher concentrations of Hyd were required to inhibit Phe-induced increases in perfusion pressure in the innervated rabbit ear artery (IC_{50} 0.29±0.1mM, n=9) and Phe-induced increases in isotonic contraction in denervated vas deferens (IC_{50} 0.30±0.12mM, n=7) and anococcygeus muscle (IC_{50} 0.25±0.09mM, n=5). In vitro denervation of the rabbit ear artery induced a biphasic response to Hyd. Low doses inhibited Phe-induced increases in perfusion pressure (IC_{50} 0.12μM) producing a plateau response (47.1±6.2% inhibition, n=6) at 1μM Hyd. This plateau was maintained until higher Hyd concentrations (>0.1mM) produced a second inhibition (IC_{50} 0.18±0.05mM, n=6) similar to that observed in the innervated artery. Hyd was without effect on ^3H release from field-stimulated vas deferens, anococcygeus muscle and perfused rat kidney preincubated in (^3H) NA, but decreased ($p<0.01$, n=5) ^3H release from (^3H)NA pretreated rabbit ear arteries at 1μM. Hyd (3μM) was without effect on endogenous NA release from perfused rabbit kidney following stimulation of the periarterial nerves, but inhibited the stimulation-induced increases in perfusion pressure. Hyd (0.3μM) was without effect on tyramine-induced ^3H release from (^3H) pretreated proximal rat tail arteries, where the drug inhibits field stimulation-induced (^3H) NA release (Chevillard et al., 1980).

The results in the rabbit ear artery confirm that the presence of sympathetic nerve terminals can modify the post-junctional actions of low (therapeutic) concentrations of Hyd, whilst other vascular tissues (rat and rabbit kidney) are sensitive to Hyd when innervated. The non-vascular tissues and the innervated rabbit ear artery were only sensitive to high (non-therapeutic) concentrations of Hyd. The relevance of the pre-junctional inhibitory effect of Hyd remains unclear.

The authors acknowledge the skilled assistance of Mss F. Daponte, M.C. Hameau and M.N. Mathieu.

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NEUROGENIC VASODILATATION IN ISOLATED BOVINE AND CANINE PENILE ARTERIES

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Pieces of isolated penile arteries from bullocks or dogs were perfused with Krebs solution (35°C) at a constant flow rate (3 ml min^{-1}); field stimulation (2-10 Hz for 10 s; 0.5 ms pulses) was applied. At first the resistance to flow was low and stimulation caused a rise in perfusion pressure which was blocked by guanethidine (4 - 10 μM). About half of the preparations from both species developed tone without further drug treatment. Tone in the remainder could be produced by either a higher concentration of guanethidine (30 μM) or by other constrictor agents, such as phenylephrine (2 μM) or ergotamine (2 μM). When tone was high, field stimulation produced dilatation manifested by a fall in perfusion pressure. Relaxation of spiral strips of the bovine penile artery in response to field stimulation was described by Klinge & Sjöstrand (1974). The fall in perfusion pressure produced by field stimulation was not blocked by atropine (0.5 μM) or propranolol (2 μM) but was abolished by tetrodotoxin (0.7 μM).

So far, three putative transmitters of the neurogenic vasodilatation have been tested. These are VIP, ATP, and the inhibitory factor extracted from the bovine retractor penis muscle (Ambache et al, 1975) which is a powerful vasodilator (Bowman et al, 1981). Additionally, two substances that block certain inhibitory mechanisms have been studied. These are apamin from bee venom, which blocks VIP and neurogenic vasodilatation in the cat small intestine (Sjöqvist et al, 1980) and a haemolysate, obtained from rat, guinea-pig or human erythrocytes, which blocks the effect of the inhibitory factor from the bovine retractor penis on several smooth muscles including some blood vessels (Bowman & Gillespie, 1981).

