ADRENAL STIMULATION BY DMPP MAY FACILITATE NORADRENALINE RELEASE, BUT PRESSOR RESPONSE IS NOT REDUCED BY P^{2x}-PURINOCEPTOR 'BLOCKADE'

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We have previously shown that repeated administration of alpha, beta-methylene-ATP (meATP) in vivo to rats achieves transient desensitisation of the pressor response, and may therefore be useful for investigating the role of the P2x-purinoceptor in mediating responses to sympathetic stimulation. Desensitisation did not displace the pressor dose-response curve to DMPP, but this could have been due either to facilitation of noradrenaline release after desensitisation of the P2x-purinoceptor, or to 're-sensitisation' of of the receptor by the time that the higher doses of DMPP were administered. We have therefore investigated [i] whether P2x-purinoceptor desensitisation by meATP attenuates the pressor response to a single high dose of DMPP; and [ii] whether desensitisation magnifies the increases in plasma catecholamines measured after DMPP administration.

6 doses of DMPP 0.06 mg were injected as a bolus into a jugular vein cannula in 5 pentobarbital anaesthetised rats, according to the following schedule: 2 sequential doses initially to measure the basal (pre-meATP) BP and plasma catecholamine responses, respectively; 2 doses to measure the BP response after 6 doses of meATP 0.4 mg/kg and vehicle, respectively; and 2 doses to measure plasma catecholamine responses after 3 further doses of meATP 0.4 mg/kg and then vehicle, respectively. For plasma catecholamine estimations, 0.25 ml blood was drawn from a carotid artery cannula before DMPP injection and at the time of peak pressor response (timed during previous doses); analysis was by double-isotope enzymatic technique (1).

The increases in BP measured after DMPP injection pre-meATP, post-meATP and post-vehicle, respectively, were 90±11/58±10, 93±13/62±2, and 104±9/63±7 mmHg.

Plasma noradrenaline increases following DMPP administration were found to be very variable, but were associated with much larger fractional increases in plasma adrenaline concentration. The rise in plasma noradrenaline concentration after P2x-purinoceptor desensitisation was consistently (up to 2-fold) greater than after the first pair of DMPP doses, and this increase was maintained for the final pair of DMPP doses administered after 3 doses of vehicle (in place of meATP).

The lack of attenuation of the pressor response to DMPP after P2x-purinoceptor desensitisation argues against a major role of ATP as a pressor neurotransmitter in this model, unless the pressor response is normally offset by presynaptic inhibition by ATP of noradrenaline release. However, the enhanced noradrenaline release was associated with adrenomedullary activation and continued after recovery from desensitisation; thus the facilitation appears more likely to be mediated by adrenaline release rather than P2x-purinoceptor desensitisation.

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CALCITONIN AND THE SYMPATHETIC NERVOUS SYSTEM IN HAEMORRHAGIC SHOCK

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Peripherally administered salmon calcitonin (sCT) exerts a pressor effect in rats rendered hypotensive by haemorrhage. This may involve an increase in sympathetic tone since chronic pretreatment with 6-hydroxydopamine (6-OHDA) greatly attenuates the response to peripheral (i.v.) sCT (Bates et al, 1984), although not to intracerebroventricular (i.c.v.) sCT (Bates et al, 1984). We have investigated the role of the sympathetic nervous system in the response to sCT during haemorrhagic shock by determining the effect of sCT after acute sympathetic blockade using guanethidine.

Sprague-Dawley rats (♂, 150-300g) were anaesthetised with urethane ($1.6g.Kg^{-1}$ i.p. and s.c.) and the jugular vein and carotid artery cannulated for drug administration and the measurement of blood pressure respectively. Animals which were to receive sCT or vehicle by i.c.v. injection were placed in a stereotaxic frame for the duration of the experiment. Animals were bled by the withdrawal of arterial blood such that mean arterial pressure (M.A.P.) was reduced by 20%.

After a 20 min. stabilisation period, guanethidine ($10mg.Kg^{-1}$ i.v.) or vehicle (0.9% NaCl) was administered. Ten minutes later, sCT ($10U.Kg^{-1}$ i.v. or $20U.Kg^{-1}$i.v. in 0.15M sodium phosphate buffer containing 0.1% BSA), sCT ($1U.Kg^{-1}$ i.c.v. or $5U.Kg^{-1}$ i.c.v. in 50mM tris-buffer containing 0.1% BSA), or appropriate vehicle were administered. M.A.P. was monitered for 60 min. Statistical analysis was by analysis of variance followed by the Students 't' test as appropriate.

Table 1. Change in M.A.P. (mmHg) after drug treatment in haemorrhaged rats.

Pretreatment	Drug	5min.	15min.	30min.	60min.
Vehicle	Vehicle (i.v.)	2±2	0±2	6±2	5±2
(n,6)	SCT ($10U.Kg^{-1}$ i.v.)	9±2*	5±1*	2±2	4±1
	SCT ($20U.Kg^{-1}$ i.v.)	18±2***	11±2**	12±3	14±4
Guanethidine	Vehicle (i.v.)	2±1	1±1	2±1	-2±2
(n,6)	SCT ($10U.Kg^{-1}$ i.v.)	3±1	4±1	3±2	1±1
	SCT ($20U.Kg^{-1}$ i.v.)	3±1	3±1	1±1	2±2
Vehicle	Vehicle (i.c.v.)	16±3	6±3	3±4	-1±3
(n,5)	SCT ($1U.Kg^{-1}$ i.c.v.)	17±3	12±5	8±5	7±5
	SCT ($5U.Kg^{-1}$ i.c.v.)	28±4*	30±5**	29±6**	20±6*
Guanethidine	Vehicle (i.c.v.)	4±2	3±1	1±2	-1±2
(n,5)	SCT ($1U.Kg^{-1}$ i.c.v.)	6±1	5±1	5±1	2±1
	SCT ($5U.Kg^{-1}$ i.c.v.)	11±3	12±3*	12±4*	9±5

mean ±s.e.m. *$p<0.05$; **$p<0.01$; ***$p<0.001$

The pressor response to i.v. sCT was completely abolished after guanethidine, whereas the response to i.c.v. sCT was reduced but not abolished. These results suggest that the central and peripheral mechanisms of the pressor response to sCT in haemorrhaged rats may not be identical. The difference between these results and those using 6-OHDA may be due to differences between acute and chronic sympathectomy.

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REGIONAL VASODILATOR ACTIVITY OF RAT ANF(5-28) IN RAT AUTOPERFUSED PREPARATIONS

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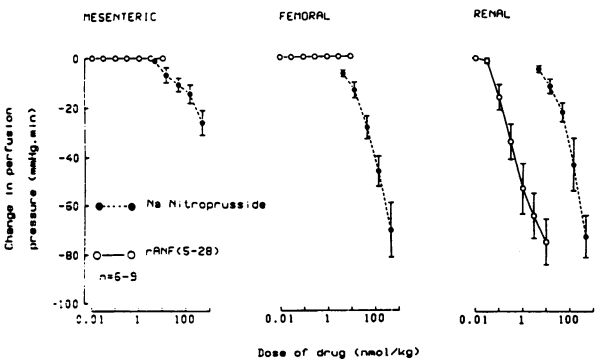
Atrial natriuretic factor (ANF) exhibits regional differences in vasorelaxant activity in isolated arterial segments in vitro (Faison et al, 1985). In isolated resistance vessels (lumen ca. 250 μm) synthetic ANF(3-28) relaxed rat renal arcuate arteries pre-contracted with K^+, noradrenaline or 5-hydroxytryptamine but had no effect on resistance vessels of the cerebral, coronary, mesenteric or femoral vasculature, while sodium nitroprusside (SNP) showed no vascular selectivity (Aalkjaer et al, 1985). In the present study we have compared the regional vasodilator effects of rat ANF (5-28) and SNP in the anaesthetised rat.

Male Sprague-Dawley rats, 280-330g, were anaesthetised with Inactin, 125 mg kg^{-1} i.p., and artificially ventilated. For autoperfusion of the femoral and mesenteric vasculature, blood was taken from the left femoral artery and delivered at a constant flow rate of 2.6 ml min^{-1} into either the right femoral or superior mesenteric artery. Systemic blood pressure was recorded from the right carotid artery. The renal vasculature was autoperfused by a method similar to that of Fink and Brody (1978) and the blood flow rate was adjusted (4.3 ± 0.25 ml min^{-1}, mean ± s.e. mean, n = 18) to give an initial renal perfusion pressure (81.2 ± 4.7 mm Hg) as near as possible to the mean systemic arterial pressure (117.2 ± 5.4) recorded from the right brachial artery. All rats received heparin 100 i.u., i.v.

SNP (4.5 to 450 nmol kg^{-1}, i.a.) elicited a transient dose-related decrease in perfusion pressure in all three vascular beds, whereas rat ANF(5-28) (0.01 to 10 nmol kg^{-1} i.a.) only produced a vasodilator response in the renal vasculature (see figure).



In rat autoperfused preparations, rANF(5-28) exerted a selective renal vasodilator action. SNP lacked regional selectivity and reduced perfusion pressure in the renal, femoral and mesenteric beds.

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ACETYLCHOLINE VASORELAXATION IN SUPERIOR MESENTERIC ARTERIAL BED OF THE RAT IS ENDOTHELIUM-DEPENDENT AND SENSITIVE TO ANTIOXIDANTS

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Although vasorelaxation due to endothelium-derived relaxant factor (EDRF) has been studied in many large vessels, there have been few investigations of EDRF in resistance vessels. Endothelium-dependent relaxation to acetylcholine (ACh) has been reported in the isolated mesenteric circulation of rabbits (Carvalho & Furchgott, 1981) and rats (Burdet et al, 1986; Byfield et al, 1986) but it has been little characterised in these small vessels.

The superior mesenteric bed of the rat was isolated from heparinised (1000i.u./kg) male Wistar rats weighing 350-400g (Bantin & Kingman, Hull) as described by McGregor (1965) and was perfused with oxygenated Krebs-Henseleit solution at 37°C and 2ml/min. Perfusion pressure was recorded by a Bell & Howell pressure transducer and a Grass 79D polygraph. The relaxant effects of bolus injections of ACh (0.3-300ng) were determined during infusion of 0.1ng/min noradrenaline (NA); the relaxations (as % of the increase in perfusion pressure caused by NA) were very similar to the % reductions observed in the pressor responses to a fixed dose of NA co-administered in single bolus injections with the same dose range of ACh. Bolus co-administration was used subsequently to assess the "relaxant" effects of ACh so the influence of inhibitors on the contractile effect could be assessed by the administration of a dose of NA alone, 6min before the combined ACh/NA bolus. In control preparations, the effects of NA and ACh remained constant over 4.5h. The % reduction of the NA response by co-administered ACh was unaffected by the dose of NA over the range 1-30µg NA; 10µg NA was used routinely to give 83±4% (n=8) of the maximum response. The maximum inhibition given by ACh was 64±4% (n=12) of the control NA response and occurred at 100ng; the ED_{50} was 8ng.

ACh no longer affected the NA response after perfusion for 90s with an 0.3% solution of the detergent CHAPS (Sigma) but 10ug nitroprusside still reduced the NA response by 45±4% (n=4) on co-administration. Removal of endothelium was confirmed histologically by silver staining (Poole et al, 1958) of mesenteric samples frozen and cut on a cryostat.

When placed in the perfusion fluid, the cyclo-oxygenase inhibitors indomethacin and diclofenac up to 40µM had no effect on the reduction by ACh of the NA response and the cyclo-oxygenase/lipoxygenase inhibitor BW755C (200µM) was also ineffective. However, the antioxidant nordihydroguaiaretic acid (2.5µM) totally and irreversibly inhibited the effects of ACh in a time-dependent manner (maximum effect after administration for 30min). All these inhibitors depressed the contractile response to NA (which was 25±4% of the control after BW755C at 200µM). Another antioxidant ascorbic acid (5mM and 10mM) also abolished the vasorelaxant effect of ACh.

The effects of ACh as a vasorelaxant in this preparation are similar to those described in larger vessels, both in regard to their dependence on endothelium, insensitivity to cyclo-oxygenase inhibitors and sensitivity to antioxidants.

This work was supported by the British Heart Foundation (Grant No. 85/39).

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THE EFFECT OF TRAVEL-INDUCED STRESS OF GUINEA-PIGS ON CARDIAC β-ADRENOCEPTOR SENSITIVITY

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It is generally accepted that β-adrenoceptors down-regulate after exposure of animals to stress such as immobilization (U'Prichard & Kvetnanski, 1979; Yamaguchi, Torda, Hirata & Kopin, 1981), possibly because of elevated levels of circulating adrenaline (Borkowski & Kelley, 1986). In the present study we have compared guinea-pigs immediately after approx. 18h exposure to the stress of transport by British Rail (approx. 150 miles) with those kept for at least 1 week in the animal house and used after the relative peace of a weekend. Isolated left atria paced at 2Hz (threshold voltage + 50% and 5ms) and spontaneous right atria were set up in Krebs solution at 37.5°C gassed with O_2:CO_2 (95:5). Cumulative concentration-response curves for the increase in left atrial tension and right atrial rate with isoprenaline (I) were obtained. After washout, a second curve for salbutamol, terbutaline or histamine was obtained. Tissue sensitivity was assessed as the EC50 from curves plotted as % of their own maxima and from the max of the partial agonists salbutamol and terbutaline relative to I.

The combined EC50 values for all experiments for the rate response to I of stressed (1.6nM, n=22) and unstressed (1.9nM, n=24) guinea-pigs were not significantly different (P>0.05). Similarly, the max of salbutamol and terbutaline in stressed (71.5±3.9 and 95.7±2.4%) and unstressed animals (66.4±3.3 and 90.2±3.3%) were not significantly different. Thus the sensitivity of right atria did not differ between travel-stressed and unstressed guinea-pigs.

In contrast, left atria from stressed animals displayed a surprising increase in sensitivity to I. The combined EC50 value was significantly less after stress (4.8nM, n=21) than in controls (13nM, n=22). The EC50 for salbutamol (4.2μM,n=10) was also significantly less than in controls (15μM, n=11) and the max response was raised from 10.6±2.9 to 17.0±4.9% but not significantly. With terbutaline, the reduction of EC50 (16 to 9.2μM) was not significant whereas the stress-induced increase in max from 37.6±4.6 to 51.9±3.0% was significant. Thus the overall trend was for an increase in sensitivity to β-adrenoceptor stimulation in left atria after transportation. This was β-adrenoceptor-specific since there was no change in sensitivity to histamine, the EC50 values in control and travelled animals being 1.2 and 1.3μM respectively. Whether this supersensitivity is stress-induced remains to be established, but it is unlikely to be due to elevated circulating catecholamines since this would be down-regulatory.

Supported by the British Heart Foundation.

