SJC

British Journal of Pharmacology

April 1990

Volume 99 Proceedings Supplement 1P–302P

1P K+ CHANNEL OPENERS DILATE BOTH LARGE AND SMALL CORONARY ARTERIES IN CONSCIOUS DOGS

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Cromakalim (C) and pinacidil (P) are two K^+ channel openers which induce coronary vasodilation through both a direct (vasorelaxation) and an indirect (increase in myocardial oxygen demand) mechanisms (Kawashima and Liang, 1985; Cook and Hof, 1988). However, if this vasodilatory effect is well documented for coronary arterioles, it has not yet been studied in large epicardial conductance vessels which are of major importance in the pathophysiology of coronary vasospasm.

The present study was thus designed to investigate the effects of C and P on large and small coronary arteries and to compare them to those of nitroglycerin (N) in conscious dogs. Large coronary artery diameter (CD) and coronary blood flow (CBF) were measured in 6 conscious dogs previously instrumented with ultrasonic crystals (sonomicrometry) and electromagnetic flow probes implanted on the circumflex coronary artery. Mean arterial pressure (MAP, intra-aortic catheter) and heart rate (HR, electrocardiogram) were monitored. Coronary vascular resistance (CVR) was calculated as the MAP/CBF ratio. Experiments were conducted at least three weeks after initial surgery and each dog received (i.v. bolus) in random order and on separate days N (0.03 to 10 µg/kg), C (1 to 10 µg/kg) and P (3 to 30 µg/kg). One day at least elapsed between two successive injections.

At doses up to 0.3 µg/kg, N selectively increased CD without simultaneously altering either hemodynamic or other coronary parameters. In contrast, C and P at all doses and N at doses higher than 0.3 µg/kg always simultaneously and dose-dependently increased CD, CBF and HR whereas MAP and CVR were decreased (all $p < 0.05$). Regardless of the dose, the N-induced decrease in MAP and CVR and increase in HR and CBF were transient and all these parameters had returned to control values when the maximal increase in CD (+5.0 ± 0.7%, from 2637 ± 49 µm, at 10 µg/kg) occurred. In contrast, with C and P, CBF and HR remained elevated and MAP and CVR were still decreased at the time of CD peak increase. The ratios of the molar doses of N, C and P required to increase CD by 2.5% were N/C = 0.15, N/P = 0.015 and C/P = 0.1. Finally, the increases in CD elicited by C were longer lasting than those induced by P (e.g. 30 min after C, 3 µg/kg, vs 10 min after P, 10 µg/kg).

We conclude that whereas N selectively dilates large coronary vessels, C and P dilate both large and small coronary arteries in conscious dogs. In this respect C has a 10-fold greater potency than P and exhibits a longer duration of action. Inasmuch as CD and CBF are simultaneously increased by C and P, it may be that in vivo K^+ channel openers-induced relaxation of large coronary vessels is not exclusively endothelium-independent.

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2P ANALYSIS OF THE MECHANISMS INVOLVED IN THE CONTRACTILE RESPONSE TO CROMAKALIM IN ISOLATED STRIPS OF RABBIT AORTA UNDER Ca^{2+}-FREECONDITIONS

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The K-channel opener cromakalim (CK) is a potent smooth muscle relaxant (Hamilton & Weston, 1989). We have recently reported a novel slow, sustained contractile response to cromakalim in rabbit isolated aorta under Ca-free conditions (Bray et al, 1989). In the present study we investigated the possible mechanisms contributing to this contractile response.

Isolated strips of rabbit aorta, denuded of endothelium, were mounted for isometric tension recording in MOPS-buffered physiological salt solution (PSS) at 37°C, pH 7.4, under 2 g resting tension. After 90 min equilibration, tissues were transferred into a Ca-free PSS containing 2 mM EGTA and 10 mM $MgCl_2$ for 15 min prior to further experimental manipulation.

Cromakalim (1-10 µM) produced a contractile response (maximum tension 2.45 ± 0.21 g; mean ± s.e.mean, n = 6). 30 min pretreatment with either ruthenium red (100 µM) which blocks intracellular Ca release (Palade, 1987) or ryanodine (100 µM) which depletes intracellular Ca stores (Hwang & van Breeman, 1987) significantly reduced this contractile response (maximal inhibition 66.5% and 42.9% respectively). Pretreatment with both these agents combined produced a greater maximal inhibition (99.2%).

As protein kinase C activation is implicated in the sustained phase of vascular smooth muscle contraction (Kahlil & van Breeman, 1988) the effect of the protein kinase C inhibitor, staurosporine, was investigated. There was no difference between the magnitude of the response to CK (1-10 µM) observed in the presence of staurosporine (200 nM) or vehicle. Cumulative addition of staurosporine (100-300 nM) also failed to relax a pre-established contraction to 1 µM CK. In contrast, staurosporine (100 nM) added simultaneously with phorbol 12-myristate 13-acetate (PMA; 10 µM) prevented the tension development produced by PMA alone (0.52 ± 0.09 g; mean ± s.e.mean, n = 5). In addition, staurosporine (100 nM) fully relaxed a pre-established contraction to 10 µM PMA.

Clearly, the contractile response to CK in Ca-free PSS is partly mediated through a mechanism sensitive to both ruthenium red and ryanodine. These results indicate an involvement of intracellular Ca release in this response to CK. Elucidation of the mechanisms involved in maintaining the contractile response is subject to further investigation although the present results suggest that protein kinase C activation is not involved.

Susan Duty is in receipt of a SERC CASE Award with Roche Products Limited.

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3P THE RELATIONSHIP BETWEEN THE EFFECTS OF CROMAKALIM AND DIAZOXIDE ON K^+ AND RB^+ EFFLUX, MEMBRANE POTENTIAL AND TENSION IN BOVINE TRACHEAL SMOOTH MUSCLE

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Some previous studies have examined the effects of cromakalim (CK) on the movement of K ions in smooth muscle tissues using Rb as a marker for K. However, Rb may not be a good substitute for K since at different concentrations CK has differential effects on the ratio of K:Rb efflux (Bray & Weston, 1989). In the present study we examined the relationship between changes in Rb and K efflux, changes in membrane potential and tension induced by CK and diazoxide in bovine tracheal smooth muscle.

Strips of bovine tracheal smooth muscle were mounted on a manifold for simultaneous K and Rb efflux studies (Edwards & Weston, 1989) or in a tissue bath for the recording of either isometric tension or membrane potential. CK and diazoxide relaxed tissues pre-contracted with 25mM KCl (IC50 values were 0.19 ± 0.02 and 20.3 ± 5.5µM respectively; mean ± s.e. mean, n=4). The effects of these drugs on 25mM KCl-induced contractions, membrane potential and K and Rb efflux are summarized in Table 1.

Table 1

Drug	Relaxation		Membrane Potential		Rb efflux		K efflux	
	%	tmax	mV	tmax	Δ%	tmax	Δ %	tmax
CK 10µM	97.0±4.0	8.7±1.9	-81.1±0.5	6.2±0.5	235±18	>24	337±43	8-12
CK 1µM	97.0±6.9	11.2±3.1	-78.1±1.6	17.0±2.7	156±13	>24	233±32	16-20
Diazoxide 300µM	100.7±5.2	7.4±1.1	-80.9±0.6	7.0±0.8	190±28	16-20	212±17	>24
Diazoxide 100µM	97.9±12.5	12.7±0.8	-76.0±0.7	14.5±2.8	154±20	16-20	173±24	>24

Mean ± s.e.mean(n=3-7). tmax denotes time taken to reach maximum response (min); denotes change in parameter

For CK the rate of rise of K efflux was greater than that for Rb and this difference was more marked at the higher concentration level. For 1 and 10µM CK, K efflux reached its maximum between 8-12 min and 16-20 min respectively whereas Rb efflux was still rising at the end of the 24 min exposure period to the drug. The maximum changes in K efflux were greater than those for Rb. In contrast, diazoxide (300µM and 100µM) caused similar rises in both K and Rb efflux and changes in K efflux paralleled those of Rb. For CK the tmax values for the changes in K efflux, membrane potential and tension were similar. These results may indicate that the effects of CK at a Rb-impermeable K channel are largely responsible for the observed electrical and mechanical changes. In contrast, the effects of diazoxide may involve a K channel permeable to Rb.

J. Longmore is supported by a grant from Rhone Poulenc.

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4P DIFFERENTIAL EFFECTS OF OUABAIN ON K^+-INDUCED CONTRACTIONS OF GUINEA-PIG TRACHEAL AND AORTIC SMOOTH MUSCLE

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Na^+,K^+-ATPase may be partially responsible for the maintenance of basal tone of guinea-pig tracheal smooth muscle (Chideckel *et al.*, 1987). In addition, this enzyme may have an important role in allergic diseases as indicated by the increase in Na^+-K^+ exchange after sensitization of the guinea-pig with antigen (Souhrada and Souhrada, 1982). The aim of this study was to compare the effects of the Na^+,K^+-ATPase inhibitor, ouabain, on K^+-induced contractions of tracheal and aortic smooth muscle preparations of the guinea-pig.

Tracheal and aortic rings (devoid of epithelium and endothelium, respectively), obtained from male Dunkin-Hartley guinea-pigs were suspended in modified Krebs solution, maintained at 37°C and gassed with CO_2 and O_2 (5:95). A load of 2g was applied to each ring. After a 90 min equilibration period ring viability was assessed by a single exposure to K^+ (56mM). A single concentration-response curve to K^+ (10-100mM) was constructed per preparation after a 20 min incubation with vehicle, ouabain or diltiazem. Results are expressed as mean ± s.e.mean.

The concentration-response curve to K^+ on the trachea, was multiphasic consisting of a small initial contraction (10-12mM; E_{max1}: 0.39 ± 0.07g, n = 12), followed by a return to basal tone (18mM) and then a second contractile phase (25-100mM; E_{max2}: 2.14 ± 0.10g). Ouabain (0.1-100µM), whilst not modifying the first phase, inhibited the contractions to 25-100mM K^+ (IC_{50}: 0.64 ± 0.1µM, n = 4). In contrast, diltiazem (0.1-10µM) inhibited both contractile phases to K^+ (IC_{50} of E_{max2}: 1.58 ± 0.1µM, n = 4). The K^+ curve on the aorta was monophasic. Ouabain displaced the K^+ curve to the left (EC_{50} : control 23.4 ± 1.5mM, n = 7; 3µM ouabain 8.2 ± 0.1mM, $P < 0.05$, n = 4) with no modification of maximum. Diltiazem induced a rightward displacement of the curve (response to 80mM K^+: control 3.17 ± 0.33g, n = 7; 10µM diltiazem 0.87 ± 0.19g, $P < 0.05$, n = 4).

In conclusion, ouabain inhibited the contractile responses to K^+ of guinea-pig tracheal smooth muscle but enhanced those of the aorta. Contractions of smooth muscle to K^+ are the consequence of voltage operated calcium channel activation as indicated by the results with diltiazem. Further studies, however, are required to determine the role, if any, of VOCs in the observed effects of ouabain.

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5P 2-DEOXY-D-GLUCOSE, AN INHIBITOR OF GLYCOLYSIS, INHIBITS CROMAKALIM BUT NOT SODIUM NITROPRUSSIDE INDUCED VASORELAXATION

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Cromakalim is a novel vasodilator thought to act by opening K^+-channels in vascular smooth muscle (Hamilton et al.,1986). Although the site(s) of action of cromakalim remains to be established recent findings have implicated the involvement of ATP-dependent K^+-channels similar to those expressed in pancreatic B-cells (Buckingham et al., 1989, Davies et al., 1989). The aim of the present study was to compare the effect of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis, with cromakalim. In addition the vasorelaxant effects of cromakalim and sodium nitroprusside were compared following inhibition of glycolysis.

Rings of rat thoracic aorta were suspended in Krebs' bicarbonate buffer at 37°C and bubbled with 95%O_2/5%CO_2. Changes in tension were recorded isometrically under a resting tension of 1g. The effects of 2-DG were assessed following incubation in Krebs' buffer in which glucose was replaced with an equimolar amount of 2-DG and supplemented with sodium acetate (5mM) to maintain oxidative phosphorylation.