Inhibitory factor (10 - 50 μl) produced an abrupt and transient vasodilatation that resembled the effect of field stimulation in its time course. The haemolysate always produced vasoconstriction and, in 9 out of 13 experiments, blocked or reduced the vasodilatation produced by field stimulation or by the inhibitory factor. The block by haemolysate was less complete and occurred less consistently than that in some other smooth muscles. ATP (5 - 50 nmol) produced vasodilatation or a biphasic response. Vasodilator responses to ATP were unaffected by haemolysate even in preparations in which responses to field stimulation and to inhibitory factor were blocked. VIP (0.2 - 0.8 nmol) produced a slow and prolonged vasodilatation in about 50% of preparations. Tachyphylaxis to repeated doses developed rapidly. When a response to VIP was no longer obtainable, nerve stimulation and inhibitory factor continued to produce normal vasodilatations. Apamin (0.1 μM) failed to block vasodilatation produced by field stimulation, by inhibitory factor, by ATP or by VIP.

The results do not support the possibility that either ATP or VIP is the transmitter of the neurogenic vasodilatation in the penile artery, but they are compatible with the possibility that the inhibitory factor obtained from the bovine retractor penis muscle serves this function.

This work was supported by the Medical Research Council.

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EVIDENCE FOR TWO DIFFERENT POSTSYNAPTIC α-ADRENOCEPTORS IN RAT PORTAL VEIN IN VITRO

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α_2-adrenoceptor agonists induce vasoconstriction in vivo (Timmermans & Van Zweiten, 1981) and contractions of dog saphenous vein to α_2-receptor agonists are blocked by diltiazem or verapamil in preference to α_1-receptor agonists (Langer & Shepperson, 1981). The possibility that α_2-receptors might operate Ca^{2+} channels in rat portal vein has been examined since myogenic activity in this vessel is dependent on extracellular Ca^{2+} ions (Sigurdsson et al, 1975).

Portal veins from male Sprague-Dawley rats (250-350g) were incubated at 37°C under 0.5g tension in Krebs solution containing propranolol 10^{-7}M and ascorbate 10^{-4}M. The tissues were aerated with 95% O_2 + 5% CO_2. Isometric recordings were made on a Grass 7 polygraph or Devices M_2-chart recorder. In some experiments extracellular recordings of electrical activity were made using the perfused capillary method of Golenhofen & Loh (1970). Low concentrations of noradrenaline (NA) increased the frequency and amplitude of the phasic activity and at higher concentrations only, these responses were superimposed on a sustained contracture of the tissue (maximum contraction = 1.4±0.2g; n = 9). The responses for phasic activity may be quantified as:-

$$\frac{\text{Change in (frequency X amplitude) of phasic waves after agonist}}{\text{(frequency X amplitude) of phasic waves before agonist}} = \frac{\Delta fa}{fa}$$

Dose-related increases in $\Delta fa/fa$ were induced by NA (6.6nM-3.3μM; n = 5-7), and were antagonised by prazosin at 10^{-7}M but only in a non-competitive manner. In contrast, the dose-response curves for the sustained contracture of the tissue to higher concentrations of NA (66nM-6.6μM) were shifted to the right in a parallel manner by prazosin at 10^{-9}, - 10^{-7}M. The pA_2 for prazosin against NA-induced contractures was 9.3 with a slope of 0.89; r = 0.96. Quantitatively similar responses for $\Delta fa/fa$ were recorded for the α_2-receptor agonist UK14304 (Cambridge, 1981) over the dose range 0.18-100μM, but UK14304 only induced a small contracture of the portal vein (maximum response = 0.2±0.1g; n = 8). The increase in $\Delta fa/fa$ for both NA and UK14304 was associated with an increase in the duration of the bursts of electrical events which preceded phasic mechanical changes. Incubation in Ca^{2+} free Krebs (10 min) abolished the phasic activity. Subsequent $\Delta fa/fa$ dose-response curves to Ca^{2+} (0.35-5mM) were constructed in the absence and presence of UK14304 (3.6-36μM; n = 6), which shifted the Ca^{2+} dose-response curve to the left and increased the maximum response. Ca^{2+} dose-response curves for $\Delta fa/fa$ in the presence of UK14304 (18μM) were selectively antagonised by yohimbine (10^{-8}, 10^{-7}M; $P < 0.01$), but not by prazosin (5×10^{-8}M). Neither yohimbine nor prazosin antagonised the increase in $\Delta fa/fa$ to Ca^{2+} in non-stimulated preparations.

