BRL 34915 INDUCED POTASSIUM CHANNEL ACTIVATION IN RABBIT ISOLATED MESENTERIC ARTERY IS NOT CALCIUM DEPENDENT

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In rat isolated portal vein, the opening of potassium channels by BRL 34915 is associated with hyperpolarisation of the cell membrane (Hamilton et al, 1985). This property of BRL 34915 probably provides the basis of its anti-hypertensive activity in animals (Buckingham et al, 1986). In the present study we have examined, in rabbit isolated mesenteric artery (RIMA), the nature of the potassium channel opened by BRL 34915.

Segments of RIMA (c.20mg) were preloaded with 86-Rb (as a marker for potassium) by preincubating with 86-Rb (2-5 mCi/mg; c.50 μCi/ml in a HEPES physiological salt solution) for 90 min. Efflux of 86-Rb was determined at 3 min intervals over the subsequent 60 min. BRL 34915 (10 μM) produced a 100% increase in the 86-Rb efflux rate. This increase was dose dependently inhibited by TEA with total inhibition occurring at 30 mM TEA. The increase in efflux brought about by 30 mM K^+ was also inhibited by TEA whilst that stimulated by NA (30 μM) was not significantly affected by TEA (30 mM). Thus the potassium channel activation produced by BRL 34915 probably occurs only through TEA sensitive K^+ channels in RIMA.

The calcium dependency of the BRL 34915 sensitive channel in RIMA was examined using nifedipine and lanthanum. Nifedipine (1 to 100 μM) produced a dose dependent inhibition of the 86-Rb efflux stimulated by BRL 34915 (10 μM) and K^+ (30 mM), whereas NA (30 μM) stimulated efflux was only partially inhibited by the highest concentration of nifedipine. NA and K^+ stimulated efflux was completely abolished by lanthanum (1 mM and 10 mM, respectively) whilst the stimulation produced by BRL 34915 was largely unaffected by lanthanum (10 mM). As BRL 34915 stimulated efflux is not inhibited by concentrations of lanthanum used to block calcium entry, and is only blocked by high concentrations of nifedipine, it would appear that BRL 34915 stimulated 86-Rb efflux is not dependent upon calcium influx in the RIMA.

The potassium channel activating effect of BRL 34915 in RIMA has been investigated further using methylene blue to inhibit cyclic GMP formation. Evidence suggests that cyclic GMP formation may be a common factor in the smooth muscle relaxant effects of a wide variety of compounds (see Ignarro & Kadowitz, 1985). Methylene blue (1-100 μM) produced a dose dependent inhibition of BRL 34915 (10 μM) stimulated 86-Rb efflux whilst methylene blue (100 μM) had little effect on basal, NA (30 μM) or K^+ (30 mM) stimulated efflux.

These preliminary findings suggest that in RIMA BRL 34915 enhances 86-Rb efflux through a TEA sensitive K^+ channel which is not dependent on calcium influx and that this effect may be associated with increased cyclic GMP formation.

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DO CONDITIONS IN VITRO, WHICH MIMIC ISCHAEMIA IN VIVO POTENTIATE THE NEGATIVE INOTROPIC EFFECT OF VERAPAMIL?

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Studies in vivo have suggested that the negative inotropic effect of verapamil is enhanced when blood flow to the myocardium is restricted (Smith et al., 1976). The negative inotropic potency of verapamil has also been shown to increase in vitro in acidotic conditions (pH 6) (Smith and Briscoe, 1985). It is unlikely that acidosis represents the only change occurring during myocardial ischaemia in vivo. We have therefore evaluated the effect of other changes, (e.g. hypoxia, depolarisation) which may occur during ischaemia, upon the negative inotropic effect of verapamil in vitro.

Male guinea-pigs (250-400g) were killed by cervical dislocation, the whole heart rapidly removed and two papillary muscles carefully dissected from the right ventricle. These were suspended between platinum stimulating electrodes in a modified Krebs' solution of the following composition (mM) NaCl 118; $NaHCO_3$, 25; KCl, 4.7; $MgSO_4.7H_2O$, 0.6; KH_2PO4, 1.2; glucose, 11; $CaCl_2$, 1.3 bubbled with 5% CO_2 in oxygen and maintained at 32°C. The pH and PO_2 of the bathing medium were measured using an ABL1 (Radiometer) and were 7.43 $\pm$ 0.01 and 560 $\pm$ 4 mmHg respectively (n=69). The preparations were electrically stimulated at supramaximal voltage (5V) with square wave pulses of 5 msec duration. Following an equilibration period of one hour during which the preparations were stimulated at 1 Hz, the frequency of stimulation was increased to 3 Hz and the voltage reduced to threshold ($\simeq$1V). The developed tension of the preparations at this point was 144 $\pm$ 8 mg (n=138). One papillary muscle was then exposed to verapamil (10nM - 10μM), added cumulatively, 30 minutes contact being allowed at each new concentration, the other preparation being left untreated and used to correct for any spontaneous change in developed tension over the course of the experiment. Under these normal conditions the geometric mean IC_{50} (95% confidence limits) for verapamil was 107nM (80-142) (n=34). The negative inotropic effect of verapamil was also determined under conditions of hypoxia, acidosis or partial depolarisation. These conditions were achieved by bubbling the bathing medium with a mixture of 5% CO_2/40% N_2/55% O_2 (PO_2 = 354 $\pm$ 3, n=12), reducing the sodium bicarbonate content of the Krebs' solution (mild acidosis pH 6.90 $\pm$ 0.007, n=10; severe acidosis pH 6.47 $\pm$ 0.01, n=10) or increasing the KCl concentration to 8mM, respectively. Each of the interventions, themselves, reduced the force of contraction (% mean reduction $\pm$ s.e.mean) as follows: hypoxia 39 $\pm$ 4%; mild acidosis 5.1 $\pm$ 10%; severe acidosis 50 $\pm$ 8%; partial depolarisation 30 $\pm$ 5%, n=8. However none of these conditions significantly changed the negative inotropic potency of verapamil (as defined by the IC_{50}). In contrast a combination of hypoxia and mild acidosis which produced a greater negative inotropic effect than either insult alone (50 $\pm$ 3%, n=10), produced a small decrease in the IC_{50} to verapamil (75nM, 51-111, n=5) although this was not statistically significant (P = 0.07). A further combination of hypoxia, mild acidosis, and partial depolarisation (% reduction in force of contraction = 70 $\pm$ 2%, n=20) significantly potentiated the effect of verapamil, IC_{50} = 28nM (17-48, P < 0.002, n=10). In contrast the negative inotropic effect of dinitrophenol was not potentiated under this same combination of conditions.

In conclusion, no potentiation of the negative inotropic response to verapamil was observed with acidosis alone. The magnitude of the acidosis and depolarisation used in the present study, are comparable to those reported to occur in acutely ischaemic cardiac muscle in vivo (see Gilmour and Zipes, 1980). Potentiation of the effect of verapamil was only observed with a combination of these interventions, and a reduction in the partial pressure of oxygen. Thus in this in vitro model, as in vivo, it is the complex interrelationship of several factors which leads to the enhanced activity of verapamil.

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CALCIUM DEPENDENCE AND THE EFFECTS OF BAY K 8644 AND METHYLXANTHINES ON RAT AND GUINEA PIG CARDIAC MUSCLE

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Calcium entry blockers have been shown to be equipotent negative inotropic agents on rat and guinea-pig cardiac muscle whilst the intracellular antagonist, ryanodine was more active in rat than guinea-pig (Clarke et al, 1985). These results can be explained by different contributions from calcium entry and intracellular release in the two species. The present study investigates the action of the calcium entry facilitator, Bay k 8644, and the intracellular calcium modulator, caffeine, on rat and guinea-pig ventricular muscle.

Right ventricular papillary muscles were dissected from either rats (Sprague Dawley, 200 - 300 g) or guinea-pigs (Dunkin-Hartley, 200 - 300 g) and superfused with physiological salt solution (2 mM calcium) at 30°C and stimulated at 0.5 Hz as previously described (Clarke et al, 1985). Inotropic effects were determined over 15 min periods (10 min for calcium dependence).

Bay k 8644 was positively inotropic in guinea-pig papillary muscle (pEC_{200} = 7.1). However, in rat tissue the compound was less active, increasing contractility by only 58% of control at 1×10^{-6} M. A similar lack of positive inotropic activity in rat tissue at 1.5 mM has been reported by Armstrong & Ferrandon (1985), whilst at 0.7 mM calcium, Bay k 8644 was positively inotropic. We investigated, therefore, the calcium-dependence of contractility in rat and guinea-pig cardiac tissue over the concentration range 0.1 - 16 mM. Rat papillary muscle was more sensitive to extracellular calcium than guinea-pig tissue; EC_{50} values were 0.4 and 6 mM respectively. In the rat, 2 mM calcium produced 93% of maximum contractility, thus increasing calcium entry with Bay k 8644 might be expected to have little effect as we have observed. Calcium stored in the sarcoplasmic reticulum (SR) can be released by caffeine in both species (Chapman & Leoty, 1976). Caffeine (1 - 10 mM) produced marked positive inotropism in guinea-pig (pEC_{200} = 2.8) but not in rat. In the presence of ryanodine (10^{-5} M), caffeine exerted a similar positive inotropic effect in the guinea-pig. This suggests that the effects of caffeine in these experiments may not be SR-mediated. Methylxanthines are also known to be phosphodiesterase (PDE) inhibitors. The positive inotropic effects of caffeine may therefore be mediated through increased cyclic AMP levels which have been shown to promote calcium entry in cardiac muscle (Irisawa & Kokubun, 1983). Both caffeine and 3-isobutyl-1-methyl-xanthine (IBMX) have been shown to be equipotent in their ability to release calcium from the SR (Miller & Thieleczek, 1977). However, IBMX was 100 times more potent than caffeine as a PDE inhibitor in cardiac muscle (Wells & Miller, 1983). IBMX was positively inotropic in guinea-pig tissue and was 100 times more potent than caffeine (pEC_{200} = 4.9).