Pre-incubation (30mins.) with cromakalim (10uM) inhibited the response to noradrenaline (NA, 1uM) by 55.3 ± 2.6% in standard Krebs' buffer. This effect was reversed by glibenclamide (10.3 ± 6.1% inhibition of NA in the presence of glibenclamide, 10uM). In contrast, pre-incubation with 2-DG Krebs' markedly enhanced the response to NA (94.9 ± 24.0%). Enhanced responses in the presence of 2-DG were unaffected by glibenclamide(10uM) or by pre-treatment with nitrendipine (1uM). In additional experiments, cumulative concentration response curves were constructed to either cromakalim or sodium nitroprusside on NA(1uM) pre-contracted tissues before and after incubation in 2-DG Krebs'. Following 2hr. incubation with 2-DG Krebs' the relaxant effect of cromakalim was markedly reduced (24.2 ± 7.3% of control activity). Under identical conditions the relaxant effect of sodium nitroprusside was unchanged.

The results clearly show that 2-DG does not mimic the effects of cromakalim. The mechanism by which 2-DG enhances the NA response is not clear but the since it was not prevented by nitrendipine it is unlikely to involve enhanced calcium uptake through voltage operated calcium channels. The finding that 2-DG inhibits cromakalim induced vasorelaxation is consistent with the hypothesis that cromakalim is acting via glycolytically regulated ATP-dependent K^+-channels. However, the involvement of other ATP-dependent processes cannot be ruled out.

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6P THE CARDIOVASCULAR EFFECTS OF SDZ PCO 400, A NOVEL K^+ CHANNEL OPENER

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SDZ PCO 400 ((-)-(3S,4R)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyloxy)-2H-1-benzopyran-6-carbonitrile) induces relaxation of vascular smooth muscle by a mechanism consistent with opening of K^+ channels (Quast et al., 1990). The present studies were carried out to define the cardiovascular profile of SDZ PCO 400 in vivo and to compare its effects with those of the prototype K^+ channel opener, cromakalim.

Male spontaneous hypertensive (SH) rats (320 - 380 g) were implanted with indwelling femoral arterial cannulae and used for experiments in the conscious state after a minimum 1 week recovery period. Other male SH rats (340 - 370 g) were anaesthetized with thiobutabarbital (Inaktin®), 150 mg/kg, and set up for recording blood pressure (BP), heart rate (HR) and, using miniaturized Doppler probes, renal, mesenteric, hindquarters and carotid blood flows (Wright & Fozard, 1988). Male and female rhesus monkeys (6 - 12 kg) were trained to sit quietly in restraining chairs and BP and HR were measured plethysmographically from the arm. Blood samples were withdrawn from a cannulated leg vein for determination of plasma renin.

In conscious SH rats acute administration of SDZ PCO 400, 0.1 - 0.3 mg/kg p.o., induced dose-dependent decreases in BP which were rapid in onset (< 15 min) and of long duration (> 5 h). Increases in HR were also seen which were, however, neither dose-dependent nor well sustained. Cromakalim, 0.3 - 1 mg/kg p.o., induced similar maximum falls in BP to those seen with SDZ PCO 400. The effects were, however, less well sustained than those of SDZ PCO 400 unlike the accompanying tachycardia which was, at the higher dose, greater and of longer duration than that induced by SDZ PCO 400. The antihypertensive effect of SDZ PCO 400, 0.1 mg/kg p.o., was well maintained on repeated daily dosing for 4 days. In anaesthetized SH rats, both SDZ PCO 400, 0.1 - 0.3 mg/kg p.o., and cromakalim, 0.3 - 1 mg/kg p.o., produced similar, sustained falls in BP associated with vasodilatation in the renal, mesenteric, hindquarters and carotid vascular beds. In conscious rhesus monkeys, SDZ PCO 400, 0.1 - 0.3 mg/kg i.v. or p.o., gave dose-related and substantial falls in BP accompanied by tachycardia and an increase in plasma renin activity. SDZ PCO 400 was some 3-fold more active than prazosin by the oral route in this model although for any given fall in BP the changes in renin activity were similar. Cromakalim was only weakly active in the rhesus monkey: 0.1 - 1 mg/kg i.v. led to only short-lived falls in BP which were nevertheless associated with tachycardia and an increase in plasma renin activity; by the oral route, cromakalim was inactive up to 3 mg/kg.

In conclusion, SDZ PCO 400 is a potent and long-lasting BP lowering agent when given orally to conscious SH rats and rhesus monkeys. Its haemodynamic profile is that of a peripheral vasodilator, consistent with an action primarily to open K^+ channels in vascular smooth muscle.

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7P THE CARDIOVASCULAR EFFECTS OF SDZ PCO 400 *IN VIVO*: COMPARISON WITH CROMAKALIM

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Potassium channel openers such as cromakalim (BRL 34915) comprise a new class of smooth muscle relaxants thought to act by increasing the membrane permeability for K^+. In this report, we describe the in vitro and in vivo vasorelaxant effects of a newly synthesized compound, SDZ PCO 400 ((-)-(3S,4R)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyloxy)-2H-1-benzopyran-6-carbonitrile). Comparisons have been made with cromakalim and/or its active (-) enantiomer, BRL 38227.

Rat portal veins were loaded with ^{86}Rb (as a marker for K^+) and mounted in a superfusion chamber for simultaneous measurement of spontaneous contractile activity and $^{86}Rb^+$ efflux (Quast, 1987). Both SDZ PCO 400 and BRL 38227 inhibited spontaneous activity with similar potency (pIC50 ca. 7.9) and increased the rate constant of $^{86}Rb^+$ efflux concentration-dependently up to a maximum of 60% (pED30 ca. 6). The respective (+) enantiomers were 100 to 200 fold weaker in eliciting these effects.

Potassium channel openers lose their vasodilator activity in depolarizing medium (see e.g. Quast & Cook, 1989). The vasorelaxant potency of SDZ PCO 400 and BRL 38227 (and their (+) enantiomers) were therefore compared on rat aortic rings precontracted with 0.1 µM noradrenaline in Krebs solution containing either 5 or 55 mM KCl (the latter in the presence of 0.1 µM isradipine). In 5 mM KCl, the (-) enantiomers relaxed the noradrenaline contraction up to 90% with a pIC45 value of 7.5. Under depolarizing conditions, both compounds were about 500 times less active in inhibiting the noradrenaline contraction, consistent with the dominant mechanism of vasorelaxation being K^+ channel opening. The inhibition was stereospecific under either condition. In the rat isolated perfused mesenteric artery bed, SDZ PCO 400 and cromakalim inhibited contractions elicited by 1 s pulses of noradrenaline (100 µM) with pIC40 values of 7.2 and 6.7 respectively, indicating that they may interfere with the ability of noradrenaline to release Ca^{2+} from intracellular stores in resistance vessels (Quast, 1989).

In normotensive anaesthetized thiobutabarbital (Inaktin®, 150 mg/kg i.p.) rats both SDZ PCO 400 and cromakalim, 0.1 mg/kg i.v., decreased blood pressure by ca. 35 mmHg and increased heart rate by ca. 30 beats/min. The effects on blood pressure were markedly reduced after pretreatment (15 min) with glibenclamide, 20 mg/kg i.v., a known inhibitor of the effects of K^+ channel openers (Quast & Cook, 1989).

In conclusion, the data show that SDZ PCO 400 induces vasorelaxation in vivo and in vitro by opening of K^+ channels in the smooth muscle membrane.

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8P INTERACTIONS BETWEEN K^+ CHANNEL OPENERS AND THE SYMPATHETIC NERVOUS SYSTEM IN PITHED SPONTANEOUSLY HYPERTENSIVE RATS

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In vitro (Hamilton et al., 1986) and in vivo studies (Buckingham, 1988) have previously shown that cromakalim attenuates adrenergically-mediated contractile and pressor responses. The aims of the present study were (1) to assess whether the previously described systemic sympathoinhibitory effect of cromakalim also develops at the regional vascular levels and if so, whether it affects homogeneously or not the different vascular beds, and (2) to investigate if this sympathoinhibitory effect is a specific property of cromakalim or if it is also shared by other K^+ channel openers, e.g. SR 44866, a newly developed drug of this group (Richer et al., 1989).

Male SHRs were anesthetized with pentobarbital (50 mg/kg i.p.), artificially ventilated and pithed. Blood pressure and heart rate were measured from the cannulated carotid artery. Cardiac output and regional (renal, mesenteric and hindlimb) flows were measured from Doppler velocity probes placed on the upper aorta, the renal and mesenteric arteries and the terminal aorta respectively. Corresponding vascular resistances were calculated. Cromakalim (1 µg/kg/min, 15 min), SR 44866 (0.25 µg/kg/min, 15 min) or their solvent (0.09 ml/kg/min, 15 min) were then infused and their effects on the systemic and regional vascular responses (a) to increasing i.v. doses of either cirazoline, a selective α1-adrenoceptor agonist (0.3 to 10 µg/kg) or UK-14,304, a selective α2-adrenoceptor agonist (3 to 100 µg/kg), and (b) to increasing frequencies of electrical stimulation of the spinal cord (0.25 to 4 Hz) were investigated and compared. Finally, the effects of the K^+ channel openers vs spinal cord stimulation were also assessed after restoration of blood pressure to its pre-drug level by an infusion of either $PGF_{2\alpha}$ (30 µg/kg/min) or vasopressin (4 mU/kg/min).

The two K^+ channel openers did not affect postsynaptic α1- but slightly reduced postsynaptic α2-adrenoceptor-mediated systemic pressor and regional vasoconstrictor responses. Both drugs significantly decreased the systemic pressor and regional vasoconstrictor responses elicited by spinal cord stimulation. These sympathoinhibitory effects were not homogeneously distributed among the different vascular beds, the decreasing rank order being : mesentery > kidney > hindlimb. Simultaneously, the spinal cord stimulation-induced tachycardia remained unaffected. Restoration of blood pressure and vascular tone to pre-K^+ channel openers infusion levels by $PGF_{2\alpha}$ and vasopressin respectively did not affect and abolished the sympathoinhibitory effects of cromakalim and SR 44866.

We conclude that in SHRs the two investigated K^+ channel openers exert similar vascular sympathoinhibitory effects which might thus be a common property to this group of drugs. These sympathoinhibitory effects which do not affect homogeneously the different vascular beds are not dependent upon the arterial blood pressure level and are most likely presynaptically located.

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9P THE EFFECT OF CROMAKALIM IN GUINEA-PIG STOMACH CANNOT BE ASCRIBED SOLELY TO OPENING K^+ CHANNELS

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Cromakalim will relax spontaneous tone in all smooth muscles so far studied. A common underlying mechanism appears to be the opening of K channels, normally causing hyperpolarization of the smooth muscle plasma membrane, a decrease in membrane resistance and an increase in K or Rb fluxes (Cook, 1988). The spontaneous mechanical activity in stomach muscle consists of rhythmic phasic contractions which are associated with regular slow depolarizing potentials or slow waves (Tomita, 1981). These slow waves do not invariably generate tension, but if they are large enough either to trigger Ca based action potentials, or the opening of slow voltage sensitive Ca channels, then contraction normally results.

To investigate the actions of cromakalim on stomach muscle, circular muscle strips were dissected from the antral region, mounted as previously described (Ohba et al., 1975) and perfused with Hepes-Tris buffered ungassed physiological saline either warmed to 32-35°C or at room temperature (25°C). Mechanical activity was recorded using a home-mounted Akers transducer, and membrane potentials were recorded with conventional KCl filled microelectrodes.