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THE EFFECT OF SK&F 101468-A, A DA_2 AGONIST, ON NERVE STIMULATION TACHYCARDIA: COMPARISON BETWEEN CATS AND CYNOMOLGUS MONKEYS

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SK&F 101468-A is a potent, selective, dopamine DA_2 agonist (Gallagher, et al. 1985) with potential for therapeutic utility in conditions with an aetiology based on high sympathetic tone. This study compares the activity of SK&F 101468-A in pentobarbitone-anaesthetised cats and cynomolgus monkeys to inhibit tachycardias produced by stimulation of the cardiac accelerans nerve.
Animals were anaesthetised (sodium pentobarbitone, 60 mg/kg i.p.for cats, 15 mg/kg i.v. monkeys). The trachea was cannulated. Blood pressure was measured from a femoral artery and heart rate, from the blood pressure pulse. The stellate ganglion was exposed. The sympathetic chain was tied cranial and caudal to the ganglion and the cardiac accelerans nerve placed on shielded stimulating electrodes and covered with cotton wool soaked in mineral oil. A cannula was placed in a brachial vein for drug administration.
The cardiac accelerans nerve was stimulated (Grass stimulator) at 2 volts for cats and 6 - 8 volts for monkeys, pulse width 4 msec. at frequencies of 0.5, 1.0, 4.0 and 8.0 Hz. Cumulative frequency response curves were constructed before and after intravenous treatment with saline, SK&F 101468-A, 10 and 50 μg/kg in cats (n=3) and 10, 50 and 150 μg/kg in monkeys (n=3). L-Sulpiride, 0.5 mg/kg i.v., a selective DA_2 antagonist was administered to a second group of cats (n=3) 30 minutes after treatment with 10 μg/kg SK&F 101468-A. Data was analysed using Dunnett's multiple range test.

Table 1. Change in heart rate 15 minutes after dosing (beats/min mean $\pm$ s.e.m.)

Treatment		Stimulation frequencies 0.5 Hz	1.0 Hz	4.0 Hz	8.0Hz
Control	(cat)	18.7 $\pm$ 3.3	37.0 $\pm$ 4.0	94.3 $\pm$ 2.8	108.7 $\pm$ 1.5
	(monkey)	16.0 $\pm$ 7.4	34.0 $\pm$ 12.2	59.7 $\pm$ 12.8	65.3 $\pm$ 12.3
Saline	(cat)	19.7 $\pm$ 4.3	40.0 $\pm$ 7.6	89.0 $\pm$ 6.8	100.7 $\pm$ 2.4
	(monkey)	11.3 $\pm$ 5.8	26.0 $\pm$ 9.2	53.3 $\pm$ 13.0	60.7 $\pm$ 12.7
10 μg/kg	(cat)	6.0 $\pm$ 3.2*	15.7 $\pm$ 7.8*	62.7 $\pm$ 20.7*	89.3 $\pm$ 12.5
	(monkey)	5.7 $\pm$ 0.9	20.7 $\pm$ 1.2	55.7 $\pm$ 3.5	61.3 $\pm$ 3.3
50 μg/kg	(cat)	0.0 $\pm$ 0.0*	0.3 $\pm$ 0.3*	20.3 $\pm$ 16.4*	57.3 $\pm$ 21.8*
	(monkey)	1.3 $\pm$ 0.3*	9.0 $\pm$ 3.5*	60.7 $\pm$ 2.0	77.3 $\pm$ 4.1
150 μg/kg	(monkey)	1.3 $\pm$ 1.3*	4.7 $\pm$ 1.5*	40.3 $\pm$ 14.4	59.3 $\pm$ 18.2

* Statistically significantly different from control value, $P<0.05$.

In the cat, SK&F 101468-A caused a dose related shift to the right in the frequency response curve. Statistically significant ($P<0.05$) reductions in the stimulation induced tachycardias were recorded except at 8 Hz after the 10 μg/kg dose. There was no effect on resting heart rate. The shift caused by 10 μg/kg was completely reversed by L-Sulpiride, 0.5 mg/kg. In the monkey SK&F 101468-A, 10 μg/kg, had no effect on either the resting heart rate or the frequency response curve. At the higher doses of 50 and 150 μg/kg, the compound caused a non-significant fall in resting heart rate of approximately 20 beats/min and abolished the tachycardia to the lower frequency stimulation. Responses to the higher frequencies stimulation were not significantly changed.
SK&F 101468-A inhibited tachycardias during sympathetic nerve stimulation in both species but was more potent in the cat than the cynomolgus monkey. There was a greater effect at the lower frequency stimulation than the higher.

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CENTRAL ADMINISTRATION OF METHOXAMINE EVOKES CARDIOVASCULAR RESPONSES IN THE RAT MEDIATED VIA THE ANTERIOR HYPOTHALAMUS

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The selective α_1- adrenoceptor agonist methoxamine, ME, (Kobinger & Pichler,1981) has previously been reported to decrease diastolic blood pressure (DBP) and heart rate (HR) following administration into the lateral cerebral ventricles (i.c.v.) of anaesthetised rats. These decreases appeared to be mediated via α_1-adrenoceptors as they could be reversed by i.c.v. administration of the α_1-adrenoceptor antagonists indoramin or Wy23925, but not the the α_2-adrenoceptor antagonist Wy26392 (Pierce & Shepperson, 1986).

Various brain structures of the rat correspond to areas rich in noradrenaline e.g. the nucleus solitarius, the locus coeruleus and the hypothalamus (Ungerstedt, 1971). Electrophysiological studies have identified these regions as cardiovascular control centres (Chiu,1982). Regions of the hypothalamus are reported to be either depressor or pressor areas (Chiu,1982). Additionally, Morris & Woodcock (1982) have identified both α_1 and α_2-adrenoceptors in the anterior (AHA) and posterior (PHA) hypothalamus. The site of action of ME has therefore been further investigated in the rat by examining the effects of ME after administration into the AHA or PHA.

Groups of 5 female Sprague Dawley rats (245-260g) were anaesthetised with pentobarbitone (50mgkg^{-1} i.p.). DBP was recorded from the femoral artery and HR derived from the pulse pressure. Either saline (1µl) or ME (5,15 or 45µg in 1 µl of saline) were injected into the AHA or PHA via a stereotaxically implanted cannula. (Pellegrino et al.,1980). DBP and HR were recorded at 5 minute intervals for 1 hour.

Following injection into the AHA all doses of methoxamine evoked significant reductions in DBP and HR, which were sustained for 1 hour, in comparison with their vehicle controls. The peak observed reductions in DBP and HR evoked by ME were 9±2,17±5 and 19±5mmHg; and 36 ± 13, 83 ± 12 and 57 ± 18 beats min^{-1}, following doses of 5, 15 and 45 µg respectively. There were no significant pressor responses at any time point, following the administration of any of the doses of ME.

Administration of ME into the PHA had no significant effect on DBP or HR. The peak observed changes in DBP and HR being a reduction of 9 ± 7 mmHg and an increase of 1 ± 2 mmHg; and reductions of 20 ± 11 and 18 ± 6 beats minute^{-1}, following doses of 5 and 15 µg respectively.

In conclusion, in anaesthetised rats administration of ME into the AHA, but not the PHA evokes a fall in DBP and HR. These findings confirm the report of Struyker Boudier et al.(1974) that α-adrenoceptors mediate hypotensive and bradycardic response in the AHA, and furthermore suggest a role for α_1-adrenoceptors in this response.

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INFLUENCE OF ENDOTHELIUM ON THE CONTRACTILE EFFECTS OF HAEMOGLOBIN IN DOG BASILAR ARTERY

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Although haemoglobin has little effect on resting tone in many peripheral blood vessels, it contracts isolated cerebral arteries and may be involved in the development of cerebral vasospasm (Tanishima, 1980). Since the ability of haemoglobin to inhibit the effects of 'endothelium-derived relaxing factor' (EDRF) may account for its constrictor effects in the cerebral circulation (e.g. Bowman et al., 1985; Martin et al., 1985), we have investigated whether or not the integrity of the endothelium is important for haemoglobin to produce contraction in dog isolated basilar artery. Beagle dogs were sacrified with pentobarbitone sodium (200 $mgkg^{-1}$ i.v.) and basilar arteries removed and stored overnight at 4°C. A segment of artery was perfused with Triton X-100 (0.1%, 0.5 ml min^{-1} for 1 min) to remove the endothelium; an adjacent, unperfused artery section was used as a control preparation. Ring segments (3-4 mm length) were suspended in modified Krebs-Henseleit solution (Apperley et al., 1976) at a final resting tension of 1g and isometric contractions recorded. Haemoglobin was prepared as described by Martin et al. (1985).

The contractile responses to potassium chloride (KCl, 30 mM) were not significantly different in untreated and Triton X-100 perfused preparations (2.20 ±0.19g and 1.64 ± 0.18g respectively, mean ± S.E.M., n=7) whilst those to 1 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were significantly increased from 1.04±0.19g to 2.00±0.27g respectively, n=7 ($P<0.05$, paired Student's t-test). Substance P (0.1-10 nM), which produces an endothelial dependent relaxation (Furchgott, 1983), caused marked relaxation of untreated preparations which had been contracted with $PGF_{2\alpha}$ (1 μM), producing a maximum effect at 10 nM of -115.7 ± 5.9% $PGF_{2\alpha}$ tone (n=7). In contrast, in Triton X-100 perfused preparations, substance P in concentrations up to 100 nM caused little or no relaxation (-1.0 ± 8.3% $PGF_{2\alpha}$ tone at 10 nM, n=7). However sodium nitroprusside (10 nM - 1 μM) caused a similar degree of relaxation in untreated and Triton X-100 perfused preparations (-93.6 ± 7.3 and -110.5 ± 13.0% $PGF_{2\alpha}$ tone respectively at 1 μM, n=3). Haemoglobin (10 nM - 3 μM) produced a concentration dependent contraction in untreated dog basilar artery. The magnitude of this response did not appear to be modified by Triton X-100 treatment; contractile responses produced by 3 μM haemoglobin in untreated and Triton X-100 perfused preparations were 37.1 ± 3.3% and 37.5 ± 6.6% of the KCl (30 mM) response respectively (n=4). Histological examination confirmed that in Triton X-100 perfused preparations, substantial areas of the artery lumen were denuded of endothelial cells, compared to untreated segments.

Since Triton X-100 perfusion abolished substance P relaxation whilst apparently producing little or no smooth muscle damage, these findings confirm reports by Verrecchia et al. (1986) that this is an effective way of removing endothelial cells from small arteries. However, since Triton X-100 treatment did not reduce the contractile effects of haemoglobin, these results suggest that the integrity of the endothelium is not essential for haemoglobin to produce contraction in dog basilar artery.

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DOES PRAZOSIN DISTINGUISH BETWEEN SUBGROUPS OF α_1- ADRENOCEPTORS?

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Prazosin and yohimbine are two of the most commonly used antagonists in α-adrenoceptor subclassification. Apart from their differential affinities at α_1- and α_2-adrenoceptors, there is considerable variation in their reported affinities within the α_1-adrenoceptor subclass (Drew, 1985). α_1- and α_2-adrenoceptors both mediate contraction in dog saphenous vein (De Mey and Vanhoutte, 1981) and exploratory studies suggested that the same was true in that of the pig; however, prazosin seemed to be more potent at α_1-adrenoceptors in porcine, than in canine, saphenous veins. Therefore, we have undertaken a more comprehensive analysis of the α-adrenoceptors in these tissues.

Saphenous veins were cut spirally into strips and divided into four. Each preparation was suspended (initial tension 1g) in modified Krebs solution (37°C) gassed with 95% O_2 and 5% CO_2. The solution contained cocaine ($3x10^{-5}$ M), corticosterone ($4x10^{-5}$ M), propranolol ($1x10^{-6}$ M) and indomethacin ($3x10^{-6}$ M). The agonists used were phenylephrine and M7, which are reported to be selective for α_1- and α_2-adrenoceptors respectively (Starke et al, 1975; Shepperson and Langer, 1981). In those experiments where M7 (6-(dimethyl-amino)-5,6,7,8-tetrahydro-1,2-naphthalenediol) was the agonist, prazosin ($3x10^{-7}$ M and $3x10^{-6}$ M for pig and beagle saphenous veins respectively) was present throughout. Likewise, when phenylephrine was the agonist, yohimbine ($1x10^{-7}$M) was added to the bathing solution. Cumulative agonist concentration-response curves were repeated until constant. One preparation was retained as control and different concentrations of an antagonist were added to the other strips 45 min before construction of the final agonist concentration-response curves. Agonist concentration-ratios obtained in the presence of the antagonist were corrected for spontaneous changes in sensitivity by dividing them by the concentration-ratio obtained in the corresponding control preparation.

Yohimbine and prazosin produced parallel, concentration-dependent dextral displacements of the concentration-response curves to M7 and phenylephrine, respectively. The pA_2 (and slope) values (mean ± S.E.M.) for yohimbine ($1x10^{-7}$-$1x10^{-6}$ M) versus M7 were 7.82 ± 0.23 (0.85 ± 0.10, n=6) and 8.19 ± 0.08 (0.73 ± 0.05, n=5) in dog and pig veins, respectively. Thus, the α_2-adrenoceptors in these tissues appear similar. The pA_2 (and slope) for prazosin ($1x10^{-8}$-$1x10^{-6}$ M) versus phenylephrine in dog saphenous vein was 7.64 ± 0.18 (0.82 ± 0.04, n=4). In contrast, prazosin ($3x10^{-8}$-$3x10^{-7}$ M) was 81 times more potent in pig veins (pA_2 = 9.55 ± 0.18; slope = 0.83 ± 0.04, n=5). Similar results were obtained in pig veins with a 10-fold lower range of prazosin concentrations. The pA_2 values for the competitive antagonists WB 4101 (8.53 ± 0.14, n=6, dog: 8.37 ± 0.11, n=4, pig) and indoramin (7.65 ± 0.16, n=4, dog: 7.44 ± 0.14, n=6, pig) at α_1-adrenoceptors showed no species dependency. The results with prazosin suggest the existence of subtypes of α_1-adrenoceptor; those with WB 4101 and indoramin do not. Further work with other selective α_1-adrenoceptor agonists and antagonists is required to clarify this issue.

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CARDIOVASCULAR EFFECTS OF A NOVEL OPIOID PEPTIDE (BW 443C) IN THE ANAESTHETISED CAT

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An enkephalin analogue incorporating the basic amino acid D-arginine and having limited CNS penetration, Tyr.D.Arg.Gly.Phe.(4-NO_2).Pro.NH_2 (BW 443C), was recently described as having antinociceptive and antitussive effects, apparently via peripheral mechanisms (Adcock et al., 1986; Follenfant et al., 1986). Cardiovascular and respiratory effects of opiates have been extensively described and may be important when considering the therapeutic use of such compounds; such effects following BW 443C were investigated in the anaesthetised cat and are described here. Further experiments to elucidate the mechanisms for the observed cardiovascular effects of BW 443C are also reported.

Adult male cats were anaesthetised with α-chloralose (60-80 $mgkg^{-1}$) following induction with halothane (5-8%). Blood pressure (BP) and heart rate (HR) were recorded via the left carotid artery. Cats were allowed to breathe spontaneously and respiration was monitored via a tracheal cannula. The effects of BW 443C, given by i.v. infusion (100 min) or bolus, on systolic BP (sBP), diastolic BP (dBP) and HR are summarised below.