These data suggest that the methylxanthines exert positive inotropism in these tissues through PDE inhibition. Different sensitivities of rat and guinea-pig tissue to extracellular calcium may explain the marked positive inotropic activity of methylxanthines and Bay k 8644 in guinea-pig cardiac tissue while in rat tissue (2 mM calcium) increases in calcium entry result in relatively small increases in contractility.

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INHIBITORY EFFECT OF NIMODIPINE ON THE RELEASE OF GABA FROM RAT CEREBRAL CORTEX.

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Introduced by M.J. Neal.

Dihydropyridines potently antagonise voltage-sensitive calcium-dependent contraction in smooth muscle. Although they have been shown to antagonise calcium fluxes in neuronal preparations, micromolar concentrations are required to reduce the depolarisation-induced release of neurotransmitter, e.g. acetylcholine (Porter et al, 1985). This indicates that a different form of calcium channel may predominate on neurones with different drug sensitivity to that in muscle, but that it can still be functionally identified with dihydropyridines (Schwartz et al, 1985). We report here that nimodipine also has a dose-dependent effect on the release of GABA from cerebral cortex.

The effect of nimodipine was tested on the 34 mM K^+-evoked release of [^{14}C]-GABA from tissue slices of rat cerebral cortex. Tissue slices were preincubated with [^{14}C]-GABA (1.1 μM; 224 mCi/mmol) for 10 min, washed with isotope-free medium for 30 min and then incubated in control and test conditions as previously described (deBelleroche et al, 1982). The 34mM K^+-evoked release of [^{14}C]-GABA was significantly reduced (by 26%) by the presence of nimodipine at a concentration of 20 μM, and maximally (34%) at 56 μM (Fig. 1). The inhibitory effect of nimodipine was not seen at non-depolarising concentrations of K^+, eg 5 mM, and was much attenuated at 20 mM K^+.

These results indicate that nimodipine (20 μM and above) does have a significant effect on the calcium-dependent release of [^{14}C] in the cerebral cortex.

We are grateful to Bayer AG for the supply of nimodipine and to Dr A.D. Mitchell and Dr L. Porto (Bayer UK) for useful discussion.

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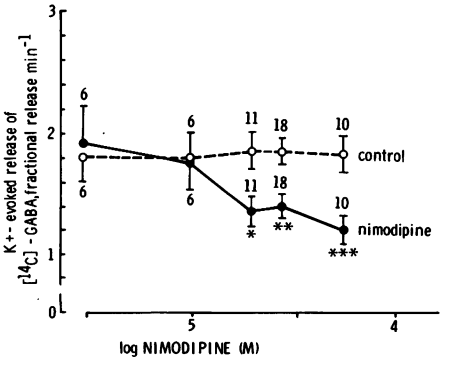

Fig.1. Values (K^+-evoked release of [^{14}C]-GABA expressed as a percentage of total remaining in the tissue at the beginning of each incubation period/min) are means±SEMs for the number of experiments indicated. Nimodipine significantly reduced the K^+-evoked release,*$p < 0.05$,**$p < 0.02$,***$p < 0.01$.

PLATELET ANTI-AGGREGATORY PROPERTIES OF BOVINE RETRACTOR PENIS MUSCLE EXTRACT.

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The inhibitory factor (IF) extracted from bovine retractor penis muscle (Ambache et al., 1975) and endothelium-derived relaxing factor (EDRF) have common physico-chemical and pharmacological properties. Both are unstable (Gillespie, Hunter & Martin, 1980; Griffith et al., 1984), anionic (Griffith et al., 1984; Cocks et al., 1985), inactivated by potassium borohydride (Gillespie, Hunter & Martin, 1980; Griffith et al., 1984) and, like nitrovasodilators, cause vascular smooth muscle relaxation by elevating cyclic GMP (Bowman & Drummond, 1984; Rapoport & Murad, 1983), which can be inhibited by methylene blue and haemoglobin (Bowman & Drummond, 1984; Martin et al., 1984). In the present study we investigated the effects of IF on platelet aggregability and compared its effects with those of sodium nitroprusside, a known inhibitor of platelet aggregation (Saxon & Kattlove, 1976). IF was prepared and acid-activated by the method of Gillespie & Martin (1980).

Human platelets were washed, prepared (Moncada et al., 1982) and suspended in Tyrode's solution to a final platelet count of 1.9-2.1 x 10^8/ml. Aggregation induced with collagen was measured by standard techniques (Born, 1962). Results are given in the Table (% increase in light transmission). Interventions were added 4 min before the collagen.

	Control	UNACT IF	ACT IF	BOILED ACT IF	ACT IF + Hb	CONTROL + Hb	NP	NP + Hb
Mean ± s.e. mean	73.2 ± 20.2	89 ± 7.8	16.8 ± 14.6	86.5 ± 11.5	33.5 ± 10.1	53.4 ± 13.7	33.4 ± 13.1	61.2 ± 4.5
n	13	6	13	6	6	8	8	6
p value		NS cf Control	<0.001 cf Control	NS cf Control	<0.05 cf ACT IF	<0.05 cf Control	<0.001 cf Control	<0.001 cf NP

Table shows % increase in light transmission in experiments with collagen alone (4 µg/ml) (Control); in the presence of activated (ACT), unactivated (UNACT) or boiled ACT IF (0.1 ml/ml) or sodium nitroprusside (NP) (10^{-5}M) with and without haemoglobin (Hb) (10^{-5}M).

Collagen-induced platelet aggregation is inhibited by acid-activated IF and NP. This inhibition is reversed by Hb. The similarities between IF and EDRF suggest therefore that EDRF has important platelet anti-aggregatory properties in addition to its effect on vascular smooth muscle.

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EFFECTS OF EXPERIMENTAL CONDITIONS ON THE ASSESSMENT OF DILATOR ACTIVITY IN ISOLATED BLOOD VESSLES.

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Rat mesentery and portal vein are frequently used 'in vitro' models of resistance vessels. We have previously shown that dilator responses of these vessels differ and that the relaxation produced depends on the agent initially used to elevate tone (El Muradi and McCurrie 1985). The present work demonstrates that the extent of relaxation depends both on the magnitude of contraction induced prior to application of dilator and on the time of its application.

Rat portal veins were suspended under 0.5g tension in oxygenated Krebs-bicarbonate containing 0.1mM ascorbic acid at 37°C. Isolated rat mesenteries were perfused with the same solution at 4ml min^{-1} at 37°C (McGregor, 1965). Three groups of experiments were performed. One constrictor and one dilator was used in each experiment. Preparations were (1) maximally constricted by noradrenaline (NA_{max}) or KCl (KCl_{max}) before application of dilator (nitroprusside, NP, 0.1-4μM for portal vein, 0.06-1μM for mesentery; Dantrolene, DANT, 30-100μM or verapamil, VP, 0.01-1μM), or (2) submaximally constricted by NA_{sub} or KCl_{sub} (approximately ED_{70}) before application of dilators, or (3) incubated with dilator for 20 mins before addition of NA_{max} or KCl_{max}. Dilatation was assessed as % reduction in vasoconstriction and as the ratio: % reduction in NA /% reduction in KCl induced constriction. This NA/KCl ratio compares dilator action on receptor and non-receptor-mediated constriction.

TABLE 1. Comparison of relaxation of maximal and submaximal contractions induced by NA and KCl.

Vasodilator	Max reduction in vasoconstriction %		Ratio of NA/KCl_{max}	Max reduction in vasoconstriction %		Ratio of NA/KCl_{sub}
Portal vein	NA_{max}	KCl_{max}		NA_{sub}	KCl_{sub}	
NP	56.2(4.8)	5.7(1.4)	9.8(1.0)	57.7(9.4)	60.2(10.8)*	1.0(0.1)
DANT	44.2(3.1)	25.6(3.5)	1.7(0.2)	77.9(11.6)*	62.0(8.7)*	1.3(0.1)
VP	86.8(14.3)	86.1(8.2)	1.0(0.2)	90.2(8.2)	86.6(4.6)	1.0(0.05)
Mesentery						
NP	79.0(12.4)	36.0(1.0)	2.2(0.4)	86.6(14.7)	49.0(3.1)*	1.8(0.2)
DANT	47.0(7.8)	9.9(3.0)	4.8(0.8)	63.2(6.9)*	7.8(1.3)	8.1(0.7)
VP	64.3(6.5)	96.5(6.8)	0.7(0.1)	74.2(9.5)*	91.6(12.4)	0.8(0.03)

Figures: mean and (S.E.M.) calculated from original data. N = 5-6.
*Different from reduction in response to maximal dose of constrictor ($P<0.05$ Mann-Whitney U-test).

The differential effects of NP and DANT on submaximal compared with maximal NA and KCl contractions are shown in Table 1. However, VP showed little change. When applied before NA_{max} and KCl_{max} DANT relaxation increased by 40% ($P<0.005$) in portal vein. However, in mesentery no relaxation could be obtained at any dose. Application of NP before KCl_{max} increased relaxation by 15% in portal vein ($P<0.001$) but decreased relaxation by 25% ($P<0.005$) in mesentery.

It is clear that the conditions in which dilators are tested have marked effects on the extent of relaxation recorded. This can complicate the assessment of dilator activity 'in vitro'.

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HEMODYNAMIC EFFECTS OF CICLOPROLOL, XAMOTEROL AND PINDOLOL IN ANAESTHETISED CATS.

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Cicloprolol (Cavero et al., 1984), xamoterol (Nuttal and Snow, 1982) and pindolol (Aellig, 1983) are compounds which can stimulate cardiac β_1-adrenoceptors and also act as β_1-adrenoceptor antagonists. The aim of this work is to compare the hemodynamic profile of these partial agonist β-blockers in cats.