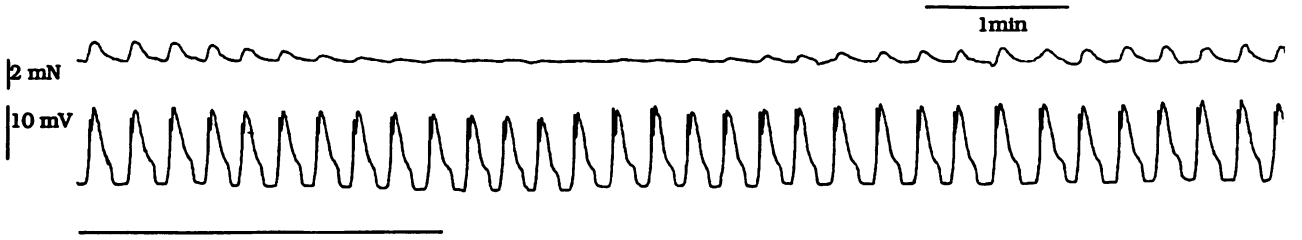

Figure 1. The effects of 3 x 10^{-6}M cromakalim on the mechanical (upper trace) and electrical (lower trace) activity of a circular muscle strip from guinea-pig stomach at 35°C. The drug was applied for the period indicated by the bar.

Figure 1 shows the effects of 3μM cromakalim at 35°C. Note the membrane hyperpolarization, and the cessation of the mechanical activity, but that the slow waves persist with a slight increase in frequency, and an enhanced early spike component. The hyperpolarization is associated with a small increase in membrane conductance, is enhanced in K-free solution and reduced as extracellular K is elevated, consistent with an increase in K permeability. The increase in slow wave frequency is more pronounced at room temperature, and not obviously related to the increased K permeability. The most obvious and unexpected finding is the clear dissociation between the mechanical and electrical events, suggesting that cromakalim has a profound effect on electro-mechanical coupling in the stomach.

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10P ANTAGONISM OF RELAXIN BY GLIBENCLAMIDE IN THE UTERUS OF THE RAT *IN VIVO*

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Relaxin is an inhibitor of uterine contractions in vivo (Porter et al., 1979) whose mechanism of action may be via increased myometrial cAMP concentrations, although these were not well correlated temporally with inhibition of contractions in vitro (Sanborn et al., 1980). K-channel openers are also uterine relaxants (Hollingsworth et al., 1987) and are antagonized in vivo by glibenclamide (Sadraei et al., 1989). The aim of this study was to determine whether relaxin was antagonized by glibenclamide compared with salbutamol, a uterine relaxant which acts via stimulation of adenylate cyclase.

Female rats, 200-250g, were anaesthetized with tribromoethanol (240mg kg-1 i.p.), bilaterally ovariectomized and equipped with an intra-uterine balloon and jugular vein cannula. After 24 h uterine responses to bolus i.v. doses of porcine relaxin (2, 5, 20μg kg-1), or salbutamol (10, 50, 200μg kg-1) were determined. Integral of uterine contractions for 60 min following relaxant bolus was expressed as % of the integral for 60 min prior to each dose. Four hours after the last relaxant dose, glibenclamide (20mg kg-1) or vehicle (0.02N NaOH in 4% glucose w/v, 5ml kg-1) was infused i.v. over 5 min followed 10 min later by a single bolus dose of relaxant (relaxin; 2-200μg kg-1, salbutamol; 10, 50 or 200μg kg-1, 20 rats per dose). The % inhibition of uterine contractions was determined over 60 min as before. Pre-infusion log ID50 values for each animal and post-infusion log ID50 values from mean data were estimated using probit analysis.

Uterine sensitivity to relaxin was unchanged in vehicle-infused rats (log ID50(μg kg-1): pre-infusion, 1.03±0.04; post-infusion, 0.99). Glibenclamide infusion significantly reduced uterine responses to all doses of relaxin ($p<0.001$, log ID50(μg kg-1): pre-infusion, 1.00±0.04; post-infusion, 2.28) and reduced the slope of the dose response curve. No significant differences in uterine sensitivity to salbutamol were observed in vehicle- (log ID50(μg kg-1): pre-infusion, 1.88±0.07; post-infusion, 1.99) or glibenclamide infused rats (log ID50(μg kg-1): pre-infusion, 1.83±0.07; post-infusion, 1.98).

In conclusion, relaxin was antagonized by glibenclamide, whereas salbutamol was unaffected, suggesting that opening of ATP-dependent K-channels may be involved in the uterine relaxant action of relaxin.

We thank Action Research for the Crippled Child for financial support, Hoechst Ltd (Hounslow) for glibenclamide and J. Dawkins (Congerstone) for pregnant sow ovaries.

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11P EVIDENCE THAT THE SENSITISING ACTION OF CAFFEINE ON MYOFIBRILS IS NOT DUE TO A DIRECT EFFECT ON THE Ca^{2+} AFFINITY OF TROPONIN C

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The "Ca^{2+}-sensitizing" drugs such as caffeine and sulmazole (AR-L 115 BS) act directly on myofibrils of striated muscle to increase the sensitivity to Ca^{2+} (e.g. Wendt & Stephenson, 1983). This may partly explain the action of these drugs to increase the force of contraction. However, we do not know the mechanism of this sensitization, or why the effect of caffeine on cardiac myofibrils is about 3 times greater that on skeletal myofibrils (Wendt & Stephenson, 1983).

To examine whether the sensitization of force by caffeine is due to an increase in the Ca^{2+} affinity of troponin C (TnC), we used isolated cardiac and skeletal TnC labelled with fluorescent probes IAANS (2-((4'-iodoacetamido)-anilino)-naphthalene-6-sulphonic acid) and DANZ (dansylaziridine), respectively. The fluorescence of these labels increases when Ca^{2+} binds to the "Ca^{2+}-specific" sites that are involved in the regulation of contraction. Ca^{2+} binding curves were produced by measuring the increase in fluorescence as $CaCl_2$ was added sequentially to a solution containing (mM): 100 BES (N,N-bis[2-hydroxyethyl]-2-aminoethane sulphonic acid), 85 K propionate, 4.4 $MgATP^{2-}$, 1 Mg^{2+}, 10 creatine phosphate, 1 dithiothreitol, 2 EGTA, 3 uM labelled TnC, pH 7.0 at 25°C. Addition of 20 mM caffeine produced no significant change in the pCa_{50} ($-\log[Ca^{2+}]$ for 50% fluorescence increase) for cardiac TnC (5.26 ± 0.07 in zero caffeine, mean ± S.E., n=4) or skeletal TnC (5.55 ± 0.02, n=4). This is in marked contrast to the effects of 20 mM caffeine on force production of skinned muscles, in which the pCa_{50} for force increased by 0.31 ± 0.04 (n=4) in detergent-skinned cardiac muscles from rat and 0.09 ± 0.01 (n=4) in glycerinated skeletal fibres from rabbit. However, we confirmed the finding of El-Saleh & Solaro (1988) that low pH (6.2) reduces the Ca^{2+} affinity of TnC, as it does for the Ca^{2+}-sensitivity of force production. Thus some interventions, such as acidity, change the Ca^{2+} sensitivity of force by directly altering the Ca^{2+} affinity of TnC, whereas caffeine does not act in this way. It seems likely that the effect of caffeine on myofibrils is via an indirect mechanism, in which caffeine acts on a site other than TnC.

Supported by the B.H.F. S.P. is a CASE student (SERC/Ciba-Geigy plc).

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12P THE PUTATIVE INTRACELLULAR CALCIUM ANTAGONIST TMB-8 NONSELECTIVELY REDUCES THE MEMBRANE CONDUCTANCES FOR CA^{2+}, NA^{+} AND K^{+} IONS

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The compound TMB-8 (8-(N,N-Diethylamino)-Octyl-3,4,5-Trimethoxybenzoate HCl) was introduced as a tool to inhibit the release of intracellularly stored calcium (Chiou & Malagodi, 1975). Observations in various non-myocardial tissues, however, suggest that TMB-8 may have additional sites of action. In guinea-pig left atria TMB-8 has a transient positive and a sustained negative inotropic effect accompanied by a prolongation in action potential duration and a decrease in $\dot{V}_{max}$ (Himmel & Ravens, 1989). These changes suggest that TMB-8 may also influence membrane currents.

We therefore studied TMB-8 in single isolated guinea-pig ventricular cardiomyocytes with the "whole cell patch clamp" technique. Conditions for measuring calcium currents, I_{Ca}, were $[Ca^{2+}]_o$ 1.8 mM, K^+ replaced by Cs^+, stimulation rate 0.5 Hz, holding potential -80 mV, 100 ms prepulse to -40 mV, 500 ms test pulse to 0 mV; for measuring sodium currents, I_{Na}: $[Na^+]_o$ 30 mM, $[Ca^{2+}]_o$ 0.5 mM, Na^+ and K^+ replaced by Cs^+, Cd^{2+} 0.1 mM, stimulation rate 0.5 Hz, holding potential -80 mV, 20 ms test pulse to -30 mV; and for measuring quasi steady-state K^+ currents: "normal" Tyrode solution, slow ramp pulses (10 mV/s) from -120 to +60 mV (rate 2/min). All experiments were carried out at 22-24 °C and with EGTA (10 mM) in the pipette solution.

TMB-8 concentration-dependently and reversibly reduces I_{Ca} (pD_2 5.0, slope -0.8), it does not affect the time course nor the potential-dependence of I_{Ca}. TMB-8 shifts the steady-state inactivation curve of I_{Ca} to more negative membrane potentials (-4.7 ± 0.3 mV with 10 µM TMB-8, n=4). The calcium channel blockade is slightly use-dependent (rest interval 120 s). TMB-8 also concentration-dependently and reversibly inhibits I_{Na} (pD_2 5.3, slope -0.8) without a change in the time-course of I_{Na}-decay or the potential-dependence of I_{Na}. The steady-state inactivation curve of I_{Na} is shifted to more negative membrane potentials (-5.6 ± 1.7 mV with 10 µM TMB-8, n=4). The K^+ current is decreased by 100 µM TMB-8 over the potential range from -120 to +60 mV.

In addition to a putative intracellular calcium antagonism TMB-8 possesses both Ca^{2+} and Na^+ channel blocking properties and reduces membrane conductance for K^+ ions. Since the effects on the different currents are found in the same concentration range, TMB-8 apparently lacks selectivity. It is speculated that the unspecific effect of TMB-8 may be traced back to its amphiphilic nature. The molecules are expected to accumulate in the lipid-water interphase and may thereby interfere nonspecifically with membrane protein function.

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13P MIOFLAZINE AND LIDOFLAZINE PROTECT ATRIAL MUSCLE AGAINST CALCIUM OVERLOAD DAMAGE: A STUDY OF ULTRASTRUCTURE, CYTOCHEMISTRY AND FUNCTION

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The protective effects of mioflazine, lidoflazine and verapamil against accumulation of cellular calcium after increasing the extracellular calcium concentration, $[Ca^{2+}]_o$, were studied in right atrial trabeculae obtained from patients undergoing open heart surgery and in left atria from rat and guinea-pig hearts. The electrically stimulated muscles (1 Hz) were exposed to two consecutive cumulative increases in $[Ca^{2+}]_o$ (1 - 25 mM) before and after addition of drug (3 µM) or solvent. At the end of the 2nd calcium challenge, they were fixed for light- and electron microscopy, and for cellular calcium localization. Force of contraction, F_c, was measured isometrically throughout the experiment. Some muscles incubated in low Ca^{2+} solution (1 mM) served as controls.

Light-microscopic examination of control human atrial trabeculae (270 min in low $[Ca^{2+}]_o$) revealed that 14.9 ± 2.7% (mean ± SEM, n=10) out of 300 cells scored were severely damaged, showing swollen mitochondria with disrupted cristae, nuclear pyknosis, contraction band necrosis and cytolysis. In solvent-treated muscles, after two consecutive Ca^{2+} challenges, 57.8 ± 3.2% of the cells were damaged. The deleterious effects of high $[Ca^{2+}]_o$ were reduced to 28.8 - 8.1% and 31.8 ± 7.7% of damaged cells by exposing the muscles to mioflazine or lidoflazine, respectively. With verapamil, 55.7 ± 6.3% of the cells were destructed. Similar results were obtained with atria from rat and guinea-pig hearts.