Table 1 Cardiovascular effects of BW 443C

Dose	No. Cats	ΔsBP(mmHg)	ΔdBP(mmHg)	ΔHR(b.p.m.)
0.1 $mgkg^{-1}$ bolus	3	↑ 67 ± 24.2*	↑ 37 ± 16.9*	↑ 23 ± 15.9*
1.0 $mgkg^{-1}$ bolus	3	↑ 65 ± 15.0* followed by: ↓ 30 ± 25.0*	↑ 43 ± 8.8* ↓ 35 ± 17.6*	Max: ↑ 25 ± 7.6*
10 $\mu gkg^{-1}min^{-1}$ infn	4	↓ 31 ± 12.5*	↓ 26 ± 12.0*	↑ 10 ± 5.5
100 $\mu gkg^{-1}min^{-1}$, infn	4	↓ 47 ± 7.7*	↓ 40 ± 6.8*	↓ 1.3 ± 14.3

* Significant change ($p < 0.05$)

Pressor responses to bolus doses were rapid in onset and transient, lasting <5 min. The depressor response to the higher bolus dose was slow in onset and sustained (>45 min). Depressor responses to infusions were sustained. In further experiments, these BP effects were shown to be reversed by naloxone (0.1-1 $mgkg^{-1}$ i.v.). Respiration was not significantly affected by BW 443C.

In 4 cats spinalised at C_1, BW 443C caused no significant effects on BP and HR. Further experiments in non-spinalised cats showed that biphasic cardiovascular effects of BW 443C (rise then fall) were caused by applying filter paper pledgets soaked in BW 443C solution (max total dose, 200μg) to the dorsal surface of the brainstem at the level of the obex. Also, the fall in BP caused by BW 443C given peripherally (by i.v. infusion) was mostly reversed by similarly applying naloxone (max. dose, 15μg) to the obex region.

These studies demonstrate significant pressor and depressor effects of BW 443C; depressor responses are likely to be the most pronounced effect of BW 443C in most situations. These effects may be due to 'leaking' of the compound into an area of the brainstem known to have a high density of opiate binding sites, via the obex - an area where the blood-brain-barrier is relatively permeable.

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CARDIOVASCULAR CHANGES FOLLOWING ADMINISTRATION OF ADRENALINE AND ISOPRENALINE TO THE HYPOTHALAMUS OF THE ANAESTHETISED RAT

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It has been shown that injection of adrenaline into the anterior hypothalamus caused an initial slight increase, followed by a longer lasting decrease, in blood pressure accompanied by a fall in heart rate (Struyker Boudier & Bekers, 1975). In these experiments we have compared the effects of adrenaline and isoprenaline injected into both the anterior and the posterior hypothalamus. Modification by prior icv propranolol was also investigated.

Male New Zealand normotensive rats (190–200g) were anaesthetised with Hypnorm/midazolam (Flecknell & Mitchell, 1984). The left carotid artery was cannulated to allow measurement of blood pressure. Guide cannulae were stereotaxically implanted according to the atlas on König & Klippel (1963). The agonists (5µg in 1µl artificial csf (Merlis, 1940) were injected into the hypothalamus over 2.5 min. Where appropriate, 30µg propranolol was injected icv in 10µl artificial csf 15 min before the agonist.

Table 1. Maximal change in mean arterial pressure (mmHg) and heart rate (bpm) following injection into the hypothalamus.
Mean $\pm$ sem (n) Difference from control * P<0.05, ** P<0.01 Student's t test

	Blood Pressure		Heart Rate	
	Control	Propranolol	Control	Propranolol
adrenaline, anterior nucleus	-9.8 $\pm$ 3.7 (12)	+4.2 $\pm$ 2.4 (6) *	-40 $\pm$ 8 (12)	+24 $\pm$ 6 (6) **
adrenaline, posterior nucleus	-19.8 $\pm$ 4.0 (8)	+25.2 $\pm$ 8.8 (6) **	-61 $\pm$ 16 (8)	-14 $\pm$ 7 (6) **
isoprenaline, anterior nucleus	-15.5 $\pm$ 3.4 (11)	-12.0 $\pm$ 6.5 (6)	+23 $\pm$ 8 (11)	+61 $\pm$ 3 (6) **
isoprenaline, posterior nucleus	-19.5 $\pm$ 4.8 (9)	-24.0 $\pm$ 8.0 (6)	+14 $\pm$ 3	+51 $\pm$ 7 (6) **

Pretreatment with propranolol abolished the depressor response to adrenaline in the anterior nucleus, and reversed the response in the posterior nucleus. The depressor response to isoprenaline was unaffected by pretreatment with propranolol, although the duration of hypotension was attenuated.

Previous studies involving icv injection of isoprenaline have indicated that the central depressor response consists of two elements, one mediated through ß-receptors and the other involving non-neuronal mechanisms (Peres-Polon & Correa, 1984; Draper et al, 1986). These results appear to support this hypothesis, since the depressor response to adrenaline is readily abolished or reversed by propranolol whereas the magnitude of the depressor response to isoprenaline in unaffected.

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THE EFFECTS OF ICI 118551 OR PROPRANOLOL ON DEPRESSOR RESPONSES TO PRAZOSIN IN PENTOBARBITONE-ANAESTHETISED RATS

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We have investigated whether non-selective β-adrenoceptor antagonism (with propranolol), or selective β_2-adrenoceptor antagonism (with ICI 118551) influence the fall in diastolic blood pressure (dbp) produced by the α_1-adrenoceptor antagonist prazosin.

Male Wistar rats (250-350g) were used. Rats were anaesthetized throughout the experiment (sodium pentobarbitone; 60mg kg^{-1} i.p.). The jugular vein and carotid artery were catheterized, and rats were left for 30-60 min to stabilize before the experiment was continued.

In the first group of rats (n=6), the effects of ICI 118551 on the heart rate and blood pressure responses to isoprenaline were studied (responses thought to be mediated by β_1- and β_2-adrenoceptors, respectively). In the second group of rats, prazosin (100μg kg^{-1} i.v.; n=6) was given, and both the fall, and the subsequent recovery of dpb were monitored. In the remaining groups of rats, propranolol (1mg kg^{-1} i.v.; n=6) or ICI 118551 (1mg kg^{-1} i.v.; n=5) were administered 30 min before prazosin.

Isoprenaline alone (3μg kg^{-1} i.v.) caused a fall in dbp of 41 ± 8 mmHg, and caused a simultaneous tachycardia (85 ± 8bmin^{-1}). ICI 118551 (1mgkg^{-1} i.v.) given 30 min later (when the effects of isoprenaline had worn off) produced a significant hypotension and bradycardia (-39 ± 4 mmHg; -20bmin^{-1}, respectively). Thirty min later, isoprenaline caused a tachycardia which was not different from that seen in the absence of ICI 118551 (+ 84 ± 8bmin^{-1}), but isoprenaline had no significant effect on dbp.

In the second group of rats, there was a significant fall in dbp within 1 min of the administration of prazosin (-39 ± 5mmHg; $P<0.05$). Although dbp recovered slightly over the next 30 min, it remained significantly ($P<0.05$) below resting levels. In the presence of propranolol, the depressor effect of prazosin was significantly greater than that seen after prazosin alone (-65 ± 4 mmHg; $P < 0.05$). DBP rose over the next 30 min; although recovery was not complete, dbp reached the same level as that seen 30 min after prazosin alone (94 ± 4mmHg after prazosin alone; 87 ± 3mmHg after prazosin given in the presence of propranolol). Thirty min after administration of ICI 118551, prazosin caused a significant fall in dbp (-52 ± 4mmHg; $P<0.05$). This was greater than that seen after prazosin alone, but less marked than was seen after prazosin in the presence of propranolol. Over the next 30 min, dbp recovered to a level not different from that seen after prazosin alone.

In conclusion, in anaesthetized rats ICI 118551 appears to be highly selective against the putative β_2-adrenoceptor mediated hypotensive action of isoprenaline, but is relatively insensitive on the putative β_1-adrenoceptor mediated tachycardia. Interestingly, it was also shown that both β_1 and β_2-adrenoceptors may influence the magnitude of the depressor response to α_1-adrenoceptor antagonism. The mechanism of the latter effect remains unclear..

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Ca^{2+} RE-ADDITION DISTINGUISHES BETWEEN PHENYLEPHERINE AND 5-HT IN RAT AORTIC RINGS: DIFFERENCES IN α_1 AND 5-HT RECEPTOR COUPLING

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The smooth muscle of rat aorta can be contracted by α-adrenoceptor agonists or by 5-hydroxytryptamine (5HT) (Krishnamurty, 1971). We have now investigated the requirement of responses to 5HT for extracellular Ca^{2+} in "Ca^{2+} re-addition" experiments, where the agonist was added in the presence of nominally "zero" Ca^{2+}. We found that 5HT produced unexpectedly small responses to the re-addition of physiological and sub-physiological concentrations of Ca^{2+}. Even when Ca^{2+} was increased to 2.5mM the response was much smaller than would be expected from its straightforward response when 5HT was added in the presence of 2.5mM Ca^{2+}. In contrast, the responses to α_1-adrenoceptor agonists such as noradrenaline or phenylephrine were consistent at each level of Ca^{2+} irrespective of whether the agonist was added before or after addition of Ca^{2+} (McGrath et al, 1984).

Male Wistar rats (250-300g) were killed by a blow to the head and exsanguination. The thoracic aorta was excised and 2-3mm ring segments cut. Endothelium was removed by gentle rubbing. Circular muscle tension was recorded isometrically in a modified Krebs' bicarbonate saline (Ca^{2+} 2.5mM, HPO_4^- 0.12mM, 95% O_2:5% CO_2, 37°C). Paired preparations were given either 5HT or phenylephrine. The tissues were first tested for sensitivity to the agonists in 2.5mM Ca^{2+} and removal of the endothelium was demonstrated by the inability of the contracted tissue to relax to 1μM acetylcholine. 3μM 5HT and 0.1μM phenylephrine were selected as concentrations which, in 2.5mM Ca^{2+}, produced similar contractions (~50% of maximum). [Ca^{2+}] was reduced to "nominal zero" for 45min, agonist was added and 3 minutes later $CaCl_2$ was added cumulatively (0.01-20mM) over a 25 minute period. The results are shown below in Figure 1.

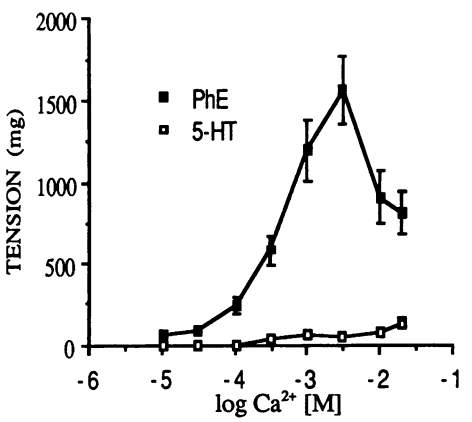

Fig 1. Responses of paired preparations of rat aortic rings to 0.1μM phenylephrine (PhE) or 3μM 5-hydroxytryptamine (5-HT) in Ca^{2+} re-addition experiments. (In 2.5mM Ca^{2+} these concentrations of agonist gave similar responses).

These results suggest that when 5HT is added in a low concentration of extracellular Ca^{2+} (thereby preventing production of a significant contractile response), activation of receptors changes the excitation-contraction coupling process in such a way that a subsequent increase in extracellular [Ca^{2+}] is no longer effective at producing contraction, possibly due to an alteration of Ca^{2+}-channel function. This does not, however, occur with activation of the α_1-adrenoceptor.

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VASOPRESSIN-INDUCED PRIMARY CHANGES IN FLOW AND SECONDARY CHANGES IN CONTRACTILITY IN PERFUSED GUINEA-PIG HEARTS

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Recent studies on the cardiodepressant actions of vasopressin have implicated respectively, metabolic alterations independent of ischaemia (Wilson et al.,1980; Dominguez-Mon et al.,1984), coronary vasoconstriction leading to ischaemia (Zito et al.,1983) and neurally mediated cardioinhibition (Hof,1986). This diversity of proposed mechanisms prompted us to re-examine the relationship between changes in flow and contractility induced by arginine-vasopressin(AVP) in isolated perfused guinea-pig hearts. A selective V_1-antagonist d$(CH_2)_5$Tyr(Me)-AVP (Kruszynski et al.,1980) was employed to indicate the receptor type mediating the effects of AVP. Direct effects of AVP on cardiac muscle were investigated in isolated atrial and ventricular muscle preparations.

Guinea-pig hearts were perfused with Krebs solution (containing indomethacin 2.8μM) by the Langendorff method at a pressure of 40 ± 1mmHg. A balloon in the left ventricle recorded pressure : dP/dT was taken as a measure of contractility. Effluent flow rate was measured by a drop counter. AVP ([Arg^8]-vasopressin:Bachem) was injected (10$\mu\ell$) into the perfusion fluid, just proximal to the heart at 30min intervals. In antagonist studies appropriate concentrations of d$(CH_2)_5$Tyr(Me)-AVP(Bachem), were present in the perfusion fluid 25min before and throughout each AVP response curve. Guinea-pig left atrial strips and papillary muscles were mounted in Krebs and electrically stimulated with point electrodes at 3Hz(atria) or 1Hz(papillaries), 0.5ms pulse width and voltage 1.2 x threshold.

Bolus injections of AVP, 1→30pmol, gave dose-related decreases in flow up to >90% inhibition at peak. Maximal effects were seen within 2min and recovery took up to 15min with the highest dose. With reductions in flow of < 40% at peak, changes in contractility were variable, but with greater reductions in flow, decreases in contractility were consistently observed, up to 60% inhibition with the 30pmol dose. However, the changes in contractility were always temporally displaced from the flow changes, occurring after a lag of a few minutes. These responses were unaltered in the presence of atropine(0.1μM) and guanethidine (10μM). The V_1 antagonist at concentrations of 3,10 and 30nM produced parallel rightward shifts of the AVP flow and contractility log dose/response curves. Schild plots were linear but of non-unity slope. Extrapolation gave pA_2 estimates in the range 8.6-8.9 for flow responses and 8.5-8.8 for the contractility responses. This compares with a pA_2 estimate of 8.6 for this antagonist in a rat vasopressor assay by Kruszynski et al.(1980). No inotropic effects were seen on atrial or papillary muscle preparations up to a concentration of 3.3μM.

We conclude that AVP acts on a V_1 receptor to give coronary vaso-constriction which, if severe, leads to markedly reduced contractility. There is no evidence for a direct effect on cardiac muscle independent of vascular factors.

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THE EFFECTS OF EPININE ON HUMAN CARDIAC ACTION POTENTIAL CHARACTERISTICS

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Ibopamine, an orally active dopamine analogue, has positive inotropic activity in man in vivo (De Vita, et al., 1986). Consistent with this epinine (n-methyl dopamine), the active metabolite of ibopamine increases the force of contraction of isolated human ventricular tissue (English, et al., 1985). We have now further investigated the effects of epinine on isolated ventricular human tissue by examining cardiac action potentials.