The results indicate that postsynaptic α_1 and α_2-receptors are present in the rat portal vein and have different response characteristics. Ca^{2+}-dependent phasic activity of this preparation can be induced by low concentrations of NA or UK14304 and preferentially antagonised by yohimbine but not by prazosin. The sustained contracture of the portal vein to NA is, however, preferentially antagonised by prazosin. These data suggest that α_2-receptors can operate Ca^{2+} ion channels in the rat portal vein, without causing a sustained contracture, but does not exclude the possibility that α_1-receptors can also operate ion channels in this preparation.

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THE RECOVERY OF α-ADRENOCEPTOR FUNCTION AND NUMBER AFTER PHENOXYBENZAMINE: AN INDEX OF RECEPTOR TURNOVER

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Phenoxybenzamine is an α adrenoceptor antagonist which binds covalently to the receptor (Harvey & Nickerson, 1954). This irreversible binding leads to interference with function. Recovery from α adrenoceptor blockade with phenoxybenzamine is slow and may depend on synthesis of new receptors.

We have previously shown that it is possible to relate changes in in vivo pressor responses to in vitro changes in specific ^{3}H prazosin and ^{3}H clonidine binding sites (Hamilton et al, 1981). These studies have been extended to measure the in vivo and in vitro rates of recovery from phenoxybenzamine treatment and to test the hypothesis that recovery is related to 'de novo' protein synthesis. Male New Zealand White rabbits received a single intravenous dose of 5 mg/kg phenoxybenzamine. In vivo pressor dose response curves to the selective α_1 adrenoceptor agonist phenylephrine, the mixed α_1/α_2 agonist noradrenaline and the α_2 adrenoceptor selective agonist guanabenz were constructed. In parallel studies in vitro ^{3}H prazosin and ^{3}H clonidine specific binding to spleen membranes was measured as previously described (Hamilton & Reid, 1981). Studies were performed 30 min, 1, 2, 3, 5, 8 and 12 days after treatment (n = 6-8/study). At each time the maximum pressor response to each agonist and the maximum number of specific prazosin and clonidine binding sites were calculated. Recovery of responses and receptor number was log linear and thus described by a simple exponential function.

Recovery of pressor responses to the α_1 agonist phenylephrine, $t_{\frac{1}{2}} = 0.94 \pm 0.20$, was significantly shorter than recovery of responses to the α_2 selective agonist guanabenz, $t_{\frac{1}{2}} = 1.36 \pm 0.05$ days ($p < 0.05$). Recovery of responses to the mixed α_1/α_2 agonist noradrenaline was intermediate, $t_{\frac{1}{2}} = 1.16 \pm 0.44$ days (mean ± SD).

$t_{\frac{1}{2}}$ for recovery of specific clonidine binding, 1.61 ± 0.9 days, was similar to recovery of in vivo guanabenz responses, but $t_{\frac{1}{2}}$ for recovery of prazosin binding, 3.65 ± 0.01 days was significantly longer ($p < 0.01$) than recovery of phenylephrine responses in vivo.

The slower recovery of binding sites compared to pressor responses would be consistent with the presence of 'spare' α_1 adrenoceptors (Hamilton et al, 1981). In addition increased responses may be a result of post receptor supersensitivity.

In studies in which rabbits were pretreated with actinomycin D (0.1 mg/kg/day) and phenoxybenzamine a reduction in the number of specific prazosin binding sites was observed compared to the number in animals only given phenoxybenzamine. Three days after phenoxybenzamine treatment there were 59 ± 20 ^{3}H prazosin binding sites in spleen compared to 148 ± 22 fM/mg protein in untreated rabbits. After actinomycin D the number was significantly lower (39 ± 4 fM/mg protein) ($p < 0.05$) than after phenoxybenzamine alone. In vivo pressor responses were also significantly reduced in the actinomycin D treated animals. Thus inhibitors of protein synthesis delay the recovery of alpha adrenoceptor binding sites after phenoxybenzamine treatment supporting the hypothesis that recovery is related to synthesis of new receptor protein.

This approach may be useful to estimate turnover of α adrenoceptors under normal and pathological conditions.