The changes in cellular calcium distribution somewhat resembled the damage observed after ischemia followed by reperfusion: The sarcolemma had lost its calcium deposits, large clusters of calcium were found in many mitochondria and often also in the nucleus, sometimes, the cytosol was full of calcium precipitate. In these cells, the mitochondria contained no calcium but amorphous densities. Mioflazine, lidoflazine, and to a lesser extent verapamil clearly prevented these shifts in cellular calcium localization. Again, there were no differences between atria from the three species investigated.

In solvent- and verapamil-treated atria, F_c was significantly suppressed during the 2nd Ca^{2+} challenge, whereas with mioflazine and lidoflazine, F_c attained the same maximum albeit at higher $[Ca^{2+}]_o$. Diastolic tension increased and after-contractions occurred at high $[Ca^{2+}]_o$. In rat and guinea-pig atria, these changes were significantly reduced by mioflazine and lidoflazine. In human muscle, however, the differences did not reach statistical significance.

Our results demonstrate that mioflazine and lidoflazine are protective against Ca^{2+} overload damage in atrial muscle. Since the Ca^{2+} channel blocker verapamil is much less effective, it is concluded that the bulk of calcium reaches the intracellular space via pathways other than Ca^{2+} channels.

14P THE EFFECTS OF A NUCLEOSIDE TRANSPORT INHIBITOR, R75231, ON ISCHAEMIC ARRHYTHMIAS IN ANAESTHETISED PIGS

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Previous studies demonstrating an antiarrhythmic effect of adenosine (Wainwright & Parratt, 1988) have led to the suggestion that adenosine may act as an "endogenous antiarrhythmic agent" Accumulation of adenosine within the myocardium occurs within seconds of a sudden reduction in coronary perfusion, and raised levels of adenosine are evident in coronary venous blood within 15 min of coronary occlusion. The aim of this study was to determine the effects of a nucleoside transport inhibitor, R75231, (2-(aminocarbonyl)-N-(4-amino-2,6-dichlorophenyl)-4-[5,5-bis-(4-fluorophenyl)pentyl]-1-piperazine acetamide (free base); Ijzerman et al, 1989) on the arrhythmias that occur in a pig model of myocardial ischaemia.

Castrated male pigs (22-30 kg) were sedated with azaperone and anaesthetised with chloralose (100 mg kg^{-1} i.v.). A mid-sternal thoracotomy was performed and the left anterior descending coronary artery (LAD) prepared for occlusion. Following stabilisation, R75231 (50 µg kg^{-1} or 100 µg kg^{-1}), or solvent vehicle, was administered. Fifteen minutes later the LAD was occluded and the subsequent arrhythmias were monitored from a lead II ECG. Standard haemodynamic parameters (ABP, LVP, PAP, HR) and electrocardiographic measurements (ST-segment, QRS, QT and RR intervals) were continuously recorded by a computerised data logging system. Prior to coronary artery occlusion only the higher dose of R75231 (100 µg kg^{-1}) had any significant haemodynamic effects (a gradual decrease in both mean ABP and LV end-diastolic pressure (LVEDP)). Following occlusion both doses exerted significant protection against deterioration of contractility (dP/dt/P) and HR seen in controls. At the end of the 30 min occlusion period (or just prior to the onset of ventricular fibrillation; VF) the arterial blood pressure was significantly lower in both treated groups (by 29% and 34%) than in vehicle treated controls. The effects of R75231 on the arrhythmias resulting from coronary occlusion are shown below.

TREATMENT	n	PVB's	% VT	% VF	Mean time to VF (min)	
Control	10	115±12	30	90	23.1±1.5	
R75231 (50µgkg^{-1})	6	85±40	33	83	16.0±3.2*	*P<0.05 compared
R75231 (100 µg kg^{-1})	10	131±30	50	20*	22.9±3.9	to control group

These results suggest that R75231 can suppress ischaemia-induced ventricular fibrillation. There is no evidence that R75231 exerts a direct effect on either cardiac muscle or vascular smooth muscle, suggesting that inhibition of adenosine uptake is the mechanism underlying this effect.

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15P UK-68,798, A POTENT AND SELECTIVE CLASS III ANTIARRHYTHMIC AGENT, REDUCES DISPERSION OF REPOLARISATION IN CANINE HEARTS *IN SITU* INDUCED BY RAPID PACING

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The vulnerability of the ventricles to fibrillation and the dispersion of repolarisation across them may be linked (Han & Moe, 1964). Drugs which reduce this non-uniformity of activity within the heart may reduce the tendency of advancing activation wavefronts to fractionate and thus suppress reentrant activation. We have induced dispersion of repolarisation in the dog heart by pacing at short cycle lengths (120-150ms) so that each cardiac excitation occurs during the relative refractory period of the preceding beat, such as may occur during a rapid ventricular tachycardia and have examined the effects of UK-68,798, a novel and highly selective class III antiarrhythmic agent (Gwilt *et al*, 1989a,b) on this dispersion. These results are compared to the effects of quinidine, a widely used antiarrhythmic drug which also prolongs the action potential.

Simultaneous epicardial recordings from a single beat (26-31 sites) were made from 15 open chest dogs anaesthetised with pentobarbitone sodium (60mg/kg iv). Digitised signals were stored on a floppy disk for subsequent analysis (King *et al*, 1990) to yield the activation time (AT), repolarisation time (RT) and activation-repolarisation interval (ARI; a measure of local action potential duration) at each site. Each array of signals provided a median value for each parameter plus its temporal dispersion (interquartile range), later averaged to provide means ± s.e.mean for each treatment group.

UK-68,798 (3-100μg/kg iv) increased the ARI dose dependently from 115±4 to 154±12ms (n=5), as did quinidine (1 - 10mg/kg) from 117±2 - 152±7ms (n=5), suggesting comparable degrees of action potential prolongation. UK-68,798 increased RT by a similar extent (156±5 to 203±10ms) without affecting mean AT or its temporal dispersion, which is consistent with its class III properties. UK-68,798 reduced the mean dispersion of repolarisation to between 43% and 55% of mean dispersions in vehicle treated dogs (n=5) at corresponding time points; differences at every dose were significant ($p<0.05$, unpaired t-test). Quinidine induced a larger prolongation of RT (159±2 to 236±7ms), explained by an increase in AT (48±2 to 85±4ms). This slowing of impulse conduction through the myocardium was not uniform, as the dispersion of AT increased by about 2-fold. No significant reductions in dispersion of repolarisation were seen.

Therefore, UK-68,798 improved the degree of electrical homogeneity across the myocardium by reducing the dispersion of repolarisation observed during stimulation at short cycle lengths. As such an action would tend to reduce the potential for fractionation of activation wavefronts and subsequent reentry (see above), this *in vivo* mechanism may significantly contribute to the antiarrhythmic and antifibrillatory properties of selective class III antiarrhythmic drugs such as UK-68,798.

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16P CORONARY VASCULAR RESPONSIVENESS TO ANGIOTENSIN IN PACING-INDUCED HEART FAILURE: EFFECT OF ENALAPRIL

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Rapid ventricular pacing in the dog produces clinical signs of congestive heart failure (CHF; Armstrong et al., 1986). In addition, vascular smooth muscle reactivity is altered at CHF (Forster et al., 1988). Given the relevant clinical data as to the efficacy of enalapril (EN) in improving survival in advanced CHF (The Concensus Trial Study Group, 1987), we undertook an examination of EN's effects on coronary vascular activity in the dog with CHF.

Treatment was initiated by blinded random assignment of dogs to placebo (PL; n=5) and EN (n=11) 1 wk following the onset of pacing, at which time the animals had mild cardiomegaly. Each group was paced to a conventional biologic end point (Armstrong et al.,1986) and sacrificed. The circumflex (CRX) coronary artery was removed, cut into rings (5mm) and mounted in organ baths. General vascular and endothelium function was assessed with KCl (20mM) and acetylcholine (Ach) respectively. Concentration-effect curves were then constructed first to either angiotensin I (AI) or angiotensin II (AII) in an individual (single concentration/ring) manner and secondly, following at least a 3h washout, concentration-effect curves to AI or AII were constructed in a cumulative manner. The results are shown below where tension developed (KCl and Amax; X±se) is in $g.mm^{-2}$, Ach results are %relaxation (X±se) and EC_{50} is [nM] (X with 95% confidence limits in parenthesis) and * $P<0.05$.

Table 1 Concentration-effect to AI and AII on canine CRX following chronic PL or EN treatment

			Individual				Cumulative			
	KCl	ACh	AImax	AIImax	$AIEC_{50}$	$AIIEC_{50}$	AImax	AIImax	$AIEC_{50}$	$AIIEC_{50}$
PL	6.1±0.6	42±2	3.6±0.4	4.6±1.1	40(11-150)	8.4(2.8-24)	2.9±0.7	3.8±1.1	19(6.5-54)	4.9(0.8-28)
EN	5.3±0.3	46±3	1.5±0.4*	2.3±0.5	6(1.9-18)	0.09(0.03-0.13)*	2.6±1.4	2.2±0.3	8.8(3.8-20)	0.03(0.007-0.1)*

These data from CHF vessels suggest that 1) EN does not modify general responsiveness of the CRX to KCl or Ach. 2) EN caused a significant decrease in max of AI following individual administration. 3) There was no significant difference in the sensitivity of the arteries to AI following EN, but the sensitivity was greatly enhanced to AII. These results suggest that EN attenuates but does not prevent coronary artery responsiveness to AI, suggesting that AI may have direct effects or that it is converted to an active peptide independent of converting enzyme and secondly AII receptors in CRX become supersensitive.

Supported by the Heart and Stroke Foundation of Ontario.
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17P DIFFERENCES IN TISSUE ACE-INHIBITORY PROFILE BETWEEN FPL 63547 AND ENALAPRIL AFTER CHRONIC DOSING TO SPONTANEOUSLY HYPERTENSIVE RATS

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The existence of tissue compartments of the renin-angiotensin system (RAS) and their susceptibility to inhibition by inhibitors of angiotensin-converting enzyme (ACE) is now recognised (Unger et al., 1984). Relative selectivity for inhibition of the cardiac RAS after acute dosing has been described for FPL 63547 (Carr et al., 1989) and captopril and zofenopril (Cushman et al., 1989). This study examines the effects of chronic administration of therapeutically relevant and equivalent doses of FPL 63547 and enalapril on the tissue RAS and systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR).

Adult SHR (6 months old) were dosed orally with vehicle (PEG), FPL 63547 (10, 50 or 250 µg/kg/day) or enalapril (100, 500 or 2500 µg/kg/day) for approximately 6 months. SBP was measured before dosing commenced and at 3 month intervals thereafter. At the end of the study animals were killed and tissue ACE activity determined according to the method described previously (Carr et al., 1989). Tissue ACE assay data are shown in the Table. SBP was reduced significantly and by a similar degree at the highest dose level only for both ACE inhibitors (FPL 63547 -23%, enalapril -27%, $p<0.05$ t-test, n = 10).

Residual ACE activity (% control) in SHR following chronic oral dosing (µg/kg/day) with FPL 63547 and enalapril (n = 5, mean ± s.e.)

	Enalapril			FPL 63547		
	100	500	2500	10	50	250
Heart	57 ± 5	47 ± 5	31 ± 5	34 ± 6	12 ± 0	4 ± 2
Skeletal muscle	71 ± 5	60 ± 2	46 ± 1	38 ± 3	11 ± 1	5 ± 1
Plasma	46 ± 3	21 ± 4	6 ± 1	61 ± 2	20 ± 3	2 ± 0
Kidney	22 ± 2	3 ± 1	0 ± 1	21 ± 2	10 ± 4	4 ± 1

The tissue ACE inhibitory profiles of FPL 63547 and enalapril after chronic dosing were clearly different, supporting earlier acute dose comparisons (Carr et al., 1989). These differences occurred at sub-antihypertensive doses and at doses of the two compounds which caused equivalent reductions in SBP. Although the relative degree of inhibition of kidney and plasma ACE was similar, at each dose level FPL 63547 had a significantly greater effect than enalapril on cardiac and skeletal muscle ACE ($p<0.05$ Mann-Whitney U test). At the minimum antihypertensive dose FPL 63547, unlike enalapril, produced maximal inhibition of ACE in the heart and skeletal muscle, which should prove to have functional significance in these tissues. In contrast to published data on captopril and zofenopril (Cushman et al., 1989) FPL 63547 was able to inhibit cardiac ACE fully at low and therapeutically relevant doses.