Left papillary muscle samples were obtained from patients undergoing cardiac transplantation. Muscle preparations (approximate dimensions 2 mm x 6 mm), were superfused at 6 cm^3 min^{-1} 37°C with Krebs solution and electrically stimulated at 0.2 Hz throughout (threshold plus 50% voltage). Action potential measurements (made using standard microelectrode techniques [electrode resistance 20-50 M Ohms]), were: action potential amplitude (APA mV); action potential duration at 50 and 90% repolarisation (APD_{50} and APD_{90} ms); maximal rate of depolarisation of phase 0 (MRD V/S). After an initial stabilisation period (3 hours), the tissue was exposed to 10^{-7}M and 10^{-6}M isoprenaline. The isoprenaline was washed out (1 hour) and the tissue exposed to ascending concentrations of epinine. All drugs were given cumulatively.

Epinine did not alter the measured parameters (see Table). However, in each experiment small concentration dependent increases in plateau amplitude were observed. Similar results were obtained with isoprenaline.

Table The effects of epinine on the action potential characteristics of human ventricular tissue. (Figures represent means ± s.e.m. N=3)

GROUP	APAmV	APD_{50}ms	APD_{90}ms	MRDV/s
Control	104±2	254±23	326±24	262±13
10^{-6}M "	106±3	254±32	323±34	261±9
10^{-5}M "	107±7	261±37	328±44	262±9
10^{-4}M "	107±6	275±44	347±44	260±9

These results indicate that epinine at concentrations that have previously been shown to cause increases in force of contraction in human ventricular muscle in excess of 50% (English, et al., 1985) failed to significantly influence measured cardiac action potential parameters.

The results indicate that epinine, like isoprenaline does not have marked effects on action potentials from human ventricular tissue. The small changes which were consistently observed (plateau phase enhancement) suggest that the inotropic effect of epinine (as well as isoprenaline) in human ventricular tissue is related to an enhancement of calcium entry via the second inward current (Isi).

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REPERFUSION ARRHYTHMIAS IN THE ANAESTHETISED RAT: BENEFICIAL ACTION OF CILAZAPRIL

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Recently, several of the angiotensin converting enzyme (ACE) inhibitors have been shown to reduce the incidence of reperfusion-induced arrhythmias in the isolated perfused rat heart preparation (Van Gilst et al, 1986). In order to determine if the new ACE inhibitor, cilazapril, is equally effective we have investigated its action on reperfusion-induced arrhythmias and mortality in an in vivo anaesthetised rat preparation with transient coronary artery occlusion (CAO).

An anaesthetised rat preparation with transient (7 min) CAO followed by reperfusion was used as described previously (Crome et al, 1985). Cilazapril or vehicle control was administered i.v. 10 min prior to occlusion.

Table 1. Effect of cilazapril on reperfusion-induced arrhythmias in the anaesthetised rat

	Incidence VF (%)	Incidence VT (%)	Mortality (%)	Total PVC's	Arrhythmia Score
Control (n = 10)	70	90	50	302 ± 65	5.0 ± 0.5
Cilazapril (n = 13) (0.1 mg/kg)	62	85	22	182 ± 30	4.2 ± 0.5
Cilazapril (n = 10) (1.0 mg/kg)	40	80	20	126 ± 23*	3.2 ± 0.5*

* $p<0.05$

Table 1 shows that although the lower dose (0.1 mg/kg) of cilazapril reduced mortality from 50% to 22% and total premature ventricular contractions (PVC's) number from 302 ± 65 to 182 ± 30, resulting in a partial reduction in mean arrhythmia score (based on criteria described by Johnston et al, 1983) of 5.0 ± 0.5 to 4.2 ± 0.5, these changes did not reach a level of statistical significance. However at the higher dose of cilazapril (1.0 mg/kg) the reduction in arrhythmia score became statistically significant (5.0 ± 0.5 to 3.2 ± 0.5; $p<0.05$). At this dose there was a large reduction in total PVC number (302 ± 65 to 126 ± 23; $p<0.05$) and a lower incidence of reperfusion-induced ventricular fibrillation (70% compared to 40%, p = N.S.). Furthermore no significant effects of cilazapril were noted on either mean arterial blood pressure or heart rate, either prior to CAO or during the ischaemic period.

Thus, cilazapril like several other ACE inhibitors can cause a significant reduction in the incidence of reperfusion-induced rhythm disturbances and if this beneficial action also was shown to occur clinically then this would suggest an additional mechanism by which ACE inhibitors may reduce the incidence of sudden cardiac death in high risk patients.

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UK-61, 260-INDUCED CARDIAC STIMULATION IN THE CONSCIOUS DOG; A PHARMACOLOGICAL AND PHARMACOKINETIC STUDY

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UK-61,260, a novel compound with inotropic activity in vitro and in vivo (Ellis, Henderson and Samuels 1987) was assessed for positive inotropic activity in the conscious dog by the non-invasive measurement of QA interval (Alabaster & Henderson, 1982).

Eight conscious male beagle dogs were used. The arterial pressure pulse, from an exteriorised carotid artery, and Lead II ECG were recorded and QA interval and heart rate (HR) derived. Measurements were made every 10 minutes for 30 minutes before and for 7 hours after the oral administration of UK-61,260 (by gavage in 5 ml tap water) at doses of 0.0625, 0.125 and 0.25 mg kg^{-1}. In a separate experiment, 3 dogs were given 0.125 mg kg^{-1} UK-61,260, in soft gelatin capsules, every 8 hours for 5 days. QA and HR measurements were made, as above, pre-dose and for 4 hours after each morning dose on days 1 to 4 and for a further period from 5 to 7 hours post the morning dose on days 1 and 4. 5 ml of peripheral venous blood was withdrawn immediately before every dose and at 4 hours post-dose on days 1 to 5, with additional samples (0.5, 1, 2, 6 and 8 hours) after the final dose on day 5. The blood was centrifuged, the plasma separated and assayed for unchanged UK-61,260 by specfic HPLC assay.

Acute oral administration of UK-61,260 produced a dose dependent decrease in QA interval which was evident within the first hour after dosing and was well maintained over the 7 hour period. There was not, even at the highest dose level, an appreciable alteration in HR (Table showing mean change from control).

Dose $mg\ kg^{-1}$	2 hours post-dose		7 hours post-dose	
	QA interval (msec)	HR (bt/min)	QA interval (msec)	HR (bt/min)
0.0625	- 9.5	- 7.0	- 8.0	- 4.0
0.125	-11.0	-12.0	- 9.5	- 9.0
0.25	-14.5	1.0	-12.0	2.0

Repetitive oral dosing of 0.125 mg kg^{-1} UK-61,260 every 8 hours produced an initial change in QA interval (-14 msec) in accord with these data and responses on days 2 to 4 (-14, -12, -12 msec respectively) were very similar. A small residual effect was noted before the morning dose on days 2 to 4. Plasma concentrations measured at 4 and 8 hours post-dose were also well maintained throughout the study (average 22 and 11 ng ml^{-1} respectively) in agreement with the consistent pharmacological response. Data indicate rapid attainment of steady state with little drug accumulation; elimination half-life on day 5 was about 4 hours.

UK-61,260 is therefore a potent, orally-active, force-selective cardiac stimulant in the conscious dog following both acute and chronic dosing.

Acknowledgements: Miss D.E. Balderson, Mr. G.D. Bowers.

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UK-61,260: A NOVEL CARDIAC STIMULANT AND VASODILATOR AGENT

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In isolated electrically-driven, canine right ventricular trabeculae (n=3) UK-61,260[6-(2,4-dimethyl-1H-imidazol-1-yl)-8-methyl-2(1H)-quinolinone] produced dose-related increases in contractile force over the dose range 4 X 10^{-8}M to 10^{-5}M. In dog heart/lung preparations (n=3) UK-61,260 (0.01 to 1.28 mg) also elicited dose-related increases in cardiac contractility (dP/dt max) with substantially less concomitant tachycardia than seen after isoprenaline, thus indicating a selective action on the force of contraction.

In normal pentobarbitone-anaesthetised dogs (n=3) consecutive 10 min intravenous infusions of UK-61,260 (0.25, 0.5, 1, 2 and 4 µg/kg/min) produced dose-related increases in dP/dt max (up to 62%) accompanied at the three highest dose levels by reductions in systemic vascular resistance (max: decrease 24%) and in mean BP (up to 29mm Hg). No significant changes were observed in heart rate, cardiac output or stroke volume. This haemodynamic profile is consistent with that of a cardiotonic agent with additional vasodilator properties. Furthermore, when administered close-arterially to a constant flow, pump-perfused, hind-limb vascular bed in the anaesthetised dog (n=4), UK-61,260 (over the range 18 ng/kg/min to 5.2 µg/kg/min for 5 min, equivalent to blood concentrations of 3ng/ml to 1µg/ml) produced dose-related decreases in hind-limb perfusion pressure (up to 34 mm Hg decrease).

UK-61,260 (up to 3µM) is without significant effect on Na^+,K^+-ATPase and cardiac adenylate cyclase nor does it interact with the following receptor systems: $alpha_1$ and beta-adrenoceptors, histamine, muscarine or dopamine D_2 (as assessed in vitro by pharmacological evaluation in isolated smooth muscle preparations or by radioligand binding studies).

UK-61,260 is also active on oral administration to conscious dogs (Alabaster and Rance).

The results presented above indicate that UK-61,260 is a potent, force-rate selective, non-adrenergic, non-glycosidic cardiac stimulant with additional vasodilator properties. Its precise mechanism of action is not known and remains under investigation.

We wish to thank S.I. Davis, T.L. Kidd and A.J. Pannell for their invaluable technical assistance.

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THE EFFECT OF SOME SELECTIVE PHOSPHODIESTERASE INHIBITORS ON VASOCONSTRICTOR RESPONSES TO STIMULATION OF SYMPATHETIC NERVES

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The elevation of intra-cellular cyclic nucleotide concentrations is generally associated with the relaxation of vascular smooth muscle (Kukovetz et al 1981). We recently reported (Owen et al 1986) that different isoenzyme selective phosphodiesterase (PDE) inhibitors resulted in different degrees of relaxation in rabbit ear arteries constricted by perfusion with potassium. Non-selective inhibition of PDEs by isobutylmethylxanthine (IBMX) resulted in the greatest vasodilatation, followed by M&B 22948, selective for the Ca^{2+}/calmodulin stimulated PDE, primarily responsible for the metabolism of cyclic GMP. Inhibition of the low Km selective cyclic AMP selective PDE by SK&F 94120 resulted in minimal vasodilatation in this preparation.

In the present investigation we have examined the effects of these three differentially selective PDE inhibitors on the contraction of vascular smooth muscle in response to stimulation of the perivascular sympathetic nerves. Changes in perfusion pressure, of rabbit isolated central ear arteries perfused at constant flow with Krebs solution, were used to assess changes in vascular smooth muscle tone.

Supramaximal electrical stimulation (20 volts, 5 msec, 1-100 Hz) of the proximal end of the artery using bipolar silver electrodes 2 mm apart, resulted in a frequency dependent increase in perfusion pressure indicating vasoconstriction. The threshold frequency for a response was about 1 Hz and the maximum occurred during stimulation at 20 or 50 Hz. The frequency required to produce a 50 mmHg rise in perfusion pressure (EF50) was 4.4 ± 0.3 Hz (n=24). Perfusion with Krebs solution containing $1x10^{-4}$ M IBMX caused a large significant ($p < 0.05$) inhibition of constrictor responses; EF50 increased from 4.1 ± 0.5 to 27.3 ± 7.1 Hz and the maximum response was reduced from 180.6 ± 28.1 to 87.4 ± 29.2 mmHg (n=8). M&B 22948 $1x10^{-4}$ M increased EF50 from 3.7 ± 0.5 to 8.3 ± 1.7 Hz with a large reduction in maximum response from 195.5 ± 21.7 to 106.8 ± 16.5 mmHg, (n=8) both significant. In contrast, SK&F 94120 $1x10^{-4}$ M caused a small insignificant increase in EF50 from 5.4 ± 0.5 to 9.3 ± 2.1 Hz and small reduction in maximum response from 182.4 ± 30.7 to 146.3 ± 28.7 mmHg (n=8).

These results indicate that inhibition of cyclic nucleotide metabolism by PDE inhibitors can reduce the vasoconstrictor responses to perivascular nerve stimulation. Non-selective inhibition by IBMX caused a large reduction in maximum response and increase in EF50. Inhibition of the Ca^{2+}/calmodulin stimulated PDE by M&B 22948 had a large effect on the maximum response with a modest increase in EF50. Inhibition of the low Km selective cyclic AMP selective PDE by SK&F 94120 had no significant effect on the vasoconstrictor response. The order of potency found for inhibition of sympathetic vasoconstrictor responses, IBMX > M&B 22948 > SK&F 94120, is similar to that found for the direct vasodilator effects of the inhibitors on potassium constricted arteries.

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ON THE ELECTROPHYSIOLOGICAL MECHANISMS OF ACTION OF A NOVEL ANTIARRHYTHMIC AGENT - RS 87337

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We have investigated the effects of N-[3,5-Dichlorophenyl]4-[4-hydroxy-2-methoxy phenyl]-piperazine-1-carboxamidine (RS 87337) on the rate of depolarisation and duration of action potentials recorded from the ventricular myocardium. Membrane potential (Em) was recorded from papillary muscles isolated from guinea-pig right ventricle, continuously superfused with physiological saline at 37°C as described by Patmore (1982). The maximum rate of depolarisation (Vmax) was determined by anologue differentiation of Em and gives an estimate of the magnitude of the sodium current (Fozzard et al., 1986). RS 87337 caused a concentration-dependent inhibition of Vmax. The inhibition was frequency-dependent, with an IC_{50} of 1.8×10^{-5}M at 3.3 Hz as compared with IC_{50}s of 5×10^{-6}M for flecainide and 8×10^{-5}M for disopyramide. These properties are characteristic of sodium channel blocking antiarrhythmic agents (Class I-Vaughan Williams, 1975). The Class I drugs have also been subclassified by their onset and offset kinetics (Campbell, 1983). The rate of onset of inhibition of Vmax by RS 87337 was 0.1; the maximum effect was achieved from quiescence in 10 action potentials. This suggests an intermediate onset of action (Class Ia) similar to disopyramide (rate = 0.1). These values can be compared with the fast onset displayed by lignocaine (rate = 0.6; maximum within 2 beats: Class Ib) and the slow onset of flecainide (rate = 0.03; maximum effect in around 30 beats; Class Ic). Offset kinetics were studied; muscles were stimulated at 1.65 Hz until maximum blockade of Vmax was achieved and then stimulation interrupted for increasing periods of time. The recovery of Vmax plotted against interrupt time yielded a time constant of recovery for RS 87337 of 5.6 s. This was faster than disopyramide (11 s) and flecainide (15 s) but not as rapid as lignocaine (1.1 s). The kinetics of sodium channel blockade suggest that RS 87337 might be considered a Class Ia intermediate onset/offset antiarrhythmic agent, similar to disopyramide. RS 87337, 10^{-5} M, significantly increased action potential duration at 3.3 Hz by 15.8% or 25 ms (mean, n=4, $p<0.001$ - Student's t-test). This compares with increases of 22% or 34 ms induced by 10^{-4}M sotalol. These effects were also observed at 1.0 and 1.65 Hz. In conclusion Class Ia and Class III antiarrhythmic actions may account for the efficacy of this agent in antiarrhythmic models 'in vivo' (see Armstrong et al., 1987).