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SUBSTITUTED BENZAMIDES: SELECTIVE ANTAGONISTS OF PERIPHERAL PRESYNAPTIC DOPAMINE RECEPTORS IN THE DOG

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The stimulation of presynaptic dopamine (DA) receptors on certain noradrenergic neurones and/or postsynaptic DA receptors in some vascular beds may be involved in the vasodilator, bradycardic and hypotensive effects of DA agonist drugs (see Cavero et al 1982). Recent results using a variety of DA antagonists suggest that important differences may exist between pre- and postsynaptic DA receptors (Massingham et al 1981). This paper examines the selectivity of the potent DA antagonist drug clebopride (Roberts, 1979) for pre and postsynaptic DA receptors in the cardiovascular system of the dog and compares it with some other DA antagonists of the substituted benzamide class.

Dogs (5 - 15 kg) were anaesthetized with pentobarbitone (35 mg/kg i.v. + 6 mg kg^{-1} h^{-1} i.v.) and artificially respired. Presynaptic DA receptors were evaluated at the level of the heart using antagonism of the tachycardia induced by continuous stimulation of the ansa subclavia (supramaximal voltage, 1 Hz, 1 msec). This response was reduced in a dose-dependent manner by the preferential presynaptic DA agonist N,N-di-n-propyldopamine (DPDA, 5 - 200 µg kg^{-1} min^{-1} i.v.) and the effect of DPDA could be reversed by certain DA antagonists. Postsynaptic DA receptor blocking activity was studied in a second series of α,β-adrenoceptor and ganglion-blocked dogs using mesenteric blood flow responses to close arterial injections of DA (0.1 - 10 µg/kg). The IC_{50} values shown for the DA antagonists are those required to inhibit the response to 50 µg kg^{-1} min^{-1} i.v. DPDA which in these experiments produced approximately 50% inhibition of the tachycardia induced by the electrical stimulation.

Clebopride (0.001 - 1 mg/kg i.v.) produced a dose-dependent reversal of the effects of DPDA at presynaptic DA receptors, the IC_{50} dose being 0.012 ± 0.006 mg/kg i.v. (n=5). In contrast, doses of clebopride up to 0.3 mg/kg i.v. were without significant effect on the increases in mesenteric blood flow elicited by DA. Over a similar dose range clebopride did not affect heart rate responses to adrenaline (1 µg/kg i.v.) or mesenteric blood flow responses to prostaglandin E_2 (1 µg/kg i.a.) neither did it reverse the effects of clonidine (20 µg/kg i.v.) at cardiac presynaptic α_2-adrenoceptors. Metoclopramide, like clebopride, antagonized the presynaptic effects of DPDA but was less potent in this respect (IC_{50} :0.078 ± 0.032 mg/kg i.v. n=4) and significantly reduced the postsynaptic effects of DA on mesenteric blood flow at 1 mg/kg i.v. The IC_{50} dose of (SR)-sulpiride for blocking presynaptic DA receptors was 0.012 ±0.007 mg/kg i.v. (n=3) with significant effects occurring at postsynaptic DA sites at 1 mg/kg i.v. Unlike the other DA antagonists, (SR)-sulpiride also blocked presynaptic α_2-adrenoceptors in doses of 0.1 mg/kg i.v. and above.

These results demonstrate that substituted benzamide drugs are potent antagonists at peripheral presynaptic DA receptor sites, with clebopride emerging as a very selective agent from these studies. In agreement with previous suggestions these results indicate that distinct differences exist between pre- and postsynaptic peripheral DA receptors.

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α_2-ADRENOCEPTORS MEDIATE DOPAMINE ACTION ON THE ANOLIS MELANOPHORE

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Adrenaline and noradrenaline antagonise MSH action on the melanophore and we have recently shown that their inhibitory action is mediated by α_2-adrenoceptors, despite kinetic evidence that the interaction was competitive (Carter & Shuster, 1982). Dopamine also antagonises MSH action on the Anolis melanophore (Tilders et al, 1975; Carter & Shuster, 1981) and we therefore investigated whether dopamine action is also mediated by α_2-adrenoceptors.