Cats of either sex (3.5-4 kg) were anaesthetised with pentobarbitione (35 mg/kg i.p. and 6 mg/kg/h i.v.) and prepared for aortic blood pressure measurements (MAP) and heart rate (HR). Left thoracotomy was performed to place an electromagnetic flow probe around the aortic arch. A cannula was placed into the left ventricle through the ventricle wall. Right ventricular contractile force (RVCF) was recorded using a Walton-Brody strain gauge, and a flow probe was also placed on the left renal artery. The hemodynamic parameters calculated or recorded were MAP, left ventricular end diastolic pressure (LVEDP), the rate of rise of left ventricular pressure (dLVP/dt), cardiac output (CO), total peripheral resistance (TPR), renal flow (RF) and renal vascular resistance (RVR).

In a first study, dose response curves to cicloprolol, xamoterol or pindolol (1-1000 µg/kg) were determined. Each dose was infused over 10 min and given 10 min apart. In a second study, cicloprolol (30 µg/kg i.v.), xamoterol (1 µg/kg i.v.) and pindolol (30 µg/kg i.v.) were evaluated at equieffective inotropic doses. The effects of cicloprolol were also studied after the administration of betaxolol and ICI 118,551.

Cicloprolol over the dose range 10-300 µg/kg i.v. produced small maximum increases in MAP (15%), HR (10%), CO (10-15%), dLVP/dt (40-50%) and RVCF (40-50%) with no change in TPR or RVR. LVEDP decreased (44%) at doses inducing positive inotropic effects. In contrast, xamoterol was about 30 times more potent than cicloprolol as a positive inotropic agent with a greater maximum increase in dLVP/dt (80%). The effects of xamoterol were accompanied by a small increase in CO (10-15%). MAP, TPR, RVR (30-40%) over baseline values over the dose range (10-300 µg/kg i.v.) with little change in LVEDP. In contrast pindolol produced positive inotropic effects and increased HR, without increasing CO or MAP. The increases in dLVP/dt or RVCF evoked by cicloprolol (30 µg/kg i.v.) were antagonised (80-90%) by betaxolol (0.1 mg/kg i.v.) but were not further modified by ICI 118551 (0.3 mg/kg i.v.) and betaxolol.

This study indicates that cicloprolol can moderately enhance myocardial contractility and CO in anesthetised cats and that these effects are mediated by stimulation of β_1-adrenoceptors. Xamoterol has a greater intrinsic activity than cicloprolol, but the increase in contractility was also accompanied by a large increase in TPR. If this latter effect of xamoterol were to occur in man it would not be desirable in patients with mild cardiac failure.

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THE EFFECT OF LABETALOL ON CONTRACTILITY OF HUMAN MYOMETRIUM

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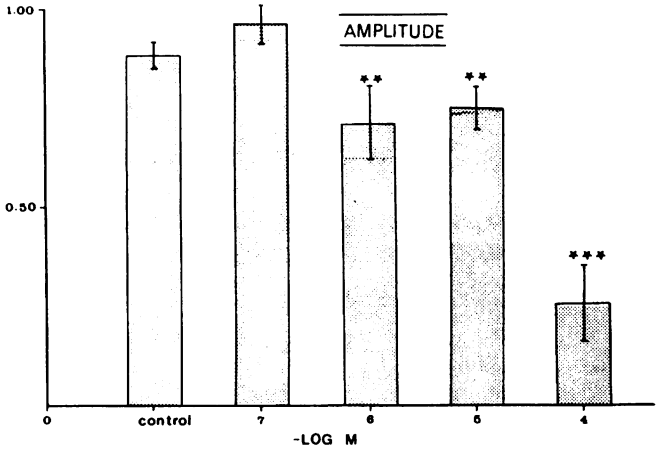
Labetalol (L) the dual alpha and beta blocker has successfully been used in the treatment of hypertension during pregnancy. The present study was designed to test the relaxant effect L on the human myometrium since it has been reported that L prolonged duration of pregnancy and labour in rats (Carey & Whalley, 1979). This effect could be explained on the basis of partial beta-2 agonist activity or alpha blockade.

Myometrial specimens were obtained at caesarean section from uncomplicated pregnancies at term. Anaesthesia was pethidine, suxamethonium, O-Tubocurarine NO_2+ O_2.

The specimens were cut into 2x6 mm sections and suspended in an organ bath filled with Krebs solution for recording of isometric tension. Rhythmic activity appeared either spontaneously or after addition of 10^{-6}M methylergometrine. L was administered in different concentrations and the effect on frequency and amplitude of contraction monitored. Control frequency was 7-14 contractions per hour. L dose-dependantly and slowly reduced amplitude but not frequency only at high concentrations with a threshold of 10^{-6}M both in spontaneously contracting and methyl-ergometrine induced experiments. Addition of the beta-2 specific beta blocker ICI 11851 (Bilski et al. 1983) at 10^{-6}M did not alter the effect of L. Phentolamine alone or in combination with L did not significantly affect uterine motility.



Labetalol has a weak tocolytic activity on human myometrium in-vitro. This effect seems unrelated to alpha- or beta- antagonism. The tocolytic effect is apparent only at high concentrations and above those used in the treatment of hypertension. It therefore seems unlikely that L should interfer with the normal process of labour when used in the treatment of pregnancy hypertension.

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α-ADRENOCEPTORS AND VASOCONSTRICTION OF THE RAT TAIL ARTERY.

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Varying degrees of the antagonism of the vasoconstrictor response to noradrenaline and other agonists by selective $alpha_1$ or $alpha_2$ antagonists have been taken as evidence for the existence of either (1) separate $alpha_1$ and $alpha_2$ adrenoreceptor subtypes (Medgett and Langer, 1984) or (2) two subtypes of the same $alpha_1$ adrenoreceptor (Marwood et al, 1985). Selective $alpha_2$ agonists have very weak effects (Hicks et al, 1984). We have investigated the potency and intrinsic activity of a series of $alpha_1$ and $alpha_2$ agonists in the perfused/superfused caudal artery of male Wistar rats (3 months of age). Arteries were perfused with Krebs bicarbonate containing cocaine ($4x10^{-6}$M), dl propranolol ($2x10^{-6}$M) and indomethacin ($2.5x10^{-6}$M) at a constant flow rate of 4 ml/min. Agonists were given in 0.1 ml bolus.

Vasoconstrictor responses were measured as changes in perfusion pressure. Bolus injections of acetylcholine (1mM) and carbachol (1mM) were unable to relax arteries constricted with infusion of noradrenaline (10^{-6}M) or phenylephrine (10^{-6}M) This is pharmacological evidence of the absence of a functional endothelium.

	Maximal vasoconstriction (mmHg)	ED_{50} (M)	Slope (logit/log)
Methoxamine (MET) ($alpha_1$, n=4)	310±16	$6.6±0.1x10^{-5}$	3.3±0.2
Phenylephrine (PHE) ($alpha_1$, n=10)	228±22	$8.4±0.3x10^{-6}$	2.3±0.3
Noradrenaline (NOR) ($alpha_1$, $alpha_2$, n=16)	226±20	$3.0±0.1x10^{-6}$	2.6±0.2
Guanfacine (GUAN) ($alpha_2$, n=6)	210±22	$6.3±0.2x10^{-5}$	3.4±0.4
Clonidine (CLO) ($alpha_2$, n=41)	114±19	$1.6±0.1x10^{-5}$	3.6±0.3
UK14304 (UK) ($alpha_2$, n=4)	23±7	$1.1±0.2x10^{-4}$	1.6±0.2
BHT933 ($alpha_2$, n=6)	no vasoconstriction up to 0.1M		

Agonists cannot be easly separated into two categories ($alpha_1$ and $alpha_2$) according their potency (NOR>PHE>CLO>GUAN>MET>UK) or intrinsic activity (MET>PHE>NOR>GUAN>CLO>UK). The agonists with the highest intrinsic activity are selective $alpha_1$ agonists. GUAN has an intrinsic activity almost as high as that of $alpha_1$ agonists, whereas CLO has weak intrinsic activity. The results obtained with clonidine were very variable. The affinities of both CLO and GUAN are greater than of MET and their dose- response curves are no shallower than those of selective $alpha_1$ agonists.

The highly specific $alpha_2$ agonist UK14304 has a low affinity, and shallow dose-response curve. Another specific $alpha_2$ agonist BHT933 did not induce vasoconstriction.

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RESPONSES OF THE ISOLATED RAT PORTAL VEIN TO α-ADRENOCEPTOR AGONISTS IN DIFFERENT O_2 TENSIONS

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We have previously shown that employing physiological arterial PO_2; "normoxia" (16%O_2: 100-120mmHg) in isolated smooth muscle preparations makes little difference to pharmacological analysis of agonist-antagonist interaction at alpha$_1$-adrenoceptors (Fasehun et al, 1985). However, there were qualitative differences in the responses of the tissues to noradrenaline in hyperoxia (bubbled with 95% O_2: PO_2 >580mmHg) compared with normoxia. The response in normoxia was usually biphasic with a phasic component lasting for about 15s and followed by a tonic contraction which was not sustained at lower concentrations, 3nM-300nM, but was at 1uM-10uM. The response in hyperoxia on the other hand was monophasic.

In the present study we have examined the responses of the isolated rat portal vein to noradrenaline over a wider range of PO_2 including a lower PO_2 (8%:70±5mmHg) which is still higher than physiological interstitial levels, and at further hyperoxic levels (32%:214±7mmHg and 64%:412±15mmHg). We have also compared the effect of selective alpha$_1$-agonists, phenylephrine and amidephrine and also alpha$_2$-agonists xylazine and UK14304 in normoxia and hyperoxia.

Male Wistar rats (245-255g wt) were used in this study. Isometric contractions were recorded from longitudinal strips of the rat isolated portal vein placed under a resting tension of 1g, in Krebs bicarbonate saline at 37°C equilibrated with 16%O_2, 5%CO_2, 79%N_2 for 90 min. The gas was then replaced by a gas mixture of required O_2 tension, 5%CO_2 (to maintain a constant pH) and the balance made up with N_2.