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EFFECTS OF A NOVEL CLASS III AND CLASS IA ANTIARRHYTHMIC AGENT RS-87337 IN RATS AND DOGS

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Pharmacological examination of a series of piperazine carboxamidine compounds revealed that some of them produced antiarrhythmic activity. RS-87337 (piperazine carboxamidine N-[3,5 dichlorophenyl] 4-[4-hydroxy-2-methoxy-phenyl] dihydrochloride) was selected for further evaluation when electrophysiological studies showed that it increased both the duration of the cardiac action potential (Class III effect) and decreased the maximum rate of depolarization with an intermediate rate of onset and offset (Class Ia effect) (see Juett and Patmore, 1987). We now report results of these studies.

In isolated working rat hearts perfused with Krebs-Ringer solution containing 1.35 mM Ca^{++} (Armstrong and Ferrandon, 1985), RS-87337 (0.01 uM - 1.0 uM) did not alter baseline values of the cardiac output (normally 62 ± 2 ml/min, n=8) or left ventricular contractility (normally 3441 ± 90 mmHg/sec, n=10) but prevented the cardiac death that occurred in 90% of hearts after release of a coronary ligature applied for 15 min. All hearts that received RS-87337 (1 uM) continued to beat producing a mean cardiac output of 45 ± 3 ml/min (n=8). At the same time, RS-87337 significantly reduced the elevated left ventricular diastolic pressure from 9.0 ± 0.7 mmHg (n=8) to 3.7 ± 0.8 mmHg (n=10, p less than 0.05, t-test). In the 10% of hearts surviving reperfusion without RS-87337, the corresponding cardiac output value was only 6 ± 3 ml/min (n=10). In ventilated, open-chest, anaesthetised rats in which hearts were induced to fibrillate by application of a a coronary ligature for a 5 min period and then the ligature removed (Manning et al., 1984), i.v. RS-87337 (3 mg/kg) reduced the incidence of fibrillation from 90% in controls to 29% (n=14) and death from 70% of controls to 28% (n=14).

In conscious dogs with a two-stage coronary ligature applied 24 hours previously (Harris 1950), RS-87337 (3 mg/kg i.v.) reduced the percentage of the ectopic ventricular complexes observed 10 min after dosing from 95 ± 2% in controls to 69 ± 12% (n=10). After 10 mg/kg had been given the percentage of ectopic beats observed was 19 ± 10% (n=10). When 30 mg/kg RS-87337 had been administered orally to 8 dogs the percentage of the ectopic complexes was reduced to 66 ± 14%, 10 min after dosing and to 19 ± 9%, 6 hours later. Abnormal ECG waves produced by i.v. infusions of ouabain (Allen et al. 1971) were also inhibited by i.v. RS-87337. For example, 5 min after injecting 3 mg/kg of the compound the percentage of abnormal complexes counted was reduced from 91 ± 5% (n=5) of those obtained initially to only 8 ± 8%.

Thus RS-87337 may be an orally effective and longlasting antiarrhythmic agent that could be particularly useful in reducing the ventricular tachycardias and fibrillation that are thought to preceed the phenomenon of sudden cardiac death.

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IN VITRO EFFECTS OF CALCIUM ANTAGONISTS ON ADRENERGIC RESPONSES IN CANINE SAPHENOUS VEIN AND RAT THORACIC AORTA

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It is generally thought that contractions of blood vessels mediated by transmural nerve stimulation (TNS) in vitro or sympathetic nerve stimulation in vivo are relatively insensitive to calcium antagonists. However recent work by Jayakody et al (1986) suggests that responses to TNS in the canine saphenous vein are sensitive to calcium entry blockade. The aim of the present study was to investigate the effects of calcium antagonists on contrctile responses to TNS in the canine saphenous vein and to compare this with their effects on noradrenaline (NA)- and KCl- mediated contractions in rat thoracic aorta.

Rings of canine saphenous vein or rat thoracic aorta were bathed in normal Kreb's solution ($[Ca^{++}]$=2.5 mM) at 37°C and at 1.5g and 1g resting tensions respectively. TNS parameters were 12V, 2.0ms pulse width at 0.5–10Hz. Contractile responses to TNS were completely abolished by phentolamine ($3x10^{-5}$M) suggesting the responses were of sympathetic origin.

In canine saphenous veins pretreated with propranolol (10^{-6}M), nifedipine (10^{-7}M–10^{-5}M) and verapamil (10^{-7}M & 10^{-6}M) had no significant effect on contractile responses to TNS. Verapamil (10^{-5}M) inhibited responses to high frequencies (5 & 10Hz) by 22±4 (mean ± s.e.mean) and 20±2 respectively (for each, $p<0.001$, n=4).

In propranolol (10^{-6}M) pretreated rat thoracic aorta, nifedipine (10^{-8}M–10^{-6}M) and verapamil (10^{-6}M & 10^{-5}M) had no significant effect on the cumulative noradrenaline dose–response curve. However nifedipine (10^{-5}M & 10^{-4}M) significantly reduced the maximum NA response to 77±7% ($p>0.05$, n=4) and 64±5% ($p<0.001$, n=4) of control respectively. Verapamil (10^{-4}M) depressed the maximum response to 58±12% ($p<0.05$, n=4) of control. In contrast, in aortae pretreated with phentolamine (10^{-5}M) and contracted with KCl (30 mM), nifedipine (10^{-8}M & 10^{-7}M) relaxed the tissues by 100% (time to 50% relaxation = 6±0.5 min and 2±0.2 min respectively). Verapamil (10^{-8}M) had no effect whilst verapamil (10^{-7}M & 10^{-6}M) relaxed the KCl–contracted tissues to 38±5%(n=4) and 7±4% (n=4) of control (time to 50% relaxation = 12±2 min and 4±0.4 min respectively).

The results demonstrate that at a $[Ca^{++}]$ of 2.5mM the responses to endogenous (TNS) and exogenous noradrenaline are relatively insensitive, whilst responses to KCl are sensitive, to the Ca^{++} antagonists nifedipine and verapamil. In contrast, Jayakody et al (1986) have shown that in canine saphenous veins bathed in a $[Ca^{++}]$ of 1.2mM, responses to TNS are sensitive to calcium entry blockade. This suggests that the sensitivity of the transmurally stimulated canine saphenous vein preparation to Ca^{++} antagonists depends on the $[Ca^{++}]$ in the bathing solution.

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POST-JUNCTIONAL α-ADRENOCEPTOR SUBTYPE(S) FOR NA ON THE RABBIT ISOLATED SAPHENOUS VEIN

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Alabaster *et al.*, (1984) recently reported that postjunctional α-adrenceptors on the rabbit isolated saphenous vein are of the α_2-subtype. In an earlier study (Purdy *et al.*, 1980), however, responses to noradrenaline (NA) in this preparation were prazosin-sensitive. We have, therefore, (re)examined the effects of the antagonists prazosin and corynanthine (selective for the α_1-subtype) and rauwolscine (selective for the α_2-subtype; McGrath 1982) against NA.

3mm wide segments of the rabbit isolated saphenous vein were placed under a resting tension of 0.5g in Krebs containing 10μM cocaine and 1μM propranolol, gassed with 95% O_2 5% CO_2 and kept at 37°C. Cumulative concentration-response curves (CRC) to NA were elicited before and 45 min after exposure to an antagonist. The agonist log concentration-ratios (LACR) at the 25%, 50% and 75% level of the peak response were determined and pA_2 values calculated (Schild 1957).

Prazosin (5nM - 3μM) and corynanthine (0.5μM - 50μM) produced concentration-dependent shifts of the NA CRC without altering the slope (similar shifts of the LACR at the 25% and 75% level). Based upon the slope of the Schild plot, however, corynanthine and prazosin effected competitive and noncompetitive antagonism, respectively (Table 1). Rauwolscine (50nM - 2.5μM) elicited a short-lived contraction (< 5% of maximum) in 30% of preparations and produced a concentration-dependent shift of the NA CRC which was associated with a significant change in the slope of the CRC - for 2.5μM rauwolscine, the LACR at the 25% level (1.37±0.32, n=6) was significantly less ($p<0.05$) than that at the 75% level (2.35±0.11). When assessed at the 75% level of the maximum response, rauwolscine was ~ 100-fold more potent than its diastereoisomer corynanthine (Table 1). Prazosin (0.1μM) selectively inhibited the "resistant" component of NA contractions in the presence of 2.5μM rauwolscine - LACR values at the 25% (2.42±0.24, n=6) and the 75% level (2.45±0.11) were similar.

Table 1: pA_2 values and slope of the Schild plot (with 95% confidence limits) for rauwolscine, corynanthine and prazosin against NA (n=22-56 individual points).

	Rauwolscine	Corynanthine	Prazosin
pA_2	8.56 (8.89-8.22)	6.36 (6.55-6.16)	8.49 (8.88-8.11)
Slope	0.85 (0.74-0.96)	0.89 (0.71-1.06)	0.58 (0.47-0.69)

Two possible explanations for our observations are; 1) there is a single population of α-adrenoceptors which exhibits characteristics of both subtypes as defined in other tissues and species or, 2) there is an α_2-subtype (potency order: rauwolscine > corynanthine) and an α_1-subtype (sensitivity to prazosin) that do not interact in a simple additive manner. We prefer the latter.

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EFFECT OF BAY K 8644 ON THE ENDOTHELIUM-DEPENDENT RELAXATION INDUCED BY ACETYLCHOLINE IN THE RAT ISOLATED AORTA

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The Endothelium-Derived Relaxing Factor (EDRF) has been shown to be released spontaneously (Griffith et al., 1984) or in response to certain agonists (Furchgott, 1983). In the rat aorta, Miller et al. (1985) have suggested that Bay K 8644 didn't increase the release of EDRF while, in the canine femoral artery, Rubanyi et al. (1985) suggested the opposite. In view of these conflicting results, we have studied the effect of Bay K 8644 on the endothelium-dependent relaxation induced by acetylcholine (Ach) in the rat aorta.

Aorta rings were taken from male Wistar rat (250 - 300 g). The rings were left intact and mounted in 50 ml organ baths filled with a physiological salt solution (PSS) at 37°C gassed with 95% O_2 : 5% CO_2. Tissues were contracted with a depolarizing solution containing 100 mM of KCl. At the maximal contraction, Ach was added in a cumulative way. Another contraction was then realized in a depolarizing solution containing 100 mM of KCl and Bay K 8644 300 nM or in a PSS containing 20 mM of KCl and Bay K 8644 300 nM. Ach was added in a cumulative way at the maximum of the contraction. Bay K 8644 (BAYER) was dissolved in acetone as a stock solution of 0.1 mM. The experiments with Bay K 8644 were realized in a dark room with a sodium lamp.

Bay K 8644 300 nM increased significantly the maximum contractile response evoked by a depolarizing solution containing 100 mM KCl ($P < 0.01$). Ach induced a concentration dependent relaxation of this contraction. The residual contraction, as expressed in % of the maximum contraction, was not significantly different in control and treated preparations (51.0 $\pm$ 3.1 % and 54.5 $\pm$ 1.4 %, n=7, $P > 0.1$, respectively). However, the control preparations were significantly more sensitive to Ach than the treated ones (EC50 : 140 $\pm$ 19 nM and 240 $\pm$ 30 nM, n=7, $P < 0.01$, respectively).
Because the contraction level was higher in treated than in control preparations, we have studied a concentration of KCl for which Bay K 8644 300 nM evoked a contraction comparable to that induced by 100 mM of KCl. This concentration was 20 mM. In these conditions, Ach induced a comparable relaxation in control (KCl 100 mM) and tested (KCl 20 mM + Bay K 8644 300 nM) preparations, i.e. the residual contraction and the EC50 were not significantly different ($P > 0.1$).

These results support the hypothesis that Bay K 8644 doesn't induce a release of EDRF in the rat isolated aorta.

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COMPARISON OF HAEMODYNAMIC EFFECTS OF VERAPAMIL AND SODIUM NITROPRUSSIDE IN ANAESTHETISED AND PITHED RATS

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Verapamil (V) and sodium nitroprusside (NP) reduce peripheral resistance by direct vasodilation but their effects on resistance vessels in vitro differ (El-Muradi and McCurrie, 1985). In the present work haemodynamic effects of V and NP were compared in vivo.

Blood flow (BF), expressed as ml min^{-1} $100g^{-1}$ tissue wet weight, was measured in anaesthetised (AR) and in pithed rats (300-350g) by the labelled microsphere technique (Sc^{46}, 15 μm NEN-TRAC). N = 6 to 14 for each group. Mean arterial blood pressure (MABP), heart rate (HR) and central venous pressure (VP) were measured immediately before V (100 or 200 μg kg^{-1}) or NP (3 or 6 μg kg^{-1}): these doses reduced MABP by 25 or 50% in AR. Total peripheral conductance (TPC) (ml min^{-1} $mmHg^{-1}$ kg^{-1}) was estimated by dividing cardiac output (CO)kg^{-1} by MABP. BF was measured 30 seconds after administering V or NP. In AR MABP was 92 ± 6, VP 1.3 ± 0.2 mmHg: HR 412 ± 14 beats min^{-1}: CO 126 ± 7 ml min^{-1}, TPC 4.3 ± 0.2. After pithing MABP was 45 ± 3, VP 3.3 ± 0.4 mmHg: HR 293 ± 17 beats min^{-1}: CO 58 ± 3 ml min^{-1}. To raise HR and MABP to values comparable with AR the sympathetic outflow was stimulated (8 Hz, 1 ms, supramaximal voltage) following vagotomy and atropine (1 mg kg^{-1}) (Gillespie and Muir, 1967). MABP increased to 113 ± 6, VP 6.4 ± 1 mmHg: HR 421 ± 19 beats min^{-1}: CO 90 ± 7 ml min^{-1}: TPC 2.4 ± 0.15.

Table 1. Effects of verapamil and nitroprusside on regional blood flow (ml min^{-1} $100g^{-1}$).

	Heart	R Kidney	L Kidney	S Intest.	Muscle	Skin
AR	723(48)	533(26)	518(26)	224(16)	9(1)	14(2)
V 100 μg kg^{-1}	1128(136)*	726(106)	652(87)	414(41)***	4(1)**	6(2)**
NP 3 μg kg^{-1}	705(79)	632(26)	622(34)	257(21)	11(2)	10(2)
Pithed (P)	213(15)	267(15)	265(17)	104(9)	6(1)	7(1)
P.Stimulated	823(103)	26(6)	26(5)	36(5)	14(2)	15(3)
V 100 μg kg^{-1}	1160(104)+	24(6)	18(4)	44(8)	20(4)	12(2)
NP 3 μg kg^{-1}	1271(139)+	21(6)	19(7)	35(6)	10(2)	12(2)

Figures: mean and (S.E.M.). * ** *** significantly different from anaesthetised control (AR) at P<0.05, 0.01 and 0.001 respectively. + significantly different from pithed, stimulated control, P<0.05 (Student's unpaired t test).

In AR V (100 μg kg^{-1}) increased flow to the heart and small intestine but decreased that to skin and skeletal muscle (Table 1). No significant flow changes occurred at the higher dose, although TPC increased by 73% (P<0.05). NP (3 μg kg^{-1}) caused no flow changes in these tissues but at 6 μg kg^{-1} flow to the heart, intestine and muscle increased; TPC increased by 102% (P<0.01). In the pithed, stimulated rat large reductions in flow to the intestine and kidney occurred; both doses of V and NP increased flow to the heart only, TPC increased after both drugs.