The rate bioassay in Anolis (Carter & Shuster, 1978) was used to obtain dose-response curves to α-MSH (log darkening speed v log dose of α-MSH). The effects of dopamine, apomorphine, yohimbine and sulpiride were studied on α-MSH action. Each drug was dissolved immediately before bioassay.

Increasing concentrations of dopamine shifted the α-MSH dose-response progressively to the right and the slopes of the curves with dopamine did not deviate significantly from that of α-MSH alone ($P>0.05$; analysis of variance). The slope of the double reciprocal plot of α-MSH increased with added dopamine, but the intercept on the abscissa remained constant. The Arunlakshana-Schild plot for dopamine was linear with a slope which did not deviate significantly from unity ($P>0.05$). The α_2-adrenoceptor blocker, yohimbine (5×10^{-7}M), did not alter α-MSH potency but completely blocked the 30% inhibition of α-MSH potency by 10^{-9}M dopamine and reduced the 80% inhibition by 10^{-8}M dopamine to 47%. Dopamine receptor involvement was studied using apomorphine, which is an agonist or a partial agonist, and sulpiride which is an antagonist to the dopamine receptor. 5×10^{-7}M or 5×10^{-9}M apomorphine had no effect on α-MSH potency and neither 5×10^{-6}M nor 5×10^{-8}M apomorphine altered the inhibitory effect of 10^{-8}M dopamine on α-MSH potency. 10^{-6}M sulpiride had no effect on α-MSH potency nor did it block the inhibitory action of 10^{-8}M dopamine. Thus there was no evidence to suggest dopamine receptors mediated the action of dopamine.

The kinetics of the interaction between α-MSH and dopamine suggested competitive antagonism by dopamine, similar to that previously shown for adrenaline and noradrenaline (Carter & Shuster, 1982). However, as with these catecholamines, yohimbine blocked dopamine action but not α-MSH action demonstrating that two separate receptors were involved. This confirms our earlier findings (Carter & Shuster, 1982) that kinetic evidence is not by itself adequate to establish the site of interaction of drugs. Since the selective α_2-adrenoceptor antagonist, yohimbine, blocked the action of low doses of dopamine, α_2-adrenoceptors mediated dopamine action. However, because yohimbine did not completely block the effect of high doses of dopamine, either yohimbine was required in higher concentrations or other receptors mediated dopamine action. Dopamine receptors were not involved since apomorphine did not act as an agonist or partial agonist and sulpiride did not block dopamine action. We conclude that the action of dopamine on the Anolis melanophore is inhibitory to MSH and acts similarly to other catecholamines through an α_2-receptor. However, whilst there is no evidence of a dopamine receptor on the melanophore we cannot exclude another, as yet unidentified, dopamine receptor mediating its action at higher concentrations.

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IS THERE MORE THAN ONE α_1-ADRENOCEPTOR OR IS THIS THE WRONG QUESTION?

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Post-junctional alpha-adrenoceptors are no longer a homogeneous group (McGrath, 1982). This heterogeneity of alpha-adrenoceptors has been easier to demonstrate in vivo than in vitro, e.g. in anococcygeus (Docherty & McGrath, 1980; Docherty & Starke, 1981). Since effector responses in smooth muscle consist of different components (mechanical, electrical or biochemical), the question arises: is the same receptor involved in different types of response, i.e. at the "first messenger" stage, or do different receptors activate each system? If this can be settled in vitro then some light might be cast on the in vivo observations.

Isometric tension of rat anococcygeus was recorded in vitro (Gillespie, 1972). Responses to noradrenaline (NA) were compared with those to amidephrine and xylazine.

The response to NA consisted of phasic and tonic components. The phasic response was produced by low concentrations of NA (< 100 nM) and thus could be seen only in the presence of an uptake-I blocker or after chemical sympathectomy. Higher concentrations of NA produced a tonic contraction. The phasic, but not the tonic, component was blocked by nifedipine (0.1 - 10 μM). The threshold for the phasic response was reduced by increasing the CO_2 content of the gas stream (5% to 8%), by reducing the Ca^{++} in the medium (2.5 mM to 1.25 mM) or by including a sub-contractile concentration of xylazine. The tonic component was reduced by lowering the O_2 content of the gas (95% to 16%).