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18P FLOSEQUINAN (BTS 49465) BEHAVES AS AN INHIBITOR OF PHOSPHODIESTERASE TYPE III

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Flosequinan (BTS 49465) is currently in clinical development for the treatment of hypertension and congestive heart failure (Yates and Hicks, 1988). The compound was originally described as a vasodilator and subsequently reported to possess additional positive inotropic activity (Yates and Hicks, 1988). The exact mechanism of action of the compound is unknown, although recent enzymological studies have indicated that flosequinan can inhibit cyclic nucleotide phosphodiesterase (PDE) activity (Frodsham et al., 1989).

We have compared the effects of flosequinan (F) with those of SK&F 94120 (SK&F), a clearly documented selective inhibitor of purified PDE type III isoenzyme (Reeves et al., 1987, Gristwood et al., 1987), on the force of contraction (Fc) of guinea-pig electrically stimulated (1 H_Z) right ventricular strips in vitro. Preparations were placed under 1 g resting tension in oxygenated Krebs solution at 37ºC.

F, like SK&F, when added cumulatively to the organ baths caused concentration related increases in Fc. The threshold concentration and the concentration causing a 50% increase in Fc (EC50) for F were 10^{-5}M and 7×10^{-5}M respectively, (n=7). F was approximately 10 times less potent than SK&F.

The positive inotropic responses to F 1×10^{-4}M were completely inhibited by carbachol 1×10^{-6}M (n=8), consistent with the involvement of increased intracellular concentrations of cyclic AMP in the response (see Gristwood et al., 1987).

It has previously been reported (Gristwood et al., 1986), that in the ventricle there is a specific synergistic interaction on force of contraction between PDE type III inhibitors and rolipram, which is selective for inhibition of the PDE type IV enzyme (Reeves et al., 1987). In our studies there was a clear synergistic interaction between F and rolipram, similar to that which occured between SK&F and rolipram. Percentage increases in Fc caused by rolipram 1×10^{-6}M in untreated and F (10^{-4}M) or SK&F (3×10^{-5}M) treated preparations being 0% (n=4), 112±13% (n=4) and 208±52% (n=5) respectively. Our interpretation of these data is that F, like SK&F, is a functional inhibitor of PDE type III.

Qualitatively similar results were obtained with the sulphone metabolite of F, BTS 53554 (BTS). Thus, for BTS the threshold concentration and EC50 for inotropic activity were 10^{-5}M and 3.4×10^{-5}M, (n=7) and increases in Fc caused by rolipram in untreated and BTS (10^{-4}M) treated preparations were 0% (n=4) and 27±3% (n=10) respectively.

We conclude that F and its major metabolite exert part of their inotropic action by virtue of their ability to inhibit PDE type III although we cannot exclude the possibility that they have additional mechanisms of action to account for their full range of effects.

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19P PRECONDITIONING OF THE ISCHAEMIC MYOCARDIUM: ROLE OF THE CYCLOOXYGENASE PATHWAY

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We demonstrate that short 'preconditioning' periods of coronary artery occlusion protect the myocardium against the serious arrhythmias that result from more prolonged periods of ischaemia and we examine whether this protection is due to the release of a substance derived from arachidonic acid via the cyclooxygenase pathway. Mongrel dogs were anaesthetised with choralose, ventilated and the chest opened. A composite electrode was sutured to the myocardium in the region supplied by the anterior descending branch of the left coronary artery and a ligature was placed around this vessel. In 20 dogs the artery was occluded for 2 preconditioning periods of 5 min (with a 20 min reperfusion period in between) then, 20 min later, the artery was occluded for 25 min. In 10 of these dogs the ligature was then released (reperfusion). The control group (10 dogs) were simply subjected to a 25 min occlusion period followed by reperfusion. A third group was given sodium meclofenamate, 2 mg kg^{-1} iv, subjected to preconditioning and then to a 25 min period of ischaemia followed by reperfusion.

Preconditioning markedly reduced the severity of the ventricular arrhythmias that occurred during the 25 min occlusion period. It reduced the incidence of ventricular tachycardia (vt; from 9/10 in the controls to 9/20; $P<0.05$) and ventricular fibrillation (vf; from 4/10 to 0/20; $P<0.05$) during ischaemia and the number of ectopic beats was reduced from a mean of 445 ± 139 to 99 ± 21; $P<0.01$). In those preconditioned dogs in which myocardial prostanoid production was suppressed by sodium meclofenamate, the protection was less marked. There were more ectopic beats (268 ± 121; $P<0.05$ cp to the preconditioned group) and, although none of the dogs fibrillated during ischaemia all did so on reperfusion. There were thus no survivors in either the control or meclofenamate groups whereas 40% of the preconditioned dogs survived.

These results suggest that not all of the protective effect of preconditioning is due to the release of substances from the heart that are derived from arachidonic acid (eg prostacyclin; Parratt et al., 1987). Other factors must be involved.

Supported by a grant from the Scottish Hospitals Research Trust.

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20P EFFECT OF NIFEDIPINE, MIOFLAZINE AND DIPYRIDAMOLE ON INTERSTITIAL ADENINE NUCLEOTIDE CATABOLITES DURING GLOBAL ISCHAEMIA IN GUINEA-PIG HEARTS

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Nifedipine (NIF), mioflazine (MIO) and dipyridamole (DIP) exerted a pronounced cardioprotective effect in guinea-pig hearts subjected to global ischaemia (GI) and reperfusion (Hugtenburg et al., 1989). During reperfusion of guinea-pig hearts after GI the nucleoside-transport inhibitors MIO and DIP, but not NIF, strongly decreased the release of adenine nucleotide catabolites (Hugtenburg et al., 1988). The question arises whether MIO and DIP inhibit the release of catabolites from myocytes or inhibit the uptake of catabolites into endothelial cells.

Guinea-pig Langendorff hearts (paced at 5Hz, voltage 15% above threshold) were perfused with Tyrode containing 1.8 mmol/l calcium. The hearts were perfused with NIF (0.02 µmol/l), MIO (0.3 µmol/l) and DIP (1 µmol/l) for 15 min, after which GI (37°C) was maintained for 60 min. Interstitial fluid (IF) was sampled from the tip of the heart after 5, 15, 30, 45, and 60 min of GI (Scheufler et al., 1988). AMP, IMP, adenosine (ADO), inosine (INO) and hypoxanthine (HYP) levels were determined by HPLC. Data are given as means ± S.E. (n=6). Statistical significance was evaluated using Student's t-test (P, 0.05).
After 17.3 ± 1.1 min of GI ischaemic contracture developed. NIF and MIO significantly ($P<0.05$) delayed the onset of ischaemic contracture to 22.7 ± 3.5 and 24.4 ± 6.4 min, respectively. DIP did not influence the onset of ischaemic contracture. In IF from normoxic hearts catabolites were not detected. After 60 min of GI AMP and ADO were not found. IMP, INO and HYP increased to 15 ± 3, 527 ± 62 and 322 ± 48 µmol/l. NIF but slightly reduced the levels of catabolites. In MIO and DIP treated hearts IMP was reduced to threshold levels. MIO and DIP significantly ($P<0.05$) reduced the level of INO to 111 ± 35 and 188 ± 140 µmol/l, respectively and of HYP to 109 ± 38 and 151 ± 55 µmol/l, respectively.

The delayed onset of ischaemic contracture in NIF and MIO treated hearts can be attributed to the calcium antagonistic effect of the drugs (Hugtenburg et al., 1989). The MIO and DIP induced reduction of INO and HYP levels in the IF suggest that under the conditions of the present study MIO and DIP reduce the release of adenine nucleotide catabolites from myocytes. The slight reduction of the INO and HYP levels in the presence of NIF seems to reflect a reduction of the degradation of ATP (Hugtenburg et al., 1989).

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21P NAFAZATROM AND PH CL 28A INHIBIT PROSTAGLANDIN E_2 CATABOLISM AND PROSTANOID SYNTHESIS IN RAT ISOLATED HEART

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The inhibitors of prostaglandin dehydrogenase (PGDH), nafazatrom and Ph CL 28A (Wong et al 1982, Berry et al, 1985) decrease PGE_2 catabolism and increase PGI_2 output in rat isolated lung (Bakhle & Pankhania, 1987 a, b). Increased synthesis of PGI_2 in the heart would be beneficial because of the anti-arrhythmic and vasodilator properties of this prostanoid (Schror, 1988). We studied the effects of nafazatrom and Ph CL 28A perfused via the coronary circulation on PG metabolism and physiological responses to exogenous arachidonic acid (AA) in rat isolated hearts.

Rat isolated hearts were perfused with warmed, oxygenated Krebs solution at 8ml/min through the coronary circulation (Langendorff preparation). Coronary perfusion pressure (CPP) and cardiac developed tension (CDT) in spontaneously beating hearts were recorded. Catabolism of PGE_2 was assessed in hearts perfused with Krebs solution containing indomethacin (3μg/ml) by measuring the PG emerging in the coronary effluent after an injection of PGE_2 (100ng, 0.1ml) into the aortic cannula by bioassay and by radioimmunoassay (RIA), before and during perfusion with nafazatrom (37μM) or Ph CL 28A (0.3μM), concentrations effective in changing prostanoid metabolism in lung (Bakhle & Pankhania, 1987 a, b). In the absence of drug, PGE_2 catabolism was about 40%; this was reduced further to 29±1% from 37±2% by nafazatrom and to 13±9% from 39±6% by Ph CL 28A ($n=4-6$). In hearts perfused without indomethacin, CPP and CDT rose transiently after injection of exogenous AA (20μg); either drug reduced the increase in CPP but nafazatrom also reduced the increased CDT. The coronary effluent following this injection contained 6-oxo-$PGF_{1\alpha}$ (51±6ng), TxB_2 (13±3ng), PGE_2 (55±10ng) and $PGF_{2\alpha}$ (5±0.4ng; $n=6-10$). Ph CL 28A decreased TxB_2 output to 59±6% of normal and nafazatrom decreased 6-oxo-$PGF_{1\alpha}$ output to 45±4% of normal ($n=6-7$), without affecting the other prostanoids.

Although nafazatrom and Ph CL 28A decreased PG catabolism in heart, as they do in lung, they did not increase output of PGI_2. Indeed, both drugs decrease output of prostanoids, particularly that of TxA_2. The decreased CPP response suggests an overall vasodilator effect for both drugs, which coupled with decreased TxA_2 output may prove useful.

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22P ETHANOL POTENTIATES THE REFLEX BRADYCARDIA ELICITED BY STIMULATING CARDIAC C-FIBRE AFFERENTS IN THE ANAESTHETISED DOG

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The effects of ethanol have been investigated on two reflexes which involve a bradycardia mediated by the vagus - namely the reflex elicited by stimulating cardiac C-fibre afferents, and that elicited by stimulating the arterial chemoreceptors (Daly *et al.*, 1988).

Experiments were performed on 5 spontaneously breathing beagle dogs (11.5-23.0 kg) anaesthetized with α-chloralose (110 $mg.kg^{-1}$ intravenously). Cardiac C-fibre afferents were stimulated with veratridine (0.66 -3.48 $\mu g.kg^{-1}$) injected into the left atrium, and the carotid bodies by intracarotid injection of sodium cyanide (21.7-41.3 $\mu g.kg^{-1}$). To exclude any respiratory effects of ethanol the stimuli were applied both during spontaneous respiration and during a period of apnoea induced reflexly by bilateral electrical stimulation of the central cut ends of the superior laryngeal nerves. Statistical comparisons were made using a Student's paired t test. Values of $P<0.05$ were taken as being significant.