These results show that for a similar reduction in MABP, V and NP differentially affected regional blood flow, these differences were absent after pithing. Neither dilator improved blood flow to the kidney or intestine during sympathetic stimulation.

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BRL 34915 INDUCED POTASSIUM CHANNEL ACTIVATION: DEPENDENCE ON CALCIUM IONS FOR CLOSING

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The novel antihypertensive agent BRL 34915 relaxes vascular smooth muscle by a mechanism associated with the activation of potassium channels (Hamilton et al, 1986), which, in rabbit isolated mesenteric artery (RIMA), are not dependent on calcium influx for opening (Coldwell and Howlett, 1986). This communication shows, however, that BRL 34915 activated potassium channels in RIMA are apparently closed by calcium and, as such, are unlike potassium channels previously described in vascular smooth muscle.

Segments of RIMA (c.20mg) were loaded with 86-rubidium (Rb) (as a marker for potassium) by preincubating with Rb (2-5mCi/mg; c.1uCi/ml) in a HEPES buffer for 90 minutes at 37° (composition in mM: NaCl 120; KCl 6.0; $CaCl_2$ 2.5; $MgCl_2$ 1.2; HEPES 5.0; glucose 11.4; pH 7.4). Rb efflux was determined at 3 min intervals over the subsequent 60 or 78 min. The buffers for the efflux period were i) normal HEPES (as above), ii) HEPES omitting the calcium but containing 2mM EGTA (OCaHEPES) or iii) calcium free HEPES containing 2mM EGTA and 10mM $MgCl_2$ (CAFH). The tissues were exposed to BRL 34915 (10μM) between minutes 30 and 48 of the efflux period. Data were calculated as % of remaining counts released during each 3 min efflux period. Basal rate was defined as the average efflux over minutes 21-30 of the efflux period.

In normal HEPES, the basal efflux rate was 2.1 ± 0.1% per 3 min (n=101), whereas in OCaHEPES, the basal rate was increased to 3.2 ± 0.3% per 3 min (n=8). This increase is thought to be associated with membrane instability and can be overcome, as in the CAFH buffer, by the addition of 10mM $MgCl_2$ to the medium (Smith & Jones, 1985). Thus in CAFH, the basal efflux rate was 2.4 ± 0.1% per 3 min (n=42).

In normal calcium containing HEPES, BRL 34915 stimulated efflux attained a maximum rate (3.2 ± 0.2% per 3 min. n=101) within 6 min of drug addition but then returned towards basal levels. In CAFH, not only was the stimulation greater than in normal HEPES, but the increase was sustained after the removal of BRL 34915. When the normal concentration of calcium (2.5mM) was readded to CAFH for the entire efflux period, the response to BRL 34915 was not maintained at the maximum level, as seen in CAFH, but produced a response similar to that observed in normal buffer. The introduction of calcium ions (2.5mM) into the CAFH efflux buffer at the end of the normal efflux period (i.e. at 60 min) reduced efflux to near basal rates.

Under conditions of high basal efflux (OCaHEPES), BRL 34915 was still able to increase efflux rate. Again, the readdition of calcium reduced the rate of efflux to near normal basal levels. In the absence of calcium, noradrenaline (NA) (30μM) and K^+ (30mM) had little effect on basal efflux rate.

These data show differences between the potassium channel opened by BRL 34915 in RIMA and the channels opened by NA or K^+ in vascular smooth muscle (Bolton and Clapp, 1984). The opening of the BRL 34915 activated channel is unaffected by calcium influx blockers such as nifedipine or lanthanum (Coldwell & Howlett, 1986) or by zero calcium buffers. Once open, however, calcium appears essential for the closing of the channel. Since the consequence of membrane hyperpolarisation by BRL 34915 is a reduction in calcium influx (Hamilton et al, 1986), our data suggest that under normal physiological conditions calcium would oppose this effect by closing the potassium channel responsible for the hyperpolarisation.

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ANTAGONISM OF THE VASORELAXANT ACTIVITY OF BRL 34915 BY K^+ CHANNEL BLOCKERS

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BRL 34915 lowers blood pressure (Buckingham et al., 1986) and relaxes pre-contracted vascular smooth muscle (Clapham & Wilson, 1986) by a mechanism which appears to involve the opening of outward K^+ channels and consequent hyperpolarisation of the smooth muscle cell membrane (Hamilton et al., 1986). In order to consolidate this hypothesis, the effects of three K^+ channel blockers, tetraethylammonium (TEA), 4-aminopyridine (4-AP) and procaine, were studied upon the vasorelaxant activity of BRL 34915 in arterial smooth muscle.

Rabbit isolated mesenteric artery rings were prepared for tension recording as described previously (Clapham & Wilson, 1986). Tissues were contracted by the addition of KCl 30mM to the organ bath. Once a steady contraction had been established, the tissues were relaxed by the cumulative addition of BRL 34915 0.01-10μM. After washout, tissues were again contracted with KCl 30mM, followed by incubation with K^+ channel blocker. Tension was again allowed to stabilise (approx. 15min) before the re-addition of BRL 34915. The results shown in the table are expressed as the mean (n=5) ± s.e.m. percentage remaining area under the BRL 34915 concentration-response curve following treatment with K^+ channel blocker.

Concentration (mM)	Procaine	4-AP	TEA
0.3	79.9 ± 6.6%	-	-
1	35.6 ± 6.4%	79.6 ± 9.5%	-
3	-	40.7 ± 7.6%	-
5	6.3 ± 5.1%	-	-
10	-	0%	73.1 ± 11.0%
30	-	-	40.1 ± 3.1%

The estimated concentrations of K^+ channel blocker required to reduce the area under the curve to 50% were 0.67mM for procaine, 2.3mM for 4-AP and 22mM for TEA. These three compounds also inhibited the relaxant effect of BRL 34915 when KCl was replaced by noradrenaline (0.1mM) as the contractile stimulus. The data are in agreement with the findings of Allen et al. (1986) who showed that the three K^+ channel blockers antagonised the effects of BRL 34915 in guinea-pig isolated trachealis.

The results of the present experiments therefore support further the hypothesis that BRL 34915 relaxes vascular smooth muscle by the activation of outward K^+ channels.

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ENDOTHELIUM DEPENDENT INHIBITION OF HUMAN PLATELET AGGREGATION IN VITRO

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The release of EDRF, a labile and potent blood vessel relaxant, from vascular endothelial cells is now well established (Furchgott & Zawadzki 1980, Furchgott, 1984). However, other pharmacological effects of EDRF have yet to be so thoroughly investigated. In this study we report preliminary data which suggests that EDRF, or a like substance, exerts platelet anti-aggregatory activity in vitro.

Human blood (9ml) was collected from the antecubital vein, of drug free volunteers and anticoagulated with 1ml of heparinized (2U/ml) 3.8% trisodium citrate. Rat aortic rings (10mg) preincubated for 20min with indomethacin (14uM), were used as the source of vascular endothelium. Release of EDRF was induced with either carbachol or histamine. A single aortic ring was equilibrated (2mins, 37°C) with 1ml anticoagulated blood in the presence of indomethacin (6uM). At the end of this period platelet aggregation was triggered with ADP (20uM) and aggregation allowed to proceed for 5min. Carbachol (0.006-6uM), histamine (0.003-13uM), or saline (1-4ul) was added to the cuvette 10sec prior to injection of ADP. In these experiments platelet aggregation in whole blood was measured using a Chronolog impedence aggregometer, model 540VS (Coulter Electronics Ltd).

Addition of saline to whole blood containing rat aortic ring which had been pre-incubated with indomethacin as described did not affect the platelet aggregation response to a submaximal dose of ADP (20uM). In contrast, a concentration dependent inhibition of ADP induced platelet aggregation was observed when either carbachol or histamine was added to the cuvette containing rat aortic ring 10sec before the injection of ADP. Carbachol (0.06uM) produced an inhibition of platelet aggregation, in the presence of rat aorta, of 11.5 ± 0.9%(n=6). At a higher concentration, carbachol (1.6uM), again in the presence of rat aorta, produced an even greater inhibition of platelet aggregation, 53.8 ± 8.7%(n=6). Similarly, histamine (0.03uM and 0.7uM) produced an inhibition of platelet aggregation, in the presence of rat aorta of, 11.6±4.4% and 46.7 ± 7.2%(n=6) respectively. Interestingly, increasing the concentration of either carbachol or histamine even further (>2uM), in the presence of rat aorta resulted in increased platelet aggregation to ADP. In control experiments addition of either carbachol (0.06-6.0uM) or histamine (0.03-13uM) to whole blood, in the absence of rat aortic ring, did not affect platelet aggregation. In some experiments de-endothelialization was carried out by gentle rubbing of the intimal surface of the aortic ring with damp cotton wool. Carbachol (1.6uM), incubated with rubbed aorta, did not inhibit ADP induced platelet aggregation (2.2±0.5% cf 53.8±8.7%, n=6). The time interval between the addition of carbachol and induction of platelet aggregation with ADP was critical. For example, carbachol (0.6uM) produced 52.9±2.3%; 38.5±8.5% and 11±7% (n=6), inhibition of platelet aggregation when the time interval before ADP addition was 5, 10 and 60sec respectively.

The results of this study provides evidence that a labile platelet anti-aggregatory agent is released from vascular endothelial cells stimulated by carbachol or histamine.

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EXPERIMENTALLY INDUCED LEUKOPENIA OF THE GUINEA-PIG AND THE ACTION OF LIPOXYGENASE AND CYCLOOXYGENASE INHIBITORS

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Neutrophils are known to respond to injury by marginating within small blood vessels close to a lesion and adhering to the vascular endothelium of the postcapillary venules. In addition, there is a migration of adherent neutrophils between the endothelial cells into the extravascular tissue. These changes could contribute towards a significant leukopenia observed in the guinea pig after neck surgery, together with other systemic changes.

Guinea pigs were anaesthetised with either sodium pentobarbitone (38 mg/kg i.p.) or halothane and cannulae inserted into both the carotid artery and the external jugular vein. Blood samples were taken at intervals over three hours and erythrocyte and differential leukocyte counts were carried out. The erythrocyte counts remained constant between 4.5-6.0 x 10^{12}/l, whereas the leukocyte counts fell from 6-7 x 10^{9}/l to less than 3 x 10^{9}/l. The leukopenia, primarily a neutropenia was evident immediately after surgery and was followed by the recovery of leukocyte numbers over the following 2 hours. The presence of either heparin (300 units/animals) or mepyramine (2 mg/kg i.p.), or a combination of both appeared to have no significant effect on the observed leukopenia. The type of anaesthetic appeared to have no effect as the leukopenia occurred whether sodium pentobarbitone or halothane was used.

The lipoxygenase inhibitors AA 861 2-(12-hydroxydodeca -5,10-diynyl) -3,5,6-trimethyl-1,4-benzoquinone (10 mg/kg p.o.) or phenidone (100 mg/kg p.o.) administered 30 minutes or 1 hour respectively before surgery, shortened the period of leukopenia to less than 60 minutes. Indomethacin (5 mg/kg p.o.) when administered one hour before gave an equivocal result suggesting only partial recovery at 2 hours when compared with the control group.

These results suggest that when neck surgery is performed, leukopenia occurs and this can be altered by the presence of inhibitors of arachidonic acid metabolism.

ASSESSMENT OF THE BIOAVAILABILITY AND SELECTIVITY OF INHIBITORS OF ARACHIDONIC ACID METABOLISM IN WHOLE BLOOD

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We previously described a method for determining the bioavailability of inhibitors of eicosanoid biosynthesis by direct radioimmunoassay (RIA) of LTB_4 and TXB_2 in plasma from calcium ionophore (A23187) stimulated human blood (Carey and Forder, 1986). While this approach can be extended to experimental animals, characterisation of immunoreactive LTB_4 has shown that cross-reactivity with large concentrations of 12HETE-like material can occur (Carey et al., 1987). We now describe a simple procedure for separating LTB_4 and 12HETE and its application to ex vivo eicosanoid measurement.

Heparinised mouse or rat blood was incubated with A23187 ($10\mu g ml^{-1}$) for 30 min at 37°C. Plasma was harvested by centrifugation and 0.04ml aliquots extracted with 0.5ml methyl formate (Carey et al.,1986). Extracts were evaporated (Speedvac concentrator) resuspended in 65:35 (v/v) hexane:diethyl ether (0.5ml)and eluted from cyanopropyl mini columns (Bond-Elut) pre-equilibrated with 2 x 1 ml hexane:ether. 12HETE was eluted with a further 2 x 0.5 ml hexane:ether. LTB_4 was then eluted with 2 x 0.5 ml methanol. The two fractions were evaporated and resuspended in 10mM sodium carbonate (1ml). Overall recoveries of $[^3H]$-LTB_4 and 12HETE from pooled mouse plasma were 63±5% and 45±5% respectively (mean±S.D., n = 3).Of the $[^3H]$ eicosanoids recovered, 88±1% of LTB_4 and 93±2% of 12HETE resided in the appropriate fraction. Overall recovery of immunoreactive (i) LTB_4 from mouse plasma was 47±4% (mean±SEM, n =6). Extraction of human plasma confirmed the absence of 12HETE-like immunoreactivity while recoveries of $[^3H]$-LTB_4 and iLTB_4 in the methanol fraction were 64±4% and 65±5% respectively (mean±SEM, n = 6).

Inhibition of eicosanoid biosynthesis was measured 1.5hr after oral dosing in mice and 5 hr post-dose in rats. BW755C inhibited iLTB_4 in LTB_4 and 12HETE fractions as well as plasma TXB_2. Respective ED_{50}'s in mice were 50,40 and $30 mg kg^{-1}$ and in rats were 40,60 and $15 mg\ kg^{-1}$. Indomethacin inhibited TXB_2 formation (ED_{50} mouse $<0.05 mg kg^{-1}$, rat $<1\ mg kg^{-1}$) but did not inhibit iLTB_4 in either fraction up to $1\ mg kg^{-1}$ (mouse) or $10\ mg kg^{-1}$ (rat). Benoxaprofen was a selective cyclooxygenase inhibitor up to $200\ mg kg^{-1}$ (mouse) and $100 mg kg^{-1}$ (rat). ED_{50}'s for TXB_2 were $15\ mg kg^{-1}$, and $<10\ mg kg^{-1}$, respectively.

While direct RIA of LTB_4 in rat plasma has previously been described (McMillan et al.,1986, Carey et. al., 1987a), the separation procedure described herein allows measurement of both LTB_4 and 12HETE. Thus, ex vivo RIA of eicosanoids in mouse and rat plasma can be used to assess the bioavailability and selectivity of inhibitors of arachidonic acid metabolism in whole blood.