Noradrenaline (3nM-10uM) induced a concentration related response in the different gas tensions - 8% to 95%. The tissue is spontaneously active and the "response" changes from being essentially an increase in this activity at low concentrations of NA to a fused contracture at higher levels. Here, we describe the changes in the height of the "spikes" or the level of contracture size since the qualitative description is similar. Responses were monophasic at 95% and 64% O_2 reaching a maintained level by 2 min. At 32% responses start to become biphasic with the separation of an early rapid component peaking by 15 sec and declining before the re-establishment of the maintained phase. This early component became steadily more apparent in moving to 16% and then 8%. The heights of maintained responses were similar at 95% to 32% but became progressively smaller at lower PO_2.

Phenylephrine and amidephrine, over the same concentration range as for NA, showed the same change in pattern of responses as did NA between 16% and 95%. In either 95% or 16%, xylazine and UK14304 caused an increased phasic activity of the tissue without a contracture , and this only at high concentrations (1uM-100uM). Two phases could not be discerned.

There is controversy on the subtype of adrenoceptors present in this tissue .It has been suggested that part of the response is due to alpha$_2$-adrenoceptors (Hicks 1983). However in our hands the responses to these agonists are susceptible to prazosin, 0.01uM. Therefore the responses induced by xylazine and UK14304 are regarded as due to alpha$_1$-adrenoceptors. In general these responses to partial agonists are unaffected in the different O_2 tensions. The difference in responses induced by noradrenaline, phenylephrine and amidephrine in normoxia and hyperoxia might be due to differences in excitation-contraction coupling in the different O_2 tensions. It is interesting that this occurred only with agonists which have high intrinsic activities and not with the partial agonists tested. Perhaps the contracture to high concentrations of full agonists should be regarded in this tissue as an unphysiological artefact induced by high O_2 tension; and so the physiological mechanism may be obscure by the use of high O_2 tension.

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EFFECTS OF PERGOLIDE ON THE TRANSIENT NEUROGENIC HYPERTENSION IN DOGS DEPRIVED OF CENTRAL INPUT TO THE ADRENAL GLANDS.

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Pergolide, a DA_2 dopamine receptor agonist, reduced blood pressure in anaesthetised dogs which had been made hypertensive by sino-aortic deafferentation. This effect was accompanied by a decrease in the plasma concentration of adrenaline and noradrenaline which were markedly enhanced by deafferentation (Cavero et al., 1985). In this communication, we present the effects of pergolide on neurogenic hypertension in dogs with intact or decentralised adrenal glands.

Dogs were anaesthetised with pentobarbitone (35.0 mg/kg, i.v.), placed under artificial respiration and prepared for aortic blood pressure and heart rate measurements. Deafferentation was achieved by section of the vagi and sinus carotid nerves. In a group of dogs, prior to deafferentation, the adrenal glands were decentralised by severing all visible splanchnic nerves around them. Plasma noradrenaline and adrenaline were measured before and 5 min after deafferentation. Pergolide (30.0 µg/kg, i.v.) or saline (0.4 ml/kg) were administered 15 min before deafferentation. In a few preparations, sulpiride (0.5 mg/kg plus 0.05 mg/kg/min i.v.) was given 10 min before pergolide.

Decentralisation of the adrenal glands did not change mean aortic blood pressure. In dogs with denervated adrenal glands, a maximal increase in blood pressure was attained 5 min after deafferentation (48 $\pm$ 3 mmHg; initial value: 134 $\pm$ 6 mmHg, n=6). This effect waned entirely within the subsequent 25 min. In contrast, in dogs with innervated adrenal glands, the increase in blood pressure was maximal (63 $\pm$ 6 mmHg; initial value: 141 $\pm$ 4 mmHg, n=8) 10 min after the deafferentation procedure and was long lasting (44 $\pm$ 4 mmHg 60 min later). The large increase (1600%) in plasma adrenaline evoked by deafferentation in intact dogs was greatly inhibited (86%) by the decentralisation of the adrenal glands. In dogs with either intact or decentralised adrenal glands, pergolide induced a similar initial rise in blood pressure (18 $\pm$ 3 mmHg, n=14) which returned to the base-line value 15 min later at which time the deafferentation procedure was performed. In dogs with decentralised adrenal glands, pergolide inhibited entirely the hypertensive response and the plasma noradrenaline and adrenaline increases evoked by deafferentation. However, in dogs with intact adrenal glands, pergolide reduced by 41 and 63% the increase in blood pressure measured 10 and 60 min after deafferentation in the control group. In this preparation only plasma adrenaline increase was significantly inhibited by pergolide. Sulpiride antagonised the inhibitory effects of pergolide in these preparations.

These results indicate that decentralisation of the adrenal glands reduces in magnitude and duration the neurogenic hypertension associated with deafferentation. Thus, an operational innervation of the adrenal glands which mediates a sustained elevated release of adrenaline is necessary to maintain neurogenic hypertension in anaesthetised dogs. Furthermore, pergolide pretreatment is more effective at inhibiting the neurogenic hypertension in dogs with decentralised than with intact adrenal glands. Finally, this effect of pergolide is mediated by DA_2 dopamine receptors since it was blocked by sulpiride.

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HYPOTENSION INDUCED BY CENTRAL INJECTION OF ISOPRENALINE AND CLENBUTEROL: MODIFICATION BY CENTRAL PROPRANOLOL.

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Hypotension has been reported to follow icv injection of isoprenaline in anaesthetised animals of various species (Gagnon & Melville, 1967; Toda et al, 1969; Bhargava et al, 1972). In these experiments we have attempted to elucidate the mechanism involved by comparing the effects of two β-adrenoceptors agonists, isoprenaline and clenbuterol, administered centrally to the anaesthetised rat. Modifications of their effects by prior central and peripheral injection of the β-adrenoreceptor antagonist, propranolol, was also investigated.

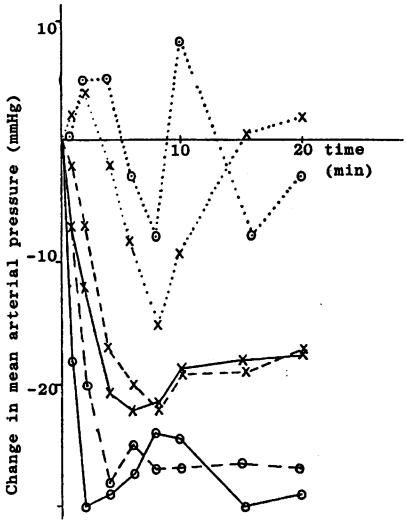

Fig. 1. Change in mean arterial pressure produced by ICV isoprenaline 5μg (x——x) and clenbuterol 5μg (o——o). Modification by propranolol 30μg ICV (x....x, o....o) and 12μg iv (x----x, o----o). (n = 6).

Male New Zealand normotensive rats (190-200g) were anaesthetised with Hypnorm/midazolam (Flecknell & Mitchell, 1984). The left carotid artery and right jugular vein were cannulated to allow measurement of blood pressure and injection of drugs respectively. A guide cannula was inserted into the left cerebral ventricle and drugs were injected icv in a volume of 5μl over 2.5min.

The dose-dependent hypotension produced by both isoprenaline and clenbuterol icv was resistant to prior injection of 12μg propranolol iv (Fig.1). Pretreatment with 30 g propranolol icv 15 min before the agonist abolished the depressor response to clenbuterol, but only partially prevented the hypotension produced by isoprenaline (Fig. 1). This confirms earlier reports (Nomura, 1976; Peres-Polon and Correa, 1984) that in the anaesthetised rat, the depressor response to central isoprenaline is resistant to propranolol, suggesting that a significant element of the depressor response of isoprenaline is not mediated through β-adrenoceptors. There is, of course, always the possibility, following icv injection, of leakage of drug to the periphery. However, central injection of ^{3}H-propranolol and ^{3}H-isoprenaline with subsequent analysis of blood and tissue isotope levels indicated that, for instance, in terms of the amount of drug available to the periphery, 12μg propranolol iv was equivalent to 30 μg propranolol icv.

Taken together, these results support the hypothesis that the central depressor response to isoprenaline comprises two elements; one mediated through β-adrenoceptors and mimicked by clenbuterol and the other probably mediated through humoral mechanisms.

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THE EFFECTS OF CADMIUM ON THE RESPONSE OF THE RAT ANOCOCCYGEUS PREPARATION TO NORADRENALINE AND ELECTRICAL STIMULATION

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Cadmium (Cd^{2+}) is a toxic ion known to accumulate in the soft tissues of man (Webb, 1975), and has been shown to have inhibitory effects on enzymes associated with noradrenaline metabolism (Cameron et al, 1985). Furthermore, the physico-chemical characteristics of Cd^{2+} are similar to those of calcium, an ion known to be involved in the transmitter release. The possibility exists, therefore, that Cd^{2+} may well alter noradrenaline action in the sympathetic nervous system, by modifying its release from nerve endings in response to electrical stimulation.

The anococcygeus preparation of the rat, first described by Gillespie (1972), provides a suitable model for the study of the effects of drugs on the sympathetic nervous system. Therefore, we have studied the effects of cadmium on the response of the rat anococcygeus to both added noradrenaline and electrical stimulation in an attempt to further elucidate the mechanism of action of this ion at the sympathetic neuroeffector junction.

Adult male Wistar rats (200-600g) were killed by cervical dislocation and exsanguination. Anococcygeus preparations were dissected out and set up under 0.5g resting tension in 50ml isolated organ baths containing either normal, or modified (4.4mM Ca^{++} or 1.1mM Ca^{++}) McEwens solution (McEwen, 1956) maintained at 37°C and gassed with 95% O_2/5% CO_2. Developed muscle tension was measured using a Grass FTO3 force-displacement transducer connected to a Grass 79D polygraph. Electrical stimulation of the preparation (140V, 500μs, 5Hz, 10s duration) was provided by parallel platinum wire electrodes attached to a Harvard stimulator.