In 11 tests veratridine produced a significant increase in heart period of 941±111 ms (mean ± s.e. mean) from 399±13 ms, a fall in mean blood pressure of 46.8±1.9 mmHg from 136.0±5.3 mmHg and a small reduction in respiratory minute volume of 0.041±0.008 from 0.184±0.014 $l.min^{-1}.kg^{-1}$. Stimulation of the superior laryngeal nerves produced an apnoea, but no consistent effects on heart period or mean arterial blood pressure. During the apnoea the response to veratridine was enhanced, heart period now increasing by 2034±377 ms and mean blood pressure falling by 60.8±7.4 mmHg. Cyanide produced an increase in respiratory minute volume of 0.201±0.071 $l.min^{-1}.kg^{-1}$ but no consistent effects on heart period during spontaneous respiration, and an increase in heart period of 3783±403 ms, but no effects on respiration during apnoea. Intravenous infusion of ethanol (30% in normal saline) produced a blood level of 130.8±10.9 mg %, a small decrease in heart period to 346±10 ms, but no effect on arterial blood pressure or respiration. Ethanol significantly potentiated the veratridine induced bradycardia both during spontaneous respiration and during apnoea (2309±176 and 4011±424 ms increase in heart period respectively). The responses to cyanide were unaffected by ethanol.

These results indicate that ethanol potentiates the bradycardia resulting from veratridine injection, but not that elicited by stimulation of the carotid bodies. These findings may suggest a mechanism for unexplained sudden deaths following small myocardial infarcts in patients with blood alcohol levels in the range 100-200 mg % (Thomas *et al.*, 1988).

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23P THE EFFECTS OF PHORBOL ESTERS ON 5-HT RELEASE FROM RAT HIPPOCAMPAL SLICES AFTER CHRONIC LITHIUM TREATMENT

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We have shown previously that acute administration of lithium caused a reduction in K^+-evoked [^{3}H]-5HT release from rat hippocampal slices (Anderson *et al.*, 1988). This reduction could be reversed by phorbol esters and diacylglycerol, both of which stimulate protein kinase C (PKC). Further studies have shown that chronic administration of lithium increased K^+-evoked [^{3}H]-5HT release from rat hippocampal slices and that these terminals became tolerant to the acute inhibitory effects of lithium described previously (Anderson *et al.*, 1989). We have now studied the effects of phorbol esters and synthetic diacylglycerol on K^+-evoked [^{3}H]-5HT release from hipocampal slices prepared from rats after chronic lithium treatment.

Male Sprague-Dawley rats (200-300g) were used; some were fed diet consisting of 0.1 % lithium carbonate for 28 d, paired controls received ordinary diet. Hippocampal slices 300 µm thick were prepared from both groups of rats and were loaded with [^{3}H]-5HT (0.01 µM; specific activity 28.3 mCi/mmol) in oxygenated HEPES-Ringer buffer containing 50 µM pargyline. The slices were then superfused in buffer containing 0.5 µM citalopram at 0.5 ml/min. A 4-min pulse of buffer containing 35 mM K^+ was given after 28 min; phorbol esters and DC8 were given 12 min prior to K^+ ; the PKC inhibitors staurosporine and Polymyxin B were added at the start of the superfusion. Fractions were collected every 4 min and [^{3}H]-5HT measured by liquid scintillation counting.

Rats which received lithium diet for 28 d showed no increase in basal [^{3}H]-5HT release but K^+-evoked release of [^{3}H]-5HT was increased by 25±6% ($p<0.05$) compared with controls. PDBU (10 nM - 1 µM) and PMA (100 nM) increased release in control slices by 30±3% ($p<0.05$) and 26±6% ($p<0.05$) respectively. After 28 d lithium treatment these phorbol esters did not enhance K^+-evoked [^{3}H]-5HT release . DC8 (100 µM) increased [^{3}H]-5HT release by 42±7% ($p<0.05$) in control hippocampal slices, however release was unchanged after 28 d lithium treatment. Staurosporine (1 µM) and Polymyxin B (10 µM) alone did not affect K^+-evoked [^{3}H]-5HT release from control slices, but the increase in K^+-evoked [^{3}H]-5HT release produced by phorbol esters and DC8 in controls was blocked by both inhibitors of PKC. The increase in release reported after 28 d lithium diet was reduced to control levels by staurosporine and Polymyxin B.

We conclude that after chronic lithium treatment K^+-evoked [^{3}H]-5HT release from hippocampal terminals is altered such that it occurs via a mechanism which is lithium insensitive and partially dependent on protein kinase C.

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24P CI-977, A NOVEL AND SELECTIVE AGONIST FOR THE KAPPA OPIOID RECEPTOR

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A number of kappa agonists based on the arylacetamide structure have previously been reported to be effective antinociceptive agents in both rodent and non-rodent species. However, despite this, none of these compounds have achieved the affinity and potency at the kappa receptor demonstrated by the original non-selective benzomorphans and oripavines.
CI-977, (5R)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide monohydrochloride, is the latest member of this series but can be separated from previous arylacetamides on the basis of a unique high affinity and efficacy at the kappa receptor.
The in vitro and in vivo methodology was as previously described (Skingle and Tyers, 1979; Dykstra and Woods, 1986; Leighton et al, 1988, Clark et al. 1988, Smith et al, 1989). In guinea-pig forebrain membranes, CI-977 had a high affinity for the kappa receptor (K_i=0.11nM) but a low affinity at both mu (K_i=105nM) and delta (K_i=1036nM) receptors. The mu/kappa ratio was approximately 950. In the guinea-pig ileum and rabbit vas deferens isolated smooth muscle preparations, CI-977 inhibited the electrically-induced contractions with IC_{50} values of 0.087nM and 3.3nM respectively. In the ileum the pK_B values for naloxone (7.6) and norbinaltorphimine (10.5) confirmed the kappa nature of these effects.
CI-977 was also an extremely potent antinoceptive agent in animal models. In the rat paw pressure test a dose as low as 30 µg/kg (i.v.) produced an effect which was 50% of the maximum possible effect (MPE_{50}). CI-977 was also effective in the mouse acetylcholine-induced abdominal constriction (MPE_{50} = 5 µg/kg) and tailclip (MPE_{50} = 30 µg/kg) tests (s.c.) but was ineffective in the mouse hotplate at doses up to 10mg/kg. In the dog tooth pulp stimulation and monkey tail immersion tests, the MPE_{50} values were 0.3 and 1.2 µg/kg (i.m.) respectively. In all behavioural studies, the antinociceptive action of CI-977 was reversed by naloxone (1mg/kg; s.c.). As would be expected from studies with other kappa agonists CI-977 produced a potent diuretic effect and also reduced locomotor activity at higher doses in both rat and mice. It did not cause any respiratory depression, inhibition of gastro-intestinal motility or physical dependence of the type produced by morphine as determined using the naloxone jumping test. These results together with the observation that the potency of CI-977 was not diminished in rats made tolerant to morphine support the conclusion that this compound produces its effects by a selective action at the kappa receptor.

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25P EFFECT OF CENTRAL AND PERIPHERAL INJECTIONS OF SUMATRIPTAN ON EXTRACELLULAR LEVELS OF 5-HT IN GUINEA-PIG FRONTAL CORTEX

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Recent studies have shown that the terminal autoreceptor on central 5-hydroxytryptamine (5-HT) neurones *in vitro* in the guinea pig and human is similar to the 5-HT_{1D} receptor binding site (Middlemiss et al., 1988). Sumatriptan (GR 43175), a drug undergoing clinical trials for the acute treatment of migraine (Doenicke et al., 1988) has high affinity and selectivity for these 5-HT_{1A}, $_{1B}$ and $_{1D}$ receptor binding sites (Peroutka and McCarthy, 1989). The purpose of this study was to determine whetner sumatriptan will reduce extracellular levels of 5-HT *in vivo*, measured by intracranial dialysis, and whether a peripheral injection will display similar effects.

Male Hartley guinea pigs (250-350 g) were anaesthetised with chloral hydrate (500mg/kg i.p.). Dialysis probes, prepared as previously described (Sleight et al., 1988), were perfused with artificial CSF and implanted into the frontal cortex. Two hours later, 4 x 20 min. samples were taken to measure basal extracellular levels of 5-HT. Separate groups of 4 animals were then given either sumatriptan (50 μg/kg i.p.), sumatriptan (500 μg/kg i.p.) or the artificial CSF was changed to incorporate sumatriptan (10^{-7} M). The flow of artificial CSF was interrupted in another group of animals to act as controls. Extracellular levels of 5-HT were measured for a further 140 mins.

An interruption in the flow of artificial CSF had no significant effect on extracellular levels of 5-HT. A constant infusion of sumatriptan (10^{-7} M) reduced extracellular levels of 5-HT by 80 $\pm$ 2% (basal levels = 21 $\pm$ 4 fmoles/20 μl). Neither peripheral injection of sumatriptan had any significant effect on extracellular 5-HT in the frontal cortex.

These results support the findings of Middlemiss et al. (1988) in that when sumatriptan was infused into the nerve terminal regions *in vivo*, extracellular levels of 5-HT were reduced. Neither the lowest peripheral dose, equivalent to the human therapeutic dose for the treatment of migraine (Doenicke et al., 1988), nor a dose 10-fold greater had any effect on extracellular levels of 5-HT suggesting that the drug penetrates the brain poorly and that the acute antimigraine effects are mediated by peripheral mechanisms (Saito et al., 1988).

We thank the NIH and the Stanley Foundation for financial support.

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26P 5-HT ATTENUATES GLUTAMATE-EVOKED RESPONSES OF RAT ENTORHINAL CORTICAL NEURONES *IN VITRO*

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Layer II/III cells of the entorhinal cortex (EC) provide one of the major inputs to the hippocampus via the perforant path (Steward & Scoville, 1976). In view of the fact that this cortical area has a high density of binding of 5-hydroxytryptamine (5-HT) receptor ligands (Pazos & Palacios, 1985; Kilpatrick *et al.*, 1987) and that numerous 5-HT immunoreactive fibres are found in the EC (Steinbusch, 1982), it was thought likely that these cells might respond either directly to 5-HT, or that 5-HT might have some modulatory effect on excitatory amino acid responses, an action recently demonstrated (Nedergaard *et al.*, 1987).

400um thick slices of EC were taken from the brains of young adult male Wistar rats and maintained at 34-35°C at the interface between artificial cerebrospinal fluid (NaCl 124mM, KCl 3.3mM, KH_2PO_4 1.2mM, $NaHCO_3$ 25.5mM, $MgSO_4$ 1.0mM, $CaCl_2$ 2.4mM and D-glucose 10mM) and warmed humidified 95%O_2 /5%CO_2. Intracellular recordings were made in layers II/III with 3M K-Acetate-filled glass microelectrodes (40-80 MOhm). Drugs were applied ionophoretically from a separate 5-barrelled pipette placed close to the recording site. Drug solutions were 5-HT bimaleate (100mM, pH 4.0) and Monosodium glutamate (200mM, pH 9.0).

5-HT, even when applied with high currents (70-150nA) for long periods, evoked no measurable changes in passive membrane properties in the majority of cells studied (13/15), although 2/15 did respond with a very slight hyperpolarization (<2mV). When short (1-2s) regular (every 30s) pulses (50-170nA) of glutamate were applied to cells and 5-HT (50-150nA) was ejected in addition, the responses to glutamate were diminished in amplitude, often by up to 70%. Full recovery was generally seen 2-3 cycles after 5-HT application ceased.

These preliminary findings are in contrast to some earlier work in neocortex and thalamus (Nedergaard *et al.*, 1987; Eaton & Salt, 1989) where facilitation of glutamate responses by 5-HT was predominantly seen, but are reminiscent of the modulatory action of 5-HT reported in the cerebellum (Lee *et al.*, 1986). Further studies are therefore needed to characterize fully this modulatory action.

A.R.S. is a Glaxo Research Scholar.