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EFFECTS OF STEROIDS ON PULMONARY OEDEMA AND PROSTAGLANDIN E_2 PHARMACOKINETICS FOLLOWING ENDOTOXIN IN RATS

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Lung injury and oedema by endotoxin (ETX) have been used as an experimental model of adult repiratory distress syndrome (ARDS) consequent on septicaemia (Brigham and Meyrick, 1986). In another model of acute lung injury induced by a-naphthyl-thiourea, the pharmacokinetics of prostaglandin E_2 (PGE_2) were changed with a time course subsequent to the physical signs of lung oedema (Bakhle, 1982). We have now studied the effects of ETX-induced lung injury on PGE_2 pharmacokinetics and how they are affected by treatment with two glucocorticosteroids, methyl prednisolone (MP) and budesonide (BUD).

Male rats (200-250 g) were given a single intraperitoneal injection of ETX (E. coli; 0111:B4; 3.5 mg/kg) dissolved in saline ((1 mg/ml). The peripheral white blood cells (WBC) fell rapidly (46±3% of normal by 2 h after ETX; mean±s.e.m., n=4-6) and remained low (59±19%) up till 16h, returning to normal levels by 28 h. The polymorphonuclear leukocytes (PMN) were particularly affected, falling to 35±3% of normal at 2 h, 64±12% at 16 h but recovering to 98±4% by 28 h. Pulmonary oedema was assessed by the lung dry:wet weight ratio. This ratio was below normal at 6 and 16 h after ETX (19.5±0.3% and 19.4±0.3% vs. normal, 20.5±0.3%; n = 4 - 9). PGE_2 pharmacokinetics were measured in isolated lungs perfused with Krebs solution containing indomethacin (3ug/ml), from rats treated with ETX as described. They were assessed by the T1/2 value (the time taken for 50% of the ^{14}C to appear in lung effluent after injection of 500 ng ^{14}C-PGE_2). ETX treatment increased this value from 4 h, reached a peak at 16 h (122±4 s vs. normal 34±1 s; n=4-6) and recovered by 28 h after ETX. Survival of PGE_2 measured by RIA at 2 h and 16 h was increased (31±6% and 43±11% respectively) relative to normal (15±2%).

Treatment of the rats with MP (30 mg/kg; s.c.) 30 min after ETX, abolished the changes in lung weight ratio and diminished the fall in WBC and PMNs. This steroid also corrected the altered T1/2 values for PGE_2 towards normal, e.g., at 16 h, 56±4 s; and over the 28 h observation period. Complete protection of the WBC and T1/2 value was not achieved as MP alone caused a small fall in WBC (maximum of 68±5% of normal at 4 h) and increase in T1/2 value (maximum of 51±2 s at 6 h). Treatment with BUD (1.2 mg/kg: s.c.), 1 h before ETX, also abolished lung weight ratio changes. However, PMNs remained low until 16 h and the changes in the T1/2 values for PGE_2 were not reversed at any time after ETX.

The early onset of changes in PGE_2 pharmacokinetics and their reversal by MP, a steroid used clinically to treat ARDS, support the suggestion that PGE_2 pharmacokinetics could be used as a biochemical index of acute lung injury (Bakhle, 1982). The lesser effects of BUD in a dose shown to be effective in another model of lung injury (Brattsand et al., 1985) probably reflects differences in steroid disposition.

We thank Upjohn for methyl prednisolone and Draco AB for budesonide generously provided.

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COMPARITIVE PHARMACOLOGY OF EDRF AND NITRIC OXIDE

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A number of pharmacological agents induce vascular relaxation in vitro which is endothelium - dependent and has been ascribed to the release of a labile agent called endothelium-derived relaxing factor (EDRF; Furchgott and Zawadzki 1980). The effect of EDRF is inhibited by Fe^{2+} and hydroquinone (HQ) and its inactivation is reduced by superoxide dismutase (SOD) which also reverses the effects of Fe^{2+} and HQ (Gryglewski et al 1986a; Moncada et al 1986). It was therefore postulated that superoxide anions (O_2^-) inactivate EDRF and that the inhibitory effects of Fe^{2+} and HQ were mediated by the generation of O_2^-. The nitro-vasodilators e.g. glyceryl trinitrate (GTN) cause endothelium-independent vasodilatation, which is thought to be mediated by the formation of nitric oxide (NO; Arnold et al 1977) which, like EDRF, stimulates smooth muscle soluble guanylate cyclase (Rapoport and Murad 1983) and produces vascular relaxation . We have compared the activity of EDRF and NO on a bioassay of vascular strips in a cascade and investigated the effects of Fe^{2+}, HQ and SOD.

Bradykinin (Bk)-induced release of EDRF from porcine aortic endothelial cells, cultured on microcarriers, and its bioassay on a cascade of spiral strips of endothelium-denuded rabbit thoracic aorta was carried out as described previously (Gryglewski et al 1986b). NO gas was dissolved in He-deoxygenated H_2O and administered as a 5µl infusion over 1 min. HQ and SOD were dissolved in saline and infused over the tissues (O.T.) which were superfused with Krebs' buffer or with the effluent from the column containing the endothelial cells.

Both EDRF (released by 20 nM Bk) and NO (44 nM) caused relaxation of the first three bioassay tissues which was progressively attenuated, at similar rates, down the cascade (half lives of EDRF and NO at biological equivalence of 3.6 ± 0.1 and 4.1 ± 0.2 s; mean ± s.e.m. n=4 respectively). Infusion of SOD (15U. ml^{-1} O.T.) reduced the rate of decay of EDRF and NO down the cascade, indicated by the increased relaxation of the first three tissues and the relaxation of the fourth tissue.

Fe^{2+} (O.T.) caused concentration-dependent inhibition of both EDRF- and NO-induced relaxation of the uppermost bioassay tissue with IC_{50}'s of 0.39 ± 0.05 and 0.41 ± 0.05µM (mean ± s.e.m. n=3) respectively. HQ (O.T.) also caused concentration-dependent inhibition of EDRF- and NO-induced relaxation (3.7 ± 0.8 and 6.2 ± 1.8 µM, mean ± s.e.m. n=3) respectively. The effect of Fe^{2+} and HQ on EDRF and NO was reduced by concomitant infusion of SOD (15U. ml^{-1} O.T.). The above concentrations of HQ, Fe^{2+} and SOD did not affect either the tone of the tissues or their response to GTN.

Thus, both EDRF and NO cause endothelium-independent relaxation and are labile with similar half lives. Furthermore both are affected by SOD, Fe^{2+} and HQ in the same way. Whether EDRF is NO remains to be demonstrated.

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FURTHER EVIDENCE FOR THE EXISTENCE OF THREE SUBTYPES OF PGE_2-SENSITIVE (EP-) RECEPTORS

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The possible existence of three subtypes of PGE_2-sensitive (EP-) receptor has been proposed (Coleman et al 1987): EP_1-receptors, which are selectively blocked by the antagonists, SC-19220 and AH6809 (Kennedy et al., 1982; Coleman et al., 1985) and two types of EP_2-receptor, where the PGE analogue, sulprostone, is a potent agonist at one, but is inactive at the other. We now report results obtained with two additional PGE_2-sensitive preparations, guinea-pig ileum circular muscle (GPIc), which is relaxed by prostanoids (Bennett et al., 1968), and guinea-pig vas deferens (GPVD), where prostanoids inhibit neurotransmitter release (Hedqvist & von Euler, 1972). These results provide further support for the above hypothesis.

The GPIc preparation was essentially as described by Bennett et al. (1968), except that rings of tissues were used and opened to form strips. GPVD was prepared according to Hedqvist & von Euler (1972). The tissues were suspended in modified Krebs solution containing indomethacin (2.8μM), maintained at 37°C and gassed with 95% O_2 in CO_2. Both GPIc and GPVD were stimulated to contract at 2 min intervals with 5s trains of pulses (frequency, 7.5Hz and 5Hz respectively; pulse width, 1ms; supramaximal voltage). The contractile responses of both preparations were inhibited in a concentration-related manner by prostanoids. In initial experiments, the potencies of PGs D_2, $F_{2\alpha}$, I_2 and the TxA_2-mimetic, U-46619 (Coleman et al., 1981), were compared with that of PGE_2, which was the most potent agonist on both preparations, exhibiting EC_{50} values of 100nM (95% C.L. 82-128nM, n=12) on GPIc, and 1.5nM (95% C.L. 1.1-2.0nM, n=12) on GPVD. PGs D_2, $F_{2\alpha}$, I_2 and U-46619 were at least 40 and 200 times less potent than PGE_2 on GPVD and GPIc respectively, consistent with them being EP-receptor- containing preparations. However, neither SC-19220 (300μM) nor AH6809 (10μM) blocked responses of either preparation to PGE_2 (n>4), thereby excluding the presence of EP_1-receptors. On GPVD, the PGE analogue, sulprostone was slightly more potent than PGE_2, with an equipotent concentration (PGE_2=1) of 0.16 (95% C.L. 0.12-0.22, n=4) However, on GPIc, sulprostone was inactive (equipotent concentration >2,000, n=4), and furthermore the highest concentration tested (21μM) did not cause antagonism, but instead a small potentiation of the PGE_2 response was observed (concentration ratio=0.44, 95% C.L. 0.41-0.48, n=4).

These results provide further support for the division of EP_2-receptors into two subtypes, and suggest that GPVD contains the same subtype as chick ileum (ChI), whilst the subtypes in GPIc is similar to that in cat trachea (CT) (see Coleman et al. 1987). We propose that the EP-receptors in CT and GPIc be termed EP_2-receptors and those in ChI and GPVD, EP_3- receptors.

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EVIDENCE FOR TWO LEUKOTRIENE RECEPTORS (LT1 & LT2) ON GUINEA-PIG ILEUM

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Leukotriene (LT) C_4 and LTD_4 both contract the guinea-pig ileum with similar potencies and maximal efficacies but with different response time courses (Holmes et al 1980, Krilis et al 1983). It seemed possible that such differences might be due to a number of causes, metabolism, diffusion or activation of different leukotriene receptors. We performed a series of studies to clarify which, if any, of these explanations was applicable.

3cm lengths of guinea-pig ileum were suspended in 10ml tissue baths containing Tyrodes (and indomethacin 3×10^{-6}M) at 37°C gassed with 5% CO_2 in O_2. Responses were measured isotonically with a load of 1g. Firstly the leukotrienes were cumulatively added to paired preparations in the presence or absence of L-serine borate (45mM) and L-cysteine (10mM) inhibitors of the enzymes known to metabolise LTC_4 and LTD_4 respectively (Snyder & Krell, 1984). These inhibitors contracted many preparations or induced spontaneous activity on others. On those preparations where no such effects occurred LTC_4 and LTD_4 were shown to produce similar responses to untreated preparations except increased duration of action for both leukotrienes. This suggested that metabolism of LTC_4 or LTD_4 was unlikely to explain their different response characteristics.

LTE_4 also contracted the ileum with similar response characteristics to LTD_4 but it only achieved 80% of the maximal LTC_4 or LTD_4 response. LTE_4 (10^{-5}M) markedly antagonised LTD_4 responses but produced relatively little antagonism of LTC_4 and no antagonism of histamine. This suggested that LTE_4 was a partial agonist on this tissue and its selective antagonism of LTD_4 further emphasised the difference in LTC_4 and LTD_4 responses.

Finally we induced tachyphylaxis to LTD_4 by extending the contact time of a high concentration (10^{-6}M) from 1 to 15 min. Such tachyphylaxis was shown on paired preparations to exist for at least 30 min. Cross tachyphylaxis also occurred to LTE_4 but only to a small extent to LTC_4. When this procedure was repeated with LTC_4 instead of LTD_4, cross tachyphylaxis occurred with LTD_4 and LTE_4. Neither LTC_4 or LTD_4 induced cross tachyphylaxis to histamine.

These results suggest that the different response characteristics of LTC_4 and LTD_4 on the ileum are probably due to their activation of two different leukotriene receptors (LT1 and LT2) as already described on the guinea-pig trachea (Snyder & Krell 1984). LTD_4 seems to act on one receptor LT1 whereas LTC_4 predominantly acts on the second receptor LT2. However, it seems likely that LTC_4 can also act on the LT1 receptor either directly or perhaps indirectly due to a small degree of metabolism to LTD_4. LTE_4 is a partial agonist of the LT1 receptor.

We are grateful for the technical assistance of Miss T. Morris.

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A SENSITVE ASSAY FOR PLATELET ACTIVATING FACTOR USING GUINEA-PIG PLATELETS

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Platelet activating factor (PAF-acether) can be conveniently assayed by its ability to aggregate platelets. The most common source of platelets is either human (Valone et al, 1982) or rabbit blood (Camussi et al, 1983). In the present study guinea-pig blood was investigated as a source of Platelet rich plasma (PRP) to assay PAF-acether, and the parameters of the assay determined.

Blood anticoagulated with citrate (3.8%) was obtained through a carotid artery cannula from pentobarbitone (60mg/kg) anaesthetised male guinea-pigs. The blood was centrifuged at 175g for 20 mins, 1560g for 2 min or 110g for 15 min (at least n=4 in each case) to determine the conditions which would result in a relatively consistent and high, platelet count. 110g for 15 min was found to be the most suitable giving a platelet count of 5.18 ± 0.20 x 10^8 platelets/ml. Platelet aggregation was measured using a 450µl volume of PRP at 37°C in a single channel Coulter aggro-meter (Model no. 335) and recorded on a Phillips (PM 8251) chart recorder. The initial PRP was diluted with platelet poor plasma (PPP) to a known count.

20mM tris buffer solution containing (0.25%$\frac{w}{v}$) bovine serum albumin (tris-BSA) was used for the administration of PAF-acether. When tested against a range of concentrations of PAF-acether the optimum platelet count was found to be 3.0 x 10^8 platelets/ml.

The ability of agents other than PAF-acether to aggregate the guinea-pig platelets was tested. Collagen (4-44.5µg/ml), ADP (250nM-5µM), thrombin (0.2-1.3 units /ml) and the thromboxane A_2 mimetic U46619 (100nM-4µM) all induced a concentration -dependent aggregation. The response of ADP (5µM) was reduced to between 22.7-38.3% of the original in the presence of both 45 units/ml creatine phosphokinase (CPK) and 22.5mM phosphocreatine (CP). Hirudin (2 units/ml) reduced the response to thrombin (1.05 units/ml) to 0-10.6% of the control. Indomethacin (1µM) was included to inhibit the response of arachidonic acid. The presence of CPK, CP, hirudin and indomethacin in the incubation did not affect the increase in the light transmission resulting from the addition of PAF-acether (0.8-8nM).

The guinea-pig PRP was sensitive to PAF-acether in a concentration range 0.8-7.8nM which was similar to that for washed human platelets (Valone et al, 1982). Studies using rabbit platelets (Camussi et al, 1983) or human PRP (McManus et al, 1981) however, found the PAF-acether concentration range to be approximately 2nM-2µM. Thus guinea-pig PRP is a very sensitive system for assay of PAF-acether.

This work was supported by the Asthma Research Council.

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THE EFFECT OF A THROMBOXANE ANTAGONIST ON UTERO-PLACENTAL BLOOD FLOW IN RATS AT DIFFERENT STAGES OF GESTATION

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Previous studies have indicated that thromboxane (TXA_2) does not contribute to oestrogen-induced uterine hyperaemia in the non-pregnant rat (Kerr and Senior, 1986). In the present study AH 23848 ([1α(Z), 2β, 5α]-(±)-7-[5-[[1,1'-biphenyl]-4-yl]methoxy]-2-(4 morpholinyl)-3-oxocytclopentyl]-4-heptanoic acid), a TXA_2 receptor antagonist, was used to assess the contribution of TXA_2 to the regulation of utero-placental blood flow in mid- and late-gestation.