Xylazine produced mainly phasic contractions and amidephrine a mixture of phasic and tonic. Nifedipine blocked the phasic responses.

Using selective alpha-1 or alpha-2 antagonists (yohimbine analogues or benzodioxan derivatives), each agonist was classifiable as alpha-1. No alpha-2 component could be detected. In the absence of uptake-I blockade NA was not competitively antagonised by alpha-blockers. While this might suggest a "resistant" receptor, it could also be due to saturation of neuronal uptake by high concentrations of NA. The only evidence from antagonists for more than one receptor was a relative resistance to corynanthine of NA (compared with xylazine or amidephrine). "Selective" alpha-2 agonists (azepexole, guanabenz, clonidine) produced only phasic contractions susceptible to alpha-1 antagonists. It is interesting that the phasic, nifedipine-sensitive contraction is elicited by "alpha-2" agonists and that it becomes more significant when the saline is modified towards "physiological" extra-cellular conditions.

It is concluded that there are sufficient discrepancies among the effects of "alpha-agonists" to suspect that two different contractile processes in anococcygeus may be initiated by different sub-groups of alpha-1 adrenoceptors: thus synthetic agonists may not faithfully mimic NA. However, so many experimental factors can switch the balance between these processes that different "types" of agonism at a single receptor cannot be excluded and data obtained in the standard Krebs' bicarbonate solution may not reflect events in vivo.

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CHARACTERIZATION AND LOCATION OF α-ADRENOCEPTORS IN THE RAT VAS DEFERENS

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Exogenous noradrenaline (NA) has three effects on the rat vas deferens: contraction of the tissue, potentiation of nerve-induced responses and inhibition of nerve-induced responses (MacDonald & McGrath, 1980). The present study examines further the effects of NA on the responses of the prostatic and epididymal halves of the rat isolated vas deferens to single pulse (0.5 ms) field stimulation in order to characterize the receptors involved and their location. In the epididymal end the contractile response to nerve stimulation is mainly adrenergic and in the prostatic end non-adrenergic (McGrath, 1978).

In the prostatic portion NA produced an α_2-adrenoceptor-mediated inhibition of nerve-induced responses (MacDonald & McGrath, 1980) which was unaffected by cocaine, 3 μM or WB 4101, 0.1 μM. The response remaining after nifedipine, 10 μM, was also inhibited by NA.

In the epididymal end the nerve-induced responses were potentiated and prolonged by cocaine, 3 μM. WB 4101, 0.1 μM, prevented this potentiation and blocked the nerve-induced adrenergic responses. Nifedipine, 10 μM, which had no effect on the adrenergic nerve-induced response (French & Scott, 1981), prevented the prolongation.

The threshold concentration of NA for contraction of the epididymal end was the same or lower than the threshold for potentiation of the nerve-induced response, in contrast to synthetic α-adrenoceptor agonists which potentiate at sub-contractile concentrations (MacDonald & McGrath, 1980). In the presence of cocaine, 3 μM, the contractile effects of NA were greater and the threshold for contraction was lowered. The effect of cocaine on the potentiating effect of NA was difficult to quantify since cocaine itself potentiated the response, but the threshold concentration was not altered. In the presence of WB 4101, 0.1 μM, the threshold for contraction was greater than that for potentiation and both contractile activity and potentiation were increased by cocaine, 3 μM.

In conclusion, the lack of effect of cocaine on the pre-junctional α_2-mediated inhibition by NA suggests that the non-adrenergic nerves are anatomically separate from the adrenergic nerve terminals. The resistance to nifedipine contrasts with its inactivation of post-junctional α_2 - mediated pressor responses (van Meel et al, 1981). The differences between the excitatory effects of NA (contraction and potentiation) suggest that different receptors may mediate these responses; each can be influenced by uptake block suggesting that the receptors are in the vicinity of the adrenergic nerve terminals. The sensitivity of both excitatory effects of exogenous NA to nifedipine contrasts with the resistance of the adrenergic nerve response suggesting either different receptors or different activation mechanisms via the same receptor. The nifedipine-sensitive component of the nerve-induced response which emerges after cocaine might correspond to the effects of exogenous NA. The concept of a single α_1 - mediated excitatory effect of NA no longer seems tenable.

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