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27P THE EFFECT OF GEPIRONE, IPSAPIRONE AND BUSPIRONE ON LOCAL CEREBRAL GLUCOSE UTILISATION IN THE RAT

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Recently the potent and selective $5HT_{1A}$ agonist, 8-OH-DPAT, has been shown to reduce glucose utilization in the rat hippocampus *in vivo* as measured by the 2-deoxyglucose quantitative autoradiography technique (Kelly et al., 1988; Grasby et al.,1989). The novel anxiolytic pyrimidinyl-piperazines, gepirone, ipsapirone and buspirone, also bind with high affinity to central $5HT_{1A}$ receptors in the rat brain (Traber & Glaser,1987). In this study we have examined whether these pyrimidinyl-piperazines have a similar effect on regional cerebral glucose utilization *in vivo* as compared to 8-OH-DPAT. Groups of male Sprague Dawley rats (250g-350g) received subcutaneously, gepirone, ipsapirone, buspirone, or saline vehicle 5 min before the i.v. injection of 40 μCi of [^{14}C]-2-deoxyglucose and 45 min later rats were sacrificed and autoradiographs prepared. For full experimental details see Kelly et al., 1988. As previously reported with 8-OH-DPAT, gepirone, ipsapirone and buspirone each reduced glucose utilization in the hippocampus and dentate gyrus (Table 1); areas with a high density of $5HT_{1A}$ binding sites. However unlike 8-OH-DPAT, these drugs caused a marked increase in glucose utilization (38-65%) in the lateral habenulae. Interestingly this latter effect is also seen with dopamine receptor antagonists (McCulloch et al., 1982). Overall, most brain regions examined did not show significant changes in glucose utilization, including many areas with a high density of $5HT_{1A}$ receptors or dense serotoninergic innervation, e.g. entorhinal cortex and globus pallidus, respectively.

TABLE 1 Effect of GEPIRONE, IPSAPIRONE and BUSPIRONE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT

Structure	Saline n=11	Gepirone n=5 (5mg/kg)	Gepirone n=4 (10mg/kg)	Ipsapirone n=5 (10mg/kg)	Buspirone n=5 (10mg/kg)
Entorhinal cortex	61 ± 2	59 ± 3	60 ± 2	56 ± 2	57 ± 3
Hippocampus	56 ± 2	49 ± 2	42 ± 2*	44 ± 1*	44 ± 1 *
Dentate Gyrus	62 ± 2	50 ± 3*	45 ± 2 *	49 ± 1*	47 ± 1 *
Globus Pallidus	45 ± 1	47 ± 4	50 ± 5	47 ± 2	49 ± 1
Lateral Habenulae	101 ± 5	149 ± 4 *	167 ±14 *	139 ± 10 *	166 ± 7*

(Mean ± s.e.mean , values in μmol/100g/min) *P<0.05 vs saline ; ANOVA with Scheffe correction.
The data suggest that a common effect of $5HT_{1A}$ receptor active drugs is to reduce glucose utilization in the hippocampus *in vivo* . This finding emphasizes the importance of the hippocampus as a site of action for $5HT_{1A}$ receptor active drugs .

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28P 8-OH-DPAT ACTS AT D_2 RECEPTORS TO INHIBIT FIRING RATE OF SUBSTANTIA NIGRA ZONA COMPACTA CELLS MAINTAINED *IN VITRO*

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It has recently been demonstrated that the $5HT_{1A}$ agonist 8-OH-DPAT stimulates dopamine D_2 receptors located presynaptically in mouse vas deferens (Smith and Cutts, 1989). We have investigated the actions of 8-OH-DPAT on central D_2 receptors located somatodentritically in the zona compacta of the substantia nigra in vitro. Brain slices 350μm thick containing the substantia nigra nuclei were prepared from Wistar-derived rats. Slices were maintained at 37°C and superfused at a rate of 0.8 ml/min. Single cell spontaneous activity was recorded using conventional extracellular techniques and the firing rate displayed on a chart recorder. All cells in this study were dopaminergic according to the criteria described by Grace and Bunney (1983). Results are n = 3 (± SEM). Dopamine (3-30μM) caused concentration-related decreases in firing rate in a manner similar to that shown by Pinnock (1983). The selective D_2 receptor agonist quinpirole (0.01-0.3μM) also inhibited cell firing. Concentration-response curves to quinpirole were shifted to the right in a parallel manner by the D_2 antagonist, sulpiride, with a pA_2 value of 7.46, and a slope not significantly different from unity. The D_1 receptor antagonist Sch23390 (1μM) had no effect.

In the presence of antagonists designed to block α_1, α_2 and D_1 receptors, 8-OH-DPAT caused concentration-related decreases in firing rate with half-maximal response occurring at 5μM. However, a maximum of only 40-50% inhibition was achieved, compared to 100% for quinpirole or dopamine. Methysergide (3μM) only weakly antagonized the response to 8-OH-DPAT giving a pK_B value of 5.87 ± 0.04. In the same 3 cells, sulpiride caused a further shift of the 8-OH-DPAT concentration-response curve, giving a pK_B value of 7.61 ± 0.06. In a separate series of experiments, sulpiride on its own antagonised 8-OH-DPAT with a mean pK_B value of 7.57.

These results show that 8-OH-DPAT can act as a partial agonist at dopamine D_2 receptors in the CNS. Sulpiride antagonised this effect with the same potency with which it antagonised the selective D_2 agonist quinpirole; methysergide weakly antagonized it with a potency approximately equal to its potency at dopamine receptors (Leysen et al., 1981). The concentration of 8-OH-DPAT necessary to activate D_2 receptors was only 10-20 times its EC_{50} at $5HT_{1A}$ receptors in septal neurones (Joëls et al., 1987) and it is thus possible that some of its behavioural effects are a result of this additional dopaminergic action.

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29P COMPARISON OF THE EFFECTS OF BUSPIRONE ANALOGUES AND 1-(2-PYRIMIDINYL)-PIPERAZINE ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS *IN VIVO*

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Buspirone, when given systemically, produces an inhibition of synaptic transmission in the hippocampus which is believed to be due to a direct action on 5-HT_{1A} receptors (O'Connor et al., 1988). It is possible that some of its activity may be due to the hepatic metabolite 1-(2-pyrimidinyl)-piperazine (1-PP, O'Connor et al., 1989). The present study compared the effects of the anxiolytics gepirone, ipsapirone and the putative 5-HT_{1A} receptor antagonist BMY 7378 (Yocca et al., 1988) with those of 1-PP on electrically evoked excitatory postsynaptic potentials (EPSP) in the hippocampus of alert rats.

Male Wistar rats (200-250g) had stimulating wire electrodes and a cannula to which two recording wire electrodes were attached, implanted in the dorsal hippocampus under pentobarbitone anaesthesia (60 mg/kg, i.p.). Animals were allowed at least 1 week for recovery before recordings were taken in a restraining hammock. Stimulation and recording was carried out in the stratum radiatum of the CA1 region. Drugs were applied either directly into the hippocampus via the cannula or systemically via the i.p. route.

Direct intrahippocampal injection of 1 µg gepirone or ipsapirone (in 0.05 µl water) produced a transient inhibition of the EPSP amplitude (30±7% inhibition±s.e.mean, n=3 and 29±10%, n=3, respectively). This was similar to that produced by 1 µg buspirone (43±8%, n=6). BMY 7378 also had an inhibitory effect on the EPSP but appeared more potent (36±8%, n=4 at 0.1 µg).When BMY 7378 was injected systemically (0.01 mg/kg) the EPSP was reduced maximally (21±9%, n=4) at 30 min and full recovery was observed within 90 min.1-PP (1mg/kg) had similar effects (32±4%, n=4) but the response was complete within approximately 60 min.

It would appear that 1-PP is an active metabolite of the buspirone-like anxiolytics in the hippocampus and may contribute to their effects when given systemically . Whether or not the activity of BMY 7378 in vivo is partly due to metabolites remains to be determined but the present results are consistent with the view that it has agonist activity on its own.

This research was supported by the Health Research Board of Ireland.

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30P CARDIORESPIRATORY EFFECTS OF MICROINJECTIONS OF 5-HT_{1A} AGONISTS INTO THE RAPHÉ OBSCURUS OF THE ANAESTHETISED RAT

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The 5-HT_{1A} agonists 8-OH-DPAT and flesinoxan have been shown to cause a fall in blood pressure (BP) by a central mechanism (Ramage & Fozard, 1987; Ramage et al., 1988). However the central site/sites at which these agonists cause this effect is unknown. It is known that central 5-HT pathways project from the raphe pallidus and raphe obscurus (RO) to preganglionic sympathetic motoneurones in the intermediolateral cell column (IML) of the rat (Loewy, 1981). Hence it was decided to examine the effects of microinjection of 5-HT_{1A} agonists into the RO on BP and heart rate (HR). Phrenic nerve activity (PNA) was also recorded as chemical (glutamate) stimulation of this nucleus in the cat causes an increase in PNA (Holtman et al, 1986).

In male Sprague-Dawley rats (250-375g) anaesthesia was induced with halothane and maintained with α-chloralose (70 mg kg^{-1}; i.v.). They were artificially ventilated following neuromuscular blockade with decamethonium iodide (1 mg per animal). Recordings were made of BP, HR and PNA. Microinjections (Sporton et al, 1989) (90 nl) of L-glutamate (2.7 nmol), 8-OH-DPAT (0.7 nmol), flesinoxan (1.3 nmol) or saline were made into the RO. One barrel was filled with Wood's metal for electrical stimulation (40Hz, 100 µA, 0.5 ms, 5s). The electrode was positioned 1-1.5 mm rostral to obex along the midline and advanced 1.5-1.75 mm down from brain surface. Injection sites were marked with pontamine sky blue for later histological verification. Electrical stimulation (n=7) of the RO caused an instantaneous increase in BP of 30 ± 6 mmHg (mean ± s.e.mean) and a bradycardia of 37 ± 9 beats min^{-1} with variable effects on PNA. Microinjections of glutamate also caused increases in BP (22 ± 6 mmHg), however had little effect on HR while PNA was increased by 53 ± 10%. Flesinoxan (n=9) or 8-OH-DPAT (n=7) also caused an instantaneous increase in BP of 9 ± 1 and 14 ± 2 mmHg respectively, lasting 5-10 min (cf. glutamate 1-2 min). However, HR and PNA were not affected. Microinjection of methiothepin (5.2 nmol, n=5) was found to cause a reversible attenuation of pressure action caused by flesinoxan.

The above observations show that activation of neurones within the RO by glutamate or 5-HT_{1A} agonists causes a rise in BP.

This work was carried out while I was on sabbatical leave from Duphar B.V., Weesp, The Netherlands.

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31P EVIDENCE THAT PROLACTIN AND ACTH RESPONSES TO 5-HTP IN THE RAT ARE MEDIATED BY 5-HT_2 RECEPTORS

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There is extensive evidence that 5-HT plays a facilitatory role in the control of prolactin (PRL) and ACTH secretion. The 5-HT precursor 5-HTP has been reported to cause increases in PRL and ACTH secretion in the rat (Meltzer et al., 1982). Recent studies using direct receptor agonists, indicate that stimulation of both the 5-HT_{1A} and 5-HT_2 subtypes may result in facilitation of ACTH (Koenig et al., 1987) and PRL (Simonovic et al., 1984; Preziosi et al., 1989) release. The present study was undertaken to determine the particular 5-HT receptor subtype(s) mediating the PRL and ACTH responses to 5-HTP administration in the rat. We report the effect of a number of 5-HT receptor antagonists on the PRL and ACTH responses to 5-HTP.

Adult male Sprague-Dawley rats were injected i.p. with the antagonist or its vehicle 30 min prior to administration of 5-HTP (100mg/kg). Animals were decapitated 45 min after administration of 5-HTP, and trunk blood was collected into Li-heparin tubes. Plasma PRL and ACTH were measured by RIA and IRMA respectively.