CD-derived pregnant rats were anaesthetised on either day 10 or day 21 of pregnancy (pentobarbitone sodium 60 $mgkg^{-1}$i.p.) and blood flow was measured using the labelled microsphere technique (15μm diameter) and expressed as ml min^{-1} $100g^{-1}$ (tissue wet weight). AH 23848 (2.5$mgkg^{-1}$ i.v.) in a sodium bicarbonate (1% w/v)/sodium chloride (0.9% w/v vehicle was administered 3h prior to blood flow determination.

Blood pressure, cardiac output and heart rate did not show any significant difference when measured on day 10 or day 21 of gestation. Treatment on day 10 with AH 23848 did not affect utero-placental blood flow and all parameters measured were similar to the untreated control group (n = 7 treated, n = 9 control). However, in day 21 pregnant rats treatment with AH 23848 produced an increase in ovarian blood flow (untreated 943±266, treated 2435±624, $P<0.05$) and in placental blood flow (untreated 99±23, treated 208±18, $P<0.01$) (n = 6 treated, n = 7 control). The uterine blood flow was not affected by treatment with the thromboxane antagonist.

AH23848 has been shown to act as a TXA_2 receptor antagonist in vitro and in vivo (Brittain et al, 1985) and at the dose used in the present experiments no partial agonist activity has been seen in this normotensive strain of rat. The results thus show that in the pregnant rat, as in the non-pregnant, thromboxane plays no part in the regulation of uterine blood flow. However, with advancing gestation thromboxane becomes involved in regulating ovarian and placental blood flow. Previous work (Zamecnik and Kennedy, 1980) has shown that the stable metabolite of TXA_2 (TXB_2) is measurable in placental tissue on day 20 of pregnancy in the rat but is undetectable on day 15. Since TXA_2 may be involved in reducing placental blood flow it is possible that it may be involved in gestational hypertension which is triggered in part by placental insufficiency.

We thank Glaxo Group Research Ltd for the supply of AH23848.

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RELEASE OF LEUKOTRIENES FROM PORCINE PULMONARY ARTERY BY PAF AND FMLP

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We have previously demonstrated the release of LTB_4, LTC_4, LTD_4 and LTE_4 from porcine pulmonary artery, upon stimulation with the calcium ionophore A23187 (Piper and Galton 1984). In these experiments we have investigated the release of leukotrienes using platelet-activating factor (PAF) and the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) as the stimuli.

Porcine pulmonary arteries, obtained from an abattoir, were weighed, chopped, suspended in Tyrode's solution (6 ml/g) and agitated at 37°C. After a 15 min equilibration period, either PAF was added to give a final concentration of 0.01 μM, or FMLP to give a final concentration of 0.02μM. After 15 min, samples were partially purified by means of C18 Sep-Paks, followed by further purification on HPLC (Spherisorb 5ODS column; solvent system MeOH:H_2O:HAc 75:25:0.02, pH 5.4; flow rate 1 ml/min). Fractions were assayed for leukotrienes by radioimmunoassay. Tritiated leukotrienes were added before purification to assess the recoveries for each experiment.

The release of leukotrienes from pulmonary artery stimulated with PAF (0.01 μM) were very variable. Eighteen experiments were performed but release was recorded in only five of these. The amount of LTC_4 released was 2.5±1.8 pmol/g of tissue. No LTD_4 or LTE_4 was detected.

FMLP (1 μg/ml) caused the release of LTC_4 and LTB_4 from porcine pulmonary artery. The amount of LTB_4 released was 0.06±0.03 pmol/g of tissue and the amount of LTC_4 was 0.18±0.01 pmol/g of tissue.

These results give support to the idea that some of the actions of PAF may be mediated by leukotrienes (Voelkel et al 1982, Piper and Stewart 1986). The release of leukotrienes by FMLP raises the question as to whether the release of leukotrienes from the vessel wall is in fact partly due to the presence of inflammatory cells.

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PHARMACOLOGICAL ACTIONS OF PAF-ACETHER IN GUINEA-PIG ISOLATED PERFUSED LUNGS AND ALVEOLAR MACROPHAGES

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PAF-acether has a presumptive role in inflammation and anaphylaxis (Benveniste & Pretolani, 1985). We have previously shown the anaphylactic release of PAF-acether from sensitized guinea-pig lungs perfused through the airways (Fitzgerald et al., 1986a) and that alveolar macrophages synthesize PAF-acether (Fitzgerald et al., 1986b). In the present study, we have first investigated the pharmacological effects of exogenous PAF-acether in guinea-pig isolated lungs and measured the concomitant release of immunoreactive TxB_2, LTB_4 and LTC_4. Secondly we have also measured the release by PAF-acether of these eicosanoids from guinea-pig alveolar macrophages in vitro.

The lungs of male Dunkin-Hartley guinea-pigs (350-450g) were removed and perfused with Krebs-bicarbonate solution warmed (37°C) and gassed (95% O_2 - 5% CO_2) at 5 ml/min either through the vasculature, or through the airways as previously described (Fitzgerald et al., 1986a). PAF-acether or lyso-PAF (100-400 ng/ml) was then infused for 5 min at 0.1 ml/min. One min samples of pulmonary effluent were collected prior to PAF-acether or lyso-PAF and then at various intervals after the start of the respective infusions for subsequent radio-immunoassay (RIA) of TxB_2, LTB_4 and LTC_4.

When infused through the pulmonary vasculature, PAF-acether caused a progressive increase in airway back pressure and vascular perfusion pressure up to a maximum of $\Delta 10.7 \pm 1.2$ and $\Delta 6.7 \pm 0.9$ mmHg respectively (mean ± s.e. mean, n=4; $p<0.01$) 7 min after the start of the 400ng/ml infusion and a dose-related release of TxB_2 of up to $\Delta 37.3 \pm 6.5$ ng/ml (n=4; $p<0.01$) at 5 min. When perfused through the trachea PAF-acether caused a dose related increase in airway perfusion pressure (APP) up to a maximum of $\Delta 24.6 \pm 5.6$ mmHg (n=4; $p<0.01$) 10 min after the start of the 400 ng/ml infusion. This increase in APP was accompanied by a dose-related release of TxB_2 up to a maximum of $\Delta 50.8 \pm 7.7$ ng/ml (n=4; $p<0.01$) 7 min after the start of the 400 ng/ml infusion. LTB_4 and LTC_4 remained below the detection limits of the RIA (0.05 ng/ml) throughout the study. Lyso-PAF (400 ng/ml, n=3) did not cause an increase in any of the parameters studied, nor did it release detectable amounts of TxA_2, LTB_4 or LTC_4.

Alveolar macrophages collected by bronchial alveolar lavage from normal guinea-pigs (Fitzgerald et al 1986b) were incubated in vitro for 5 to 30 minutes with either PAF-acether (10-200 ng/ml) or lyso-PAF (200 ng/ml). PAF-acether caused a dose-dependent release of TXB_2 which was maximum after a 5 minute incubation. The maximum release of TxB_2 was $\Delta 11.1 \pm 0.4$ ng/10^6 cells (n=3; $p<0.01$) following a 5 min incubation with 200 ng/ml PAF-acether. Lyso-PAF (200 ng/ml) did not cause TXB_2 release over the time periods studied and LTB_4 and LTC_4 remained below the detection limits of the RIA in all samples.

These results demonstrate that in guinea-pig isolated lungs, PAF-acether perfused either via the vasculature or the airways stimulates endogenous release of TXB_2 but not LTB_4 or LTC_4 and also causes vascular and airway smooth muscle contraction. PAF-acether also releases TXB_2 in significant amounts from guinea-pig alveolar macrophages. However other cells, e.g. endothelial cells may also contribute to the release of TXB_2 by PAF-acether in the whole lung, and by inference, to the contractile effects of PAF-acether in this situation.

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ADRENERGIC MODULATION OF PROSTAGLANDIN I_2 RELEASE FROM MESENTERIC ARTERIAL BED OF THE NORMOTENSIVE AND HYPERTENSIVE RAT

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Noradrenaline (NA) is known to increase the release of prostaglandin I_2 (PGI_2) from the mesenteric arterial bed (MAB) of the normotensive (WKR) and hypertensive (SHR) rat (Pipili & Poyser, 1982). In the present study the effect of α_1 and α_2 adrenoceptor antagonism on the release of PGI_2 was examined in the MAB of WKR and SHR in an attempt to establish a functional relationship between selective inhibition of α-adrenoceptor subtypes and PGI_2 release in the above experimental systems.

The MAB of the rat was set up and perfused as previously described (Pipili, 1986). Dose response curves to NA in the absence and presence of prazosin, an α_1 and rauwolcine, an α_2-antagonist (Starke, Endo & Taube, 1975, Timmermans, Kwa & Van Zwieten, 1979) were constructed as reported by Pipili, 1986. Prostaglandin I_2 was measured as 6-oxo-$PGF_{1\alpha}$ by RIA in samples of perfusate obtained 1 min before to 3 min after the administration of 1μg of NA in the absence and presence of antagonists. Results were compared by paired t-test.

NA increased the release of PGI_2 from the MAB of both WKR and SHR. Prazosin (10^{-10}-10^{-8}M) reduced the pressor responses to NA but did not reduce the NA-induced increases of PGI_2 in the WKR or SHR. Rauwolcine at 10^{-7} and 10^{-6}M reduced the pressor responses to NA and abolished the increases in PGI_2 release from the MAB of WKR. At 10^{-8}M it enhanced the pressor responses to NA and reduced the increased release of PGI_2. In the MAB of SHR rauwolcine reduced pressor responses at all three concentrations but did not affect the NA-induced increases of PGI_2 release. These results are summarized as follows:

PGI_2 release expressed as ng/min of 6-oxo-$PGF_{1\alpha}$

	WKR (n>5)			SHR (n>5)		
	before NA	after NA	p	before NA	after NA	p
Control	4.0±0.31	5.7±0.61	<0.02	2.5±0.34	3.2±0.52	<0.01
Prazosin 10^{-10}M	6.2±1.47	8.5±1.58	<0.05	2.8±0.55	3.1±0.64	n.s
Prazosin 10^{-9}M	6.0±0.76	9.0±1.18	<0.02	3.8±0.44	4.6±0.46	<0.02
Prazosin 10^{-8}M	6.2±1.04	9.5±1.15	<0.05	4.4±0.80	6.7±1.00	<0.05
Rauwolcine 10^{-8}M	5.2±1.11	6.5±2.00	n.s	1.8±0.12	2.1±0.81	<0.05
Rauwolcine 10^{-7}M	4.4±0.52	4.2±0.63	n.s	1.9±0.53	2.8±0.81	<0.05
Rauwolcine 10^{-6}M	4.4±0.91	4.5±0.83	n.s	2.1±0.52	2.6±0.54	<0.05

These results suggest either that the NA-induced release of PGI_2 is mediated through the α_2-postsynaptic adrenoceptor or that the contribution of smooth muscle and endothelium (which contains mainly α_2-adrenoceptors, Bullock, Taylor & Weston, 1986) to the basal as compared to NA-induced release of PGI_2 is not the same. The different effect of rauwolcine on the PGI_2 release in WKR and SHR may reflect the proliferation of smooth muscle cells in SHR which may produce larger amounts of PGI_2 than the endothelium thus masking any effect of rauwolcine on the α_2-receptor of the latter. This point is presently under investigation in the endothelium denuded MAB.

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THE EFFECTS OF ACUTE AND REPEATED T_3 ADMINISTRATION ON BEHAVIOURAL RESPONSES MEDIATED VIA 5-HT_1 AND 5-HT_2 RECEPTORS IN MICE

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Although acute and repeated administration of L-triiodothyronine (T_3) has previously been reported to enhance 5-HT mediated behavioural responses in rats (Atterwill, 1981), no study has been made of the actions of T_3 on behavioural changes mediated by the subtypes of 5-HT receptor. In these experiments, 5-HT_{1A} function was assessed by the hypothermic response to 8-hydroxy 2-(di-n-propyl-amino) tetralin (8-OH-DPAT), 5-HT_{1B} function by the locomotor response to 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl) IH indole (RU 24969) and 5-HT_2 function by measurement of the head-twitches induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Green and Heal, 1985; De Souza et al, 1986). Adult male C57/Bl/6Ola mice (25-30g) were used throughout. T_3 (100 µg/kg in 0.02M NaOH) or NaOH vehicle (10 µl) were injected subcutaneously once daily for either 1 or 10 days. Experiments were performed 24h after the final injection. Body temperature was measured 20 min after injection of the 5-HT_{1A} agonist, 8-OH-DPAT (0.5 mg/kg sc). The locomotor responses of groups of 2 mice were measured in the 60 min following injection of the 5-HT_{1B} agonist, RU 24969 (50ng icv), using LKB Animex meters. 5-HT_2 mediated head-twitches were counted in the 6 min following injection of 5-MeODMT (2 mg/kg ip). 5-HT_2 receptor binding was measured in membranes from pooled frontal cortices (4-5 mice) using [^{3}H]-ketanserin (0.3-5.0 nM), with specific binding defined by 10^{-6}M methysergide. Concentrations of 5-HT and 5-HIAA were measured by HPLC with electrochemical detection. 8-OH-DPAT induced hypothermia was unaffected by a single T_3 injection (Mean temperature decrease °C ± S.E., T_3 x 1 = 1.4 ± 0.13 (8); Controls 1.28 ± 0.14 (7)), but was significantly attenuated after repeated T_3 treatment (T_3 x 10 = 0.92 ± 0.09 (6); Controls = 1.45 ± 0.16 (6), $P<0.05$). The locomotor activity produced by RU 24969 was not altered by acute or repeated T_3 injection, although the latter tended to decrease the RU 24969 response. 5-MeODMT induced head twitches were increased after a single T_3 injection (Mean twitches ± S.E., T_3 x 1 = 20.1 ± 2.2 (5); Controls = 11.0 ± 1.3 (6), $P<0.01$) but not after 10 treatments (T_3 x 10 = 12.0 ± 1.0 (5); Controls = 11.8 ± 1.1 (6)). Cortical 5-HT_2 receptor number was unaffected by acute T_3 administration, but a 10% decrease ($P<0.025$) was observed after repeated T_3 treatment. Acutely, T_3 injection increased mid/hindbrain 5-HIAA concentrations by 15% ($P<0.01$) with no change in 5-HT levels. Repeated T_3 injection elevated 5-HIAA concentrations in both forebrain and mid/hindbrain by 14% ($P<0.01$) and 22% ($P<0.01$), respectively, and also increased 5-HT levels in the latter region by 24% ($P<0.01$).

In conclusion, therefore, T_3 administration increased 5-HT_2 and decreased 5-HT_{1A} mediated behavioural responses, respectively, although the time courses for these 2 effects were quite different. T_3 injection did not affect 5-HT_{1B} receptor function in mouse brain. In general, repeated T_3 treatment increased 5-HT synthesis and metabolism and this effect may account for the reduction in 5-HT_2 receptor number and the attenuated 5-HT_{1A} mediated behavioural responses observed.

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