The addition of low concentrations of Cd^{2+} (1-10μM) to the bath produced a slow-onset, concentration-dependent inhibition of the response of the rat anococcygeus to electrical stimulation which, at concentrations above 5μM reached 100% blockade after 9-12 min. However, even when the Cd^{2+} blockade of electrical stimulation was fully established the tissue response to added noradrenaline was only slightly reduced.

Incubation of the tissues in McEwens solution containing either 1.1mM or 4.4mM calcium did not significantly alter the tissue response to electrical stimulation. However, changes in the calcium concentration of the bathing medium altered the inhibition of electrically-induced twitches due to Cd^{2+}. The presence of 4.4mM calcium reduced the inhibition of the electrically induced responses produced by 2μM Cd^{2+} from 65 ± 3.6% to 17 ± 4% (n=4), whereas incubation of the tissue in 1.1mM calcium increased the inhibition produced by this ion from 65 ± 3.6% to 81 ± 8% (n=4).

The results presented here suggest that, initially, low concentrations of Cd^{2+} may alter neurotransmitter function at the sympathetic neuroeffector junction, possibly by interfering with the calcium-mediated release of noradrenaline.

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KYNURENIC ACID REDUCES MEDIAL PERFORANT PATH QUANTAL SIZE IN RAT DENTATE GYRUS

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Kynurenic acid is a tryptophan metabolite which has been shown to antagonise the effects of excitatory amino acids in the central nervous system (Perkins & Stone, 1982; Stone & Connick, 1985) and to block neuronal pathways thought to utilise an excitatory amino acid transmitter. In the present study we have sought to define the mechanism by which kynurenate blocks the medial perforant path input to dentate granule cells by obtaining intracellular records of the excitatory postsynaptic potential (e.p.s.p.) and subjecting these to quantal analysis.

Slices of hippocampus (400μm thick) were prepared from Wistar rats using a tissue chopper. The slices were maintained in a bicarbonate buffer of the following composition (mM): NaCl 134; KCl 5; KH_2PO_4 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 16; D-glucose 10, gassed with 5% CO_2 in O_2 for at least 1 hour at 36°C. After this time cells were impaled using potassium acetate filled electrodes (70-140 Mohms). The medial perforant path was stimulated through coarse glass micropipettes containing 1M NaCl (3-4μm tip diameter) using 20μs pulses at 1 Hz and an amplitude sufficient to evoke e.p.s.p.s of 5-15mV. Compounds were applied by microiontophoresis from independently mounted multibarrelled micropipettes containing 1M glutamate and 0.1M kynurenic acid. The iontophoretic pipette was moved so that a brief pulse of glutamate (<1s) induced a rapidly rising depolarisation of about 5mV.

Results from 15 cells (resting potential 59.5 ± 2.17mV) showed that the iontophoretic ejection of kynurenate reduced the e.p.s.p. amplitude with a latency of 8-10 seconds, with no evidence of any change in membrane conductance. Quantal analysis of the data using a PDP11/23 computer revealed that kynurenic acid depressed the mean quantal size from a control of 0.24 ± 0.02mV to 0.11 ± 0.01mV ($P<0.01$; $n = 15$) whereas it did not alter the number of quanta released per stimulus (control 40.2 ± 1.9; kynurenate 41.6 ± 2.2). This result implies that the blockade by kynurenate of medial perforant path e.p.s.p.s is mediated almost exclusively at postjunctional sites on the granule cells.

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RESPONSES TO TACHYKININS IN THE TETRODOTOXIN TREATED HEMISECTED NEONATAL RAT SPINAL CORD IN VITRO

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The mammalian tachykinins substance P, (SP) neurokinin A (NKA) and neurokinin B (NKB) and a tachykinin analogue [D-Arg^{1} D-Trp^{7,9}Leu^{11}]-SP (SPA-1), which is a tachykinin antagonist on smooth muscle, depolarise motoneurones of the neonatal rat in vitro (Matsuto et al. 1984; Brown et al 1985). The concentration-response curve to SP is shifted to the right in the presence of tetrodotoxin (TTX) (Otsuka & Yanagisawa, 1980) suggesting that a component of the response may be mediated indirectly via interneurones. Ninkovic et al (1984), have reported that binding sites for I^{125}-Bolton Hunter Eledoisin, a ligand for "SP-E like" receptors are localised in the dorsal horn, whereas binding sites for I^{125}-Bolton Hunter-SP, a ligand for "SP-P like" receptors are localised throughout the grey matter of the spinal cord. These observations raised the possibility that NKA and NKB, the proposed endogenous ligands for "SP-E like" receptors, might depolarize motoneurones indirectly via activation of neurones in the dorsal horn. We have therefore investigated the effect of TTX treatment on the responses to SP, NKA, NKB & SPA-1 in the hemisected neonatal rat spinal cord in vitro to determine whether there is an indirect component of the response for each compound.

Sprague-Dawley rats (1-5 days old) were decapitated and the spinal cord was dissected free along with intact ventral roots. The cord was hemisected sagitally, mounted between layers of absorbent paper and superfused with oxygenated Krebs' solution (2ml/min; 21°C). A ventral root was electrically isolated from the rest of the preparation by a layer of liquid paraffin/petroleum jelly. The D.C. potential difference across this barrier was recorded via Ag/AgCl electrodes and displayed on a chart recorder, (Brown et al 1985). Serial concentration-response curves were constructed to either SP, NKA or NKB. Blockade of synaptic transmission was then achieved by adding TTX, 4μM, to the superfusion medium for 5 mins, and maintained thereafter with TTX, 0.5μM.

Over the concentration range, 10-3000nM, SP, NKA and NKB elicited concentration dependent depolarisations of the ventral root. In the presence of TTX there was a modest parallel shift to the right of the concentration-response curves to SP, NKA and NKB. The following concentration ratios (± s.e.m.) were measured at the approximate EC_{50} level: SP, (EC_{50}, 0.15μM) 3.87±0.77, n=4; NKA, (EC_{50}, 0.45μM), 2.41±0.14, n=4; NKB (EC_{50}, 0.45μM), 8.5±1.56, n=6.

SPA-1, 10-30μM, also depolarised the ventral roots but caused a long-lasting tachyphylaxis and it was therefore not possible to obtain concentration-response curves. SPA-1, 10-30μM, failed to antagonise SP, indeed, responses to SP were often potentiated. Similar results were obtained in the presence of TTX, SPA-1, 10-30μM, depolarized the ventral roots and potentiated responses to SP.

These results confirm the previous result with SP and suggest that the indirect (TTX-sensitive) component makes a relatively minor contribution to the response of neonatal rat motoneurones in vitro to NKA, NKB and SPA-1. The agonist activity of SPA-1 provides further evidence that neuronal tachykinin receptors are different from those on smooth muscle.

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A1 ACIDIC AMINO ACID RECEPTORS: RECOGNITION SITE SPECIFICITY

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The A1 (N-methyl-D-aspartate [NMDA]-preferring) sub-type of excitatory amino acid receptor has been implicated in a wide range of brain mechanisms, including long-term potentiation, seizure activity, spasticity and neurodegeneration (Fagg, 1985). Recent studies have shown that this receptor may be labeled in vitro using L-^{3}H-glutamate (L-Glu; Fagg & Matus, 1984) or other A1 receptor radioligands (Olverman et al., 1984; Fagg & Foster, 1985) and that it is enriched in postsynaptic densities (PSDs) isolated from the rat brain. In the present study, we utilized the L-^{3}H-Glu binding technique to directly examine the molecular specificity of the A1 receptor recognition site.

L-^{3}H-Glu bound reversibly to rat brain PSD fractions with Kd 0.3 µM. Specific binding at 50 nM L-^{3}H-Glu (non-specific determined using 0.5 mM L-Glu) comprised 87% of total binding, of which 80-90% was sensitive to inhibition by A1 receptor ligands such as NMDA (Ki 6.1 µM), DL-2-amino-5-phosphonopentanoate (Ki 2.5 µM) and DL-2-amino-7-phosphonoheptanoate (Ki 11.9 µM). Amongst a series of α-amino-dicarboxylic acids, L-Glu was the most potent inhibitor at A1 binding sites (Ki 0.22 µM), followed by L- and D-aspartate (Ki's 1.8 and 2.1 µM, respectively) and D-α-aminoadipate (Ki 24 µM). D-Glu, L-α-aminoadipate and the longer chain pimelate and suberate homologues were weaker. Inhibitory activity was abolished by removal of the amino moiety (succinate, glutarate) and, in the case of D-aspartate, was reduced by N-alkylation with groups of increasing size (Ki values: methyl, 6.1 µM; ethyl, 31 µM; propyl, 45 µM). Loss, or replacement of the α-carboxyl function of DL-Glu with phosphino or phosphono groups abolished inhibitory potency, while substitution of the ω-carboxyl terminal with other acidic groups yielded weaker analogues (Ki values: carboxyl, 0.46 µM; sulphonyl, 2.4 µM; phosphino, 9.1 µM; phosphono, >50 µM). Cyclic analogues (e.g., cis-2,3-piperidine dicarboxylate, quinolinic acid) were generally weak inhibitors of A1 receptor binding, although activity was retained in compounds with optimized configuration (trans-2,3-piperidine dicarboxylate, Ki 5.6 µM). Substances active at other receptor sites (e.g., quisqualate, γ-D-glutamylaminomethylsulphonate, phencyclidine, ketamine) were weak or inactive at A1 sites. GTP inhibited the binding of L-^{3}H-Glu to A1 receptors with Ki 21 µM, whereas other nucleotides were weak or inactive.

This study demonstrates that the radioligand binding approach will facilitate both the expansion of the "structure-activity" data base for A1 excitatory receptors, and the development of novel therapeutic agents active at this site. Our data show that A1 receptors exhibit properties distinct from those of other excitatory amino acid receptors and, further, that phencyclidine-like drugs antagonize NMDA-evoked neuronal responses (Anis et al., 1983) at a site remote from the A1 receptor recognition site. Additional experiments are necessary to determine whether the inhibitory effect of GTP reflects a link to an intracellular "second messenger" system via a GTP binding protein.

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INHIBITION BY CATECHOLAMINES OF CA-SPIKE IN RAT PREGANGLIONIC CERVICAL SYMPATHETIC NERVES

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Catecholamines inhibit transmitter release in mammalian superior cervical ganglia (e.g. Dun & Karczmar, 1977). Their mechanism of action is uncertain, but one possibility is that they reduce presynaptic Ca^{2+} conductances. To test this I have assessed their effect on Ca-spikes in rat preganglionic cervical sympathetic nerves.

Extracellular recordings from desheathed preganglionic nerve trunks were made using a 3-chamber bath (Brown & Marsh, 1978). Ca-spikes were revealed by blocking the Na^{+}-spike with 0.5-1μM tetrodotoxin and adding 1mM 4-aminopyridine (Scholfield,1984). The preparation was stimulated supramaximally with 1 ms duration rectangular pulses at 0.017 Hz at room temperature. The modified Krebs solution used had the composition (mM): NaCl(118), KCl(6), $CaCl_2$(5), $MgCl_2$(1.2), ascorbate(0.5), Hepes(10) at pH 7.4 and bubbled with 100% O_2.

L-noradrenaline (NA) produced a rapid reversible depression of the Ca-spike, with an average maximal depression of 90% and an ED_{50} of 1.5μM. The potencies of some other agonists are given in Table 1. Yohimbine (1μM,n=4) and prazosin (10μM,n=2) had no effect on the response to 10μM NA. Phentolamine was the most potent antagonist tested; a Schild plot (4 points) gave a pA_2 value of 6.47±0.067 with a slope of 0.653±0.077. Propranolol had no effect at 0.5μM.

In conclusion, catecholamines inhibit the Ca-spike in preganglionic fibres. This may explain their action in presynaptic inhibition.

Table 1

Pooled data for dose-response curves analyzed by the method of Parker & Waud, (1971) (mean±s.e.mean)

Agonist	Max. depression	ED_{50}(μM)	Slope factor	n
Clonidine	76.1± 4.9	0.44± 0.11	0.739±0.246	5
L-Adrenaline	92.6±12.1	1.3 ± 0.74	0.633±0.315	7
L-Noradrenaline	88.8± 5.6	1.52± 0.32	1.057±0.414	4
Dopamine	66.9± 6.8	45.9 ±23.6	0.541±0.258	3
L-Phenylephrine	61.6± 5.7	154.3 ±50.4	0.814±0.401	3
Amidephrine	<50	>10mM		

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DIRECT EFFECTS OF DIAZEPAM AT THE NEUROMUSCULAR JUNCTION

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Benzodiazepines form a group of centrally acting drugs, which also cause skeletal muscle relaxation. The mechanism underlying the latter effect so far remains unknown, but it is generally considered to originate in the central nervous system, in contrast to the neuromuscular blocking drugs, which act primarily at the neuromuscular junction. Diazepam and other benzodiazepines are widely used as skeletal muscle relaxants in a variety of spastic states.

Little is known about eventual direct effects of benzodiazepines on neuromuscular transmission. There are some - although conflicting - reports with regard to such effects. Effects of diazepam on the transmission in in vitro nerve-muscle preparations have been described as absent (Crankshaw and Raper, 1968), potentiating (Moudgil and Pleuvry, 1970) and inhibitory (Vyskocil, 1977, 1978). Driessen et al. (1984), investigating several benzodiazepine derivatives, found a biphasic action of diazepam on the phrenic nerve-diaphragm preparation.

We investigated possible peripheral effects of diazepam on synaptic vesicle size at the presynaptic site of the phrenic nerve-diaphragm preparation, at the electronmicroscopic level. If such effects do exist indeed, they might be correlated to observations made in vitro, either by measuring the twitch tension or by testing electrophysiological properties.

Female rats (Cpb WU:WI), 170-200g, were injected with 1.0, 2.5, 5.0 or 10.0 mg/kg diazepam, respectively. These dosage levels produce dose-dependent sedation but no loss of righting reflex. Animals were killed at several intervals after treatment. Control animals were treated with propylene glycol. Hemidiaphragms were dissected, prepared for electronmicroscopic study, and the cross sectional area (CSA) of the synaptic vesicles measured as previously described (Leeuwin et al., 1983).

The results can be summarized as follows. As compared to the average control CSA value, a dose of 1 mg/kg diazepam produces a progressive and highly significant ($P<0.005$) increase of the CSA of the synaptic vesicles with time. The longer the treatment interval, the larger the vesicle size. The same tendency is observed using 2.5, 5.0 and 10.0 mg/kg diazepam. There also exists a significant ($P<0.05$) dose-effect relationship at comparable time intervals.

Using the phrenic nerve-diaphragm preparation cumulative as well as single doses of diazepam have been tested in preliminary experiments. At relatively low doses the twitch tension is increased, although depressed at higher doses and eventually completely blocked. After washing the twitch tension gradually recovered. At present it can only be concluded tentatively that diazepam does seem to interfere directly with (presynaptic) events at the neuromuscular junction. The increase in vesicle size may be correlated to the increase in twitch tension at low concentrations. The latter may be a result of the increase in quantal content, as observed by van Wilgenburg (1986).

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ENDOGENOUS ADENOSINE MODULATES NEURONAL ACTIVITY IN HIPPOCAMPAL AREA CA3

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Extracellular burst potentials recorded in area CA3 of the hippocampal slice are a useful in vitro model of epileptic activity. We have previously observed that theophylline, at concentrations that antagonize the effect of exogenous adenosine, increases the rate of epileptiform discharge in the presence of bicuculline and can induce bursting when applied to slices at a concentration of 30 μM. In these experiments we have extended our investigation of the pro-convulsant action of theophylline and compared the effects of theophylline and caffeine.

Low resistance (2-5 megohm) glass microelectrodes were used to record spontaneous burst potentials from area CA3 or population spikes evoked by stimulation of Schaffer-commissural fibres in area CA1. Slices were superfused at 1.2-1.5 ml per min with Elliot's medium (containing 3.5 mM K+, 1.3 mM Ca2+) which was maintained between 30 and 31 °C.

Kainic acid (10 nM) induced bursting at rates from 3-11 per min (n=12). When superfused in addition to kainic acid, theophylline (0.3-30 μM, n=4) or caffeine (1-100 μM, n=4) increased the rate of discharge in a dose dependent manner, with the hghest doses producing a threefold enhancement. These results were similar to those using bicuculline as a convulsant.

Slices incubated in normal medium did not exhibit spontaneous or electrically-evoked bursting. In 29 such cases, theophylline (1-30 uM) or caffeine (3-100 μM) were applied in a cumulative manner to determine the threshold dose necessary to induce bursting. From the dose response plots, it was calculated that 7 μM theophylline and 17 μM caffeine would induce bursting in 50% of slices. In contrast to the convulsant effect of theophylline in area CA3, concentrations up to 100 μM only induced a small secondary population spikein area CA1 (n=4).

These data suggest that endogenous adenosine physiologically serves to limit or suppress epileptiform activity in the hippocampus and indicate that this effect is selective for area CA3.

β_2-ADRENOCEPTORS MEDIATE THE INSULINOTROPIC AND HYPERGLYCAEMIC ACTIONS OF ISOPRENALINE IN FASTED RATS

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Beta-adrenoceptor involvement in plasma glucose metabolism and insulin release has been investigated after isoprenaline in the presence of either selective $\beta_1$adrenoceptor blockade (atenolol or betaxolol), β_2-adrenoceptor blocked (ICI 118551), or non-selective blockade (propranolol), in conscious, fasted rats.

Male albino Sprague-Dawley rats (Olac, 250-420g) were implanted with permanent aortic cannulae (Popovic and Popovic, 1960), and allowed a 48 hour minimum recovery period before being fasted for 18-22 hours. A control blood sample (0.6 ml) was taken before drug administration (s.c.); antagonists (or 0.9% saline vehicle) were injected 15 minutes before isoprenaline (or 0.9% saline vehicle) injection (0 minutes). Further blood samples were taken at 20, 40, 60 and 120 minutes after the injection of isoprenaline. Each experiment consisted of a 0.9% saline control group, an isoprenaline group and a group which received both isoprenaline and an antagonist (n = 4-7 in each). Plasma glucose was determined by an Analox GM6 analyser; and plasma insulin was measured by radioimmunoassay (Novo Rat Insulin Kit). The AUC's of responses were compared by the Neuman-Keuls parametric test for significant differences between groups.

Isoprenaline (200 µg/kg) caused a mild hyperglycaemia (+3.0 mM/1; $p < 0.01$) with a concomitant marked initial rise in plasma insulin levels (+1.5-2.5 ng/ml after 20 minutes, $p<0.01$) followed by a gradual return to baseline. Atenolol (1.0 mg/kg) and betaxolol (1.0 mg/kg) appeared to potentiate slightly these responses to isoprenaline, however the responses were not significantly different from those to isoprenaline alone. Both ICI 118551 (1.0 mg/kg) and propranolol (1.0 mg/kg) significantly attenuated the hyperglycaemic and insulinotropic responses to isoprenaline ($p<0.01$). None of the antagonists given alone significantly affected basal plasma insulin or glucose levels.

It is concluded that in conscious fasted rats, isoprenaline-induced elevations in plasma glucose and insulin levels are mediated by β_2-adrenoceptors. β_1-adrenoceptors do not appear to have a significant role under these experimental conditions.

Gareth John holds an SERC CASE award in collaboration with Reckitt and Colman plc.

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α-ADRENOCEPTORS IN THE RAT COLON

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Previous reports have suggested that the rat colon contains excitatory α-adrenoceptors (Regoli & Vane, 1964; Belisle & Gagnon, 1971). Recently it has been shown that the mouse colon contains both inhibitory postjunctional α_1-adrenoceptors and inhibitory prejunctional α_2-adrenoceptors located on non-adrenergic, non-cholinergic neurones (Fontaine et al, 1984). In the present study we investigated the effects of selective α-adrenoceptor agonists and antagonists on the longitudinal smooth muscle of the rat colon in an attempt to characterise the α-adrenoceptor subtypes present.

Segments of rat distal colon were removed and placed in 30 ml organ baths containing Krebs' medium at 37°C. In all experiments propranolol (2μM) was present in the medium throughout. In experiments involving noradrenaline the Krebs also contained cocaine (3 μM), hydrocortisone (30 μM), ascorbic acid (30 μM) and EDTA (30 μM).

Normally the rat colon exhibited little spontaneous activity. Noradrenaline (0.01-0.03 μM) and UK-14,304 (0.01-0.1 μM) both induced phasic contractions, the amplitude and frequency of which were independent of the dose of the agonists used. Cirazoline (0.01-1 μM) produced no excitatory effects in the rat colon. These phasic contractions induced by noradrenaline and UK-14,304 were antagonised by idazoxan (0.01-0.1 μM) and by rauwolscine (0.01-0.1 μM) but unaffected by prazosin (0.01-1 μM). The α-adrenoceptor antagonists alone had no effect. The ability of noradrenaline and UK-14,304 to induce phasic contractions in the rat colon persisted in the presence of guanethidine (5 μM) and hexamethonium (1-100 μM). Naloxone (0.1-10 μM) had no effect on noradrenaline-induced or UK-14,304-induced phasic activity. Higher concentrations of noradrenaline (0.1-1 μM) and all concentrations of cirazoline (0.01-1 μM) inhibited phasic contractions induced either by noradrenaline or UK-14,304 and this inhibition was reversed by prazosin (0.1 μM). Tetrodotoxin (TTX, 0.1-1 μM) also induced phasic activity similar to that produced by noradrenaline and UK-14,304. TTX-induced activity was unaffected by idazoxan (0.01-0.1 μM), prazosin (0.01-0.1 μM) and UK-14,304 (0.01-1 μM) but was abolished by cirazoline (0.01-1 μM). The inhibition of TTX-induced activity by cirazoline was reversed by prazosin (0.1 μM).

In conclusion, motility of the rat colon is under the influence of an inhibitory neural mechanism which appears to be mediated via non-adrenergic, non-cholinergic nerves. Activation of α_2-adrenoceptors situated prejunctionally on the non-adrenergic, non-cholinergic neurones reduced this inhibitory influence. This is similar to the situation in the mouse colon (Fontaine et al, 1984) and is further evidence for the presence of inhibitory prejunctional α_2-adrenoceptors on non-adrenergic, non-cholinergic nerves in the gastrointestinal tract (Fontaine et al, 1984; Dettmar et al, 1984; Dettmar et al, 1985). In addition to prejunctional α_2-adrenoceptors, inhibitory postjunctional α_1-adrenoceptors were also found in the present study. No evidence for excitatory postjunctional α_1-adrenoceptors or for postjunctional α_2-adrenoceptors, either excitatory or inhibitory was found.

J.K. holds an SERC CASE award in collaboration with Reckitt & Colman plc.

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REGULATION OF THE CELL CONTENT AND RELEASE OF OPIOID PEPTIDES AND CATECHOLAMINES IN BOVINE ADRENAL CHROMAFFIN CELLS

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Secretory granules of bovine adrenal chromaffin cells contain opioid peptides as well as noradrenaline and adrenaline. Nicotinic receptor stimulation causes the release of both catecholamines and peptides and these cells have thus been used as a model for the co-storage and co-release of these two classes of neurotransmitter. It has been reported previously that culture under conditions which raise cAMP levels increases total opioid peptide levels (Eiden et al. 1984). Here we report the effects of culturing cells in dibutyryl cAMP or forskolin on the cellular levels of noradrenaline, adrenaline and (met)-enkephalyl-Arg^6-Phe^7-immunoreactivity (IR). This radioimmunoassay cross-reacts to the same extent with both peptide B, which is the C-terminal fragment of proenkephalin, and its cleavage product, the heptapeptide (met)-enkephalyl-Arg^6-Phe^7. The two immunoreactive forms can be separated by gel permeation chromatography. The effects of treatment on the nicotine-stimulated release of each neurotransmitter were also measured.

Cells were cultured in control conditions, in dibutyryl cAMP (1 mM) or forskolin (25 μM) for 72 h. In control conditions, the cell content of noradrenaline and adrenaline was 3540 ± 197 and 7208 ± 420 pmol/well respectively (mean ± s.e. mean, n = 4). Culture in dibutyryl cAMP, but not forskolin, consistently decreased catecholamine levels by 10-30%. Total (met)-enkephalyl-Arg^6-Phe^7-IR in control cells was 3.07 ± 0.17 pmol/well, this was increased to 4.59 ± 0.35 and 5.04 ± 0.16 pmol/well following culture in dibutyryl cAMP and forskolin respectively. These increases were due to increased levels of both peptide B-like IR and (met)-enkephalyl-Arg^6-Phe^7-like IR within the cells. Nicotine-stimulated (5×10^{-5} M) release of catecholamines was increased after culture in dibutyryl cAMP or forskolin. For example, nicotine-stimulated noradrenaline release was 458 ± 51 pmol/well and 551 ± 57 pmol/well following incubation in dibutyryl cAMP or forskolin respectively, compared to the control stimulation release of 289 ± 36 pmol/well. Both treatments also caused a 2-fold increase in the release of (met)-enkephalyl-Arg^6-Phe^7-IR.

Thus, in bovine adrenal chromaffin cells, elevation of cAMP levels exerts different effects on the cellular levels of opioid peptides and catecholamines. The increase in (met)-enkephalyl-Arg^6-Phe^7-IR presumably reflects the increase in preproenkephalin mRNA seen following culture in forskolin (Eiden et al. 1984). Although no increases in cellular catecholamine levels were seen, elevation of cAMP levels does exert similar effects on the nicotine-stimulated release of both catecholamines and opioid peptides.

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β_2-ADRENOCEPTOR REGULATION OF ELECTROLYTE TRANSPORT IN RAT JEJUNUM.

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It has been reported that cholera-enterotoxin induced fluid secretion in rat ileum *in vivo* is inhibited by the β-adrenoceptor antagonist propranolol (Donowitz & Charney, 1979). In human studies, however, propranolol has been shown to reduce small intestinal ion and water absorption whilst the β-adrenoceptor agonist isoprenaline has been shown to stimulate ion and water absorption (Morris & Turnberg, 1981). *In vivo* studies have not determined whether these β-effects are exerted at a mucosal level or via an effect on blood flow to the small intestine.

We have investigated β-adrenergic effects on ion transport in rat jejunum *in vitro* using a modified Ussing Chamber. Tissues were obtained from male Wistar rats (160g-200g) anaesthetised with sodium pentobarbitone. Measurements were made of changes in resting short circuit current (SCC) induced by isoprenaline (ISO) and salbutamol (SAL) alone and in the presence of a number of adrenoceptor antagonists. Resting SCC was stable 20-30 minutes after mounting the tissues. (-1 ± 1 $\mu amp.cm^{-1}$ change over 3min test period) Antagonists were added at 30 minutes, agonists at 40 minutes. ISO ($10^{-6}M$) caused an immediate, transient increase in SCC which was maximal within 2-3 minutes of addition. The results (Table 1) show that the ISO induced increase in SCC was significantly inhibited by both propranolol and the selective β_2-antagonist ICI 118551 (O'Donnell & Wanstall, 1980) but was unaffected by phentolamine or practolol (all antagonists at a concentration of $10^{-6}M$). Salbutamol ($10^{-6}M$) also caused a transient increase in SCC which was inhibited by ICI 118551 ($10^{-6}M$). Propranolol or ICI 118551 alone caused an immediate sustained fall in SCC (14 ± 5 and 9 ± 3 $\mu amps. cm^{-1}$ respectively) but practolol and phentolamine had no effect upon SCC.

TABLE 1. Changes in SCC (mean ± s.e.) induced by ISO ($10^{-6}M$) and SAL ($10^{-6}M$) alone and in the presence of a number of adrenoceptor antagonists (n=5).

Agonist	Antagonist	SCC ($\mu amps. cm^{-2}$)	P
ISO	-	+31 ± 5	-
ISO	Propranolol	0 ± 1	<0.001
ISO	Phentolamine	+26 ± 6	NS
ISO	ICI 118551	+2 ± 1	<0.001
ISO	Practolol	+28 ± 5	NS
SAL	-	+28 ± 4	-
SAL	ICI 118551	+3 ± 1	<0.001

*student's 't'-test for upaired data:comparisons with agonist response alone.

These results provide evidence for a β_2-adrenoceptor mediated effect on electrolyte transport in the rat jejunum which probably reflects an increase in anion secretion in the rat jejunum. The immediate sustained fall in SCC observed in the presence of either propranolol or ICI 118551 may indicate the presence of β_2-adrenoceptor tone in this preparation.

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FACILITATORY PRE-SYNAPTIC RECEPTORS IN THE ISOLATED PERFUSED RAT MESENTERY.

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It is generally accepted that presynaptic receptors modulate neurotransmitter release from sympathetic nerve endings (Starke, 1981). Various neurohumoral agents (Angiotensin II and beta-adrenoceptor agonists) have been shown to facilitate this release. Demonstration of the existence of a complete renin-angiotensin system in the rat mesenteric vascular wall (Desjardins-Giasson et al, 1981) raises the possibility of an involvement of this system in the isoprenaline (Iso)-induced facilitation of adrenergic neurotransmission (Kawasaki et al, 1984). Male and female Japanese Spontaneously Hypertensive rats (SHR, m and SHR, f, respectively) and New Zealand Hypertensive rats (NZH, f) 190-260g were used. The isolated perfused mesentery was set up as described by McGregor (McGregor, 1964) and increases in perfusion pressure in response to periarterial nerve stimulation (PNS) (80V, 1msec, 30Hz for 10sec) were recorded in the presence of a range of concentration of Iso. It was found that in all three groups Iso caused a significant dose-dependent potentiation of the pressor response to PNS. This potentiation was blocked by ICI 118,551 but not by atenolol. Addition of angiotensin II (AII), 10ng/ml, to the perfusate significantly potentiated the pressor response to PNS. This potentiation was attenuated by [Sar1-Ile8]angiotensin II (SAR), an AII antagonist. The SAR itself markedly attenuated the ISO-induced potentiation of the pressor response to PNS; however, ICI 118,551 did not affect the AII potentiation of the pressor response.

TABLE 1

	% Perfusion pressure in response to PNS (control 100%)				
	ISO * ($5x10^{-8}$M)	ISO + atenolol* (10^{-7}M)	ISO + ICI118,551 ($5x10^{-7}$M)	AII * (10ng/ml)	ISO + SAR (200ng/ml)
SHR, m	150+/-4	207+/-11	95+/-5	158+/-7	104+/-4
SHR, f	140+/-5	142+/-5	99+/-2	150+/-8	103+/-5
NZH, f	179+/-3	180+/-4	97+/-2	153+/-6	101+/-10

Values are Mean +/- s.e.m. n=4 *All values significantly different from control, $p<0.01$.

The results suggest that the potentiation of the effect of PNS by Iso involves activation of presynaptic beta 2 adrenoceptors and that this in turn may require activation of the renin-angiotensin system.

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MODULATION BY ISOPRENALINE OF [^{3}H]-NORADRENALINE RELEASE FROM RABBIT ISOLATED PULMONARY ARTERY.

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Isoprenaline enhances the release of noradrenaline evoked by nerve stimulation in many sympathetically innervated tissues (Langer, 1981; Majewski, 1983). The enhancement is considered to be due to activation of presynaptic facilitatory β-adrenoceptors (Adler-Graschinsky & Langer, 1975). However, in some adrenergically innervated tissues, including the rabbit pulmonary artery (Endo et al., 1977) β-adrenoceptor agonists did not enhance the depolarization-evoked release of transmitter (Majewski, 1983). This raises the question whether or not presynaptic facilitatory β-adrenoceptors exist at all peripheral adrenergic nerve endings. The aim of the present work was to demonstrate the presence of presynaptic β-adrenoceptors in the rabbit isolated pulmonary artery by investigating the effect of isoprenaline on [^{3}H]-noradrenaline ([^{3}H]-NA) release evoked by electrical-field stimulation. This was done under experimental conditions designed to optimize the chance to reveal the possible presence of this subtype of adrenoceptors. The method described previously (Nedergaard, 1980) was used.

(-)-Isoprenaline (10^{-7}-10^{-6}M) had no effect on the [^{3}H]-overflow evoked by stimulation (3 Hz) of the pulmonary artery preloaded with [^{3}H]-NA. At 10^{-5} and $3x10^{-5}$M, (-)-isoprenaline reduced the [^{3}H]-overflow by maximally 39%. (-)-Isoprenaline (10^{-5}M) caused a block that remained almost constant with time. The same results were obtained with (-)-isoprenaline (10^{-7}-$3x10^{-5}$M) in the presence of either cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M) or the catechol-O-methyltransferase inhibitor U-0521 (3',4'-dihydroxy-2-methylpropiophenone). In the presence of these uptake inhibitors, (-)-isoprenaline (10^{-10}-10^{-7}M) had no effect on the [^{3}H]-overflow evoked by stimulation at 1 Hz. At 10^{-6}M, (-)-isoprenaline slightly reduced the [^{3}H]-overflow. At 10 Hz, (-)-isoprenaline (10^{-6}-$3x10^{-5}$M) decreased the [^{3}H]-overflow and had no effect at 10^{-7}M.

In the presence of the selective α_2-adrenoceptor antagonist rauwolscine (10^{-6}M), (-)-isoprenaline (10^{-7}-10^{-6}M) did not enhance the stimulation-evoked [^{3}H]-overflow. However, rauwolscine antagonized the inhibitory effect of (-)-isoprenaline (10^{-5}-$3x10^{-5}$M). In the presence of the non-selective α_2-adrenoceptor blocking agent phentolamine (10^{-6}M), the effect of (-)-isoprenaline (10^{-7}-$3x10^{-5}$M) was not altered.

(±)-Propranolol (10^{-7}-10^{-5}M) did not alter the stimulation-evoked [^{3}H]-overflow. At 3×10^{-5}M, this β-adrenoceptor antagonist reduced the neurogenic response by 50%. (±)-Propranolol (10^{-6}M) did not alter the effect of (-)-isoprenaline (10^{-7}-$3x10^{-5}$M) on the stimulation-evoked [^{3}H]-overflow.

The dextro isomer of isoprenaline (10^{-7}-$3x10^{-5}$M) enhanced slightly the stimulation-evoked [^{3}H]-overflow. No enhancement was seen in the presence of either (±)-propranolol (10^{-6}M) or rauwolscine (10^{-6}M).

The results suggest that the sympathetic nerve terminals in rabbit pulmonary artery do not possess facilitatory β-adrenoceptors. Furthermore, that (-)-isoprenaline in high concentrations activates presynaptic inhibitory α_2-adrenoceptors.

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IN VIVO RECOVERY OF β-ADRENOCEPTORS FOLLOWING IRREVERSIBLE BLOCKADE IN A RAT MODEL OF HUMAN HYPOXIC DISEASE.

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Exposure to chronic hypoxia is associated with a modulation of beta-adrenoceptor density in myocardial tissue (Voelkel et al., 1981) and in lung with altered response to internalization by agonist (Winter et al., 1986). In this study bromoacetylalprenololmethane (BAAM), an alkylating beta-blocker (Baker & Pitha, 1982), has been used to study whether the de-novo synthesis of beta-adrenoceptors in vivo is altered by chronic hypoxia.

Albino male Wistar rats (weighing 80-120g) were placed in an environmental chamber where the inspired oxygen tension was maintained at 10%. Control animals were kept in normoxia (n = 42 in each environment). After 28 days, half the animals were treated with BAAM (25 mg/kg intraperitoneally), the remainder received vehicle. At 7 time points (range 4-500 hrs) following treatment, rats were killed and heart and lungs removed and stored at -70° until use.

Sarcolemmal and plasma membranes of heart and lung were made in the conventional manner. In brief, tissues were homogenised in 10 vols of 50mM Tris HCl, pH 7.4, 5mM EDTA, passed through two layers of muslin, centrifuged and washed once in Tris buffer. Pellets were taken up in 50mM Tris HCl, pH 7.4, 0.5mM EDTA, centrifuged and resuspended in 50mM Tris HCl, pH 7.4. Beta-adrenoceptor number was measured by radioligand binding techniques using the specific radioligand ^{125}I-iodocyanopindolol (^{125}I-CYP). Specific binding was defined as that displaceable by 200μM (-) isoprenaline.

There was no change in the dissociation constant (Kd) of the radioligand in heart or lung as a result of hypoxia or treatment with BAAM. After 15 hours, treatment resulted in a 55% decrease in binding site maxima (Bmax, measured as fmoles/mg protein) in heart and lung in normoxia. Treatment in hypoxia was also associated with a decrease in Bmax of 55% in both tissues. In both tissues beta-adrenoceptor number had returned to control values by about 200 hours. They also displayed a similar time course. Chronic hypoxia did not change the rate of regeneration of beta-adrenoceptors in heart or lung.

We conclude that de-novo synthesis of beta-adrenoceptors is not altered in chronic hypoxia. Changes in receptor density and susceptability to internalization seen, therefore, probably reflect an alteration in the dynamic equilibrium between cell membrane bound receptors and sequestered pools.

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EFFECT OF DIAZEPAM ON GRWTH.AND DECAY PHASES OF MINIATURE ENDPLATE CURRENTS IN THE ISOLATED RAT DIAPHRAGM PREPARATION.

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Although the skeletal muscle relaxing effects from benzodiazepines are generally considered to originate in the central nervous system, there is also evidence that peripheral components may be involved. Conflicting results have been reported on the effects of diazepam on the *in vitro* peripheral neuromuscular function: absent, potentiating (1) or inhibitory (2). The concentrations of diazepam used, however, were different in these studies. A biphasic response on the indirectly evoked twitch contractions becomes clear after cumulative as well as single doses of diazepam when applied to the phrenic-nerve diaphragm preparation *in vitro* in the range of 0.0035 to 0.35 mmol/litre (3, 4). Potentiation of twitch tension at lower diazepam concentrations is followed by depression of twitch tension at concentrations of 0.175 mmol/litre and higher.

In the present study the peripheral effects of diazepam on miniature endplate currents (mepc's) and quantal content have been studied. If neuromuscular transmission is directly affected by diazepam, either by presynaptic or postsynaptic effects, this should be reflected by changes in the growth and decay phases of the mepc's and/or the release parameters of mepc's.

Cut muscle preparations of phrenic nerve-hemidiaphragms of female rats (Cpb WU/WI, 170-200 g) were kept in a bath of 2 ml, and superfused continuously with Krebs solution. The temperature was kept at 32 $\pm$ 0.2°C. The voltageclamp had a gain of 2000 ($\pm$ 15 V swing) for passing current. Time constants were measured by a computer. Diazepam was added to the Krebs solution before superfusion.

The results may be summarized as follows. At diazepam concentrations up to 0.0825 mmol/litre the time-constants of the decay phase of the mepc's were reduced to 62% of the control values, while no significant effect on the growth phase was seen. At a diazepam concentration of 0.175 mmol/litre the rising time of the growth phase increased significantly by a factor 2 and more to values higher than 1 msec. The quantal content was increased by diazepam.

The results of the present experiments are in good agreement with the findings that twitch tension is reduced at diazepam concentrations of 0.175 mmol/litre and higher (3). An increase in the time constant of the growth phase will cause a decrease in current density, which might result in neuromuscular blockade. The potentiation of twitch tension seen at lower diazepam concentrations might be caused by an increase in quantum content (5) as well as by an increase in vesicle size as observed by Leeuwin et al. (4). Both effects can compensate for the decrease in the time constant of the decay phase of the mepc's.

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