Table 1 Effects of 5-HT receptor antagonists on the PRL and ACTH responses to 5-HTP

	PRL (ng/ml)	ACTH (pg/ml)
Vehicle	131 ± 11.2 (12)	1142 ± 96.9 (13)
Metergoline (0.5mg/kg)	50 ± 9.0 (7)**	661 ± 125.4 (7)**
Ritanserin (0.4mg/kg)	30 ± 18.8 (6)**	407 ± 179.4 (6)*
Ketanserin (2.5mg/kg)	15 ± 2.9 (6)**	211 ± 32.1 (6)**
ICI 170,809 (5.0mg/kg)	28 ± 15.0 (6)**	168 ± 86.8 (6)**
Spiperone (1.0mg/kg)	72 ± 25.3 (5)*	484 ± 174.5 (5)*
(±)Pindolol (4.0mg/kg)	146 ± 25.0 (6)	1664 ± 121.6 (6)

Data are mean ± s.e.mean (N) *p<0.05; **p<0.01 vs vehicle pre-treated control (Student's t-test)

The PRL and ACTH responses to 5-HTP were significantly attenuated by pre-treatment with the 5-HT antagonists, metergoline, ritanserin, ketanserin, ICI 170,809, and spiperone, all of which have high affinity for the 5-HT_2 receptor subtype. The selective 5-HT_{1A}/5-HT_{1B} receptor antagonist pindolol failed to antagonize both the PRL and ACTH responses to 5-HTP (Table 1). The results obtained strongly suggest that the PRL and ACTH responses to 5-HTP administration in the rat are mediated by 5-HT_2 receptors.

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32P M-CHLORO-PHENYLBIGUANIDE IS A POTENT HIGH AFFINITY 5-HT_3 RECEPTOR AGONIST

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Several potent and highly selective 5-HT_3 receptor antagonists have been identified, these include MDL 72222, ICS 205-930 and ondansetron (GR38032). However, there are no very potent and selective 5-HT_3 receptor agonists. The agonists phenylbiguanide (PBG) and 2-methyl-5-HT are thought to have some selectivity for 5-HT_3 receptors (Richardson et al., 1985, Wallis & Nash, 1981, Ireland & Tyers, 1987) but both of these compounds are less potent than 5-HT. We now report on a potent 5-HT_3 receptor agonist with a high affinity for 5-HT_3 receptors, m-chloro-phenylbiguanide (mCPBG)

mCPBG was compared with 5-HT, PBG and 2-methyl-5-HT for ability to inhibit [^{3}H]GR67330 binding to rat entorhinal cortex homogenates, depolarize the rat isolated vagus nerve (RVN) and evoke the Bezold-Jarisch reflex in the anaesthetised cat. Experiments were performed exactly as described previously (Kilpatrick et al., 1987; Ireland & Tyers, 1987, Butler et al, 1988 respectively). The results are presented in Table 1. mCPBG potently inhibited [^{3}H]GR67330 binding to 5-HT_3 receptors with an affinity two orders of magnitude higher than the other agonists tested; the Hill number, as with other 5-HT_3 agonists, was greater than unity. mCPBG depolarized the RVN with a pD_2 one order of magnitude higher than 5-HT although the maximum response was only 51% of that to 5-HT. GR38032 ($1x10^{-7}$M) antagonised the depolarization evoked by mCPBG with a pK_B of 8.6±0.1 (mean±S.E., n=7). mCPBG potently evoked the Bezold-Jarisch reflex in the anaesthetised cat with an i.v. ED_{50} approximately one third of that to 2-methyl-5-HT. mCPBG ($1x10^{-5}$M) had no effect on preparations containing 5-HT_1-like (dog saphenous vein), 5-HT_{1A} (^{3}H-8-OH DPAT binding) and 5-HT_2 (rat aorta) receptors.

Table 1 The activities of mCPBG and other 5-HT_3 agonists in three 5-HT_3 receptor assays

	5-HT	2-methyl-5-HT	PBG	mCPBG
[^{3}H]GR67330 binding pKi (Hill number)	7.4 (1.51)	7.2 (1.71)	7.4 (1.64)	9.5 (1.33)
RVN pD_2 (% of 5-HT maximum)	6.2 (100)	5.5 (58)	6.0 (83)	7.3 (51)
Cat Bezold Jarisch reflex ED_{50} (nmol/kg)	-	36	102	13

Thus it appears that mCPBG is a potent, high affinity agonist at 5-HT_3 receptors both in vitro and in vivo, the efficacy of this agonist is similar to 2-methyl-5-HT. It is likely that mCPBG will be a useful pharmacological tool for studying 5-HT_3 receptors.

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33P MOLECULAR TARGET SIZE OF 5-HT_3 RECEPTORS IN N1E115 NEUROBLASTOMA CELLS AND BRAIN DIFFER BY A FACTOR OF TWO

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Activation of 5-HT_3 receptors causes rapid depolarization in both certain neuronal cell lines and vertebrate neurones, suggesting they are members of the superfamily of ligand-gated ion channels (Peters and Lambert,1989; Derkach et al.,1989). The best characterized receptors in this family, those for acetylcholine, GABA and glycine, are multimeric proteins, with subunit molecular weights of between 45,000 and 60,000. Here we compare the molecular target size of 5-HT_3 receptors in N1E115 cells and mammalian brain.

The molecular target size of multimeric receptors can vary depending on experimental conditions, but irradiation of whole frozen tissue yields the best estimate of the smallest functional molecular mass (Nielsen and Braestrup,1988). Thus N1E115 cells, grown to confluency in Dulbeccos modified Eagles medium supplemented with 10% foetal calf serum and 3mM glutamine, were gently scrapped into physiological buffer and then frozen. Samples of rat cortex were also frozen without any prior treatment. Enzymes of known molecular weight were added to cells and cortex prior to freezing. Frozen samples were irradiated at -15°C with the 10MeV linear accelerator at Riso, Denmark. 5-HT_3 receptors were assayed as previously described using [^{3}H]GR65630, with 30μM metoclopramide to define non specific binding (Kilpatrick et al.,1987).

[^{3}H]GR65630 binding sites in confluent cultures of N1E115 cells have an affinity (K_d =0.69 nM) and pharmacological characteristics similar to those in brain. Specific [^{3}H]GR65630 binding in N1E115 cells decreased monoexponentially with increasing radiation dose. The target size determined from the enzyme calibration curve was 98,620 ± 11,310 (mean ± SE, n=6). Similar experiments in cortex samples revealed a target size of 49,150 ± 8,530 (mean ± SE, n=5). Thus we conclude the 5-HT_3 receptor in brain has subunits of approximate molecular weight 50,000, whereas the smallest functional unit of 5-HT_3 receptors in N1E115 cells is twice this size.

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34P STEREOSELECTIVE EFFECT OF 5-HT_{1A} LIGAND, MDL 72832, ON NON-OPIOID DEFEAT ANALGESIA IN MICE

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Non-opioid defeat analgesia in male mice is blocked by 5-HT_{1A} receptor agonists, such as 8-OH-DPAT, buspirone, gepirone and ipsapirone (Rodgers & Shepherd, 1989). The present study examined the effects of MDL 72832, a potent, selective and stereospecific ligand for the 5-HT_{1A} receptor (Fozard et al.,1987) on basal nociception and defeat analgesia. Group-housed adult male DBA/2 mice (Bantin & Kingman, Hull) were maintained under a reversed LD cycle in a temperature-controlled room (24±1°C). Testing was conducted under dim red light during the dark phase of the cycle. Tail-flick latencies (temperature controlled to give 2-3 sec baselines) were determined both before and after defeat. For defeat experience, intruder mice were placed into the home cage of an aggressive resident conspecific and removed immediately upon display of species-typical defeat behaviour.(+) and (-)MDL 72832 were ultrasonically dispersed in a distilled water/Tween 80 mixture (2drops/10ml) and administered i.p. 30 min before testing. Data were analysed by ANOVA and appropriate follow-up tests.

TABLE 1 Effects of (-) and (+) MDL 72832 on defeat analgesia in male mice. Data are presented as Mean ±(SE) tail-flick latencies (sec). Pre=baseline, Post=post-defeat. *p<0.001 vs baseline; + p<0.05, ++p<0.01, +++ p<0.005 vs vehicle.

		(-) MDL 72832			(+) MDL 72832					
	VEH	0.01	0.10	0.50	0.01	0.10	0.50	1.0	3.0	5.0 mg/kg
Pre	1.91(0.1)	2.29(0.2)	1.96(0.2)	2.02(0.2)	2.35(0.1)	2.01(0.2)	2.00(0.2)	1.88(0.2)	1.86(0.1)	1.95(0.1)
Post	4.20(0.4)	4.17(0.6)	3.03(0.5)	2.48(0.1)	4.24(0.6)	3.47(0.4)	3.39(0.6)	3.45(0.3)	2.69(0.2)	3.13(0.3)
	*	*	* +	++	*	*	*	*	* +++	* +

0.01-0.5 mg/kg (-)MDL 72832 and 0.01-5.0 mg/kg (+)MDL 72832 were without significant intrinsic activity on the tail-flick assay. In defeat studies (see Table 1), the strong analgesic response of vehicle-treated mice (p<0.001) was partially and totally inhibited by 0.1 mg/kg and 0.5 mg/kg (-)MDL 72832,respectively (higher doses were not assessed due to significant locomotor impairment). A comparable partial inhibition was produced only with much higher doses (3.0-5.0 mg/kg) of the positive isomer, (+)MDL 72832. These data are consistent with previous reports on the stereospecificity of the compound, with an approximately 30-fold difference in activity between the two isomers, at the 5-HT_{1A} receptor (Fozard et al.,1987). Present findings add further support to the involvement of 5-HT_{1A} receptors in the mediation of defeat analgesia.

This work was supported by a Yorkshire Regional Health Authority grant. We thank Merrell Dow, Strasbourg for their kind gift of MDL 72832.

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35P BINDING CHARACTERISTICS OF 8-OH-DPAT ANALOGUES SUITABLE FOR IODINATION

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5HT has been shown to be important in a variety of major psychiatric disorders (Goodwin and Post, 1983). The observation that binding sites for 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) are reduced post mortem by 50% in Alzheimer-type dementia (Middlemiss et al, 1986) also led us to look at ligands for $5HT_{1A}$ receptors as putative SPET (single photon emission tomography) ligands.

We now report on a number of compounds structurally related to 8-OH-DPAT, a potent $5HT_{1A}$ agonist (Middlemiss and Fozard, 1983) with and without the incorporation of an iodine atom. Since for SPET studies it will be important to have a ligand with high affinity (K_D ~ 10^{-9}M) and selectivity we have so far tested these compounds on radioligand binding assays and determined K_D values at the $5HT_{1A}$ site and also their selectivity with respect to the other $5HT_1$ receptor subtypes (Peroutka, 1988).

Using a standard filtration assay the N,N-dipropyl-5-carboxamidotryptamine (DP-5CT, 30nM) sensitive component of [^{3}H]-5HT binding to rat cerebrocortical membranes was defined as the binding to $5HT_{1A}$ sites. The insensitive component was used as a measure of 1B and 1C subtypes. Using a similar assay with pig brain membranes, mesulergine (100nM) sensitive and insensitive components of the [^{3}H]-5HT binding were used to define the relative proportions of the 1C and 1D subtypes respectively. [^{3}H]-8-OH-DPAT (0.25nM) was used to measure $5HT_{1A}$ binding sites directly.

The methyl and propenyl ether derivatives of the 8-hydroxyl substituent showed high affinity (K_D 2.5nM) for the $5HT_{1A}$ site. Modification of one of the propyl chains to either a propenyl (K_D 3nM) or an n-propyl phenyl (K_D 15nM) also retained activity. While the ratio of selectivity towards the other $5HT_1$ sites varied, all showed at least a 1000-fold higher affinity for the $5HT_{1A}$ site compared with the other $5HT_1$ subtypes.

Iodination of 8-OH-DPAT and its methoxy derivative in the 5 position caused a reduction in potency with K_D values from 1nM to 250nM and from 2.5nM to 50nM for the 8-OH-DPAT and 8-MeO-DPAT structures respectively. The binding affinities for the other $5HT_1$ sites was unaltered. An iodopropenyl substitution either on the 8-hydroxyl group of the tetralin ring or on the nitrogen both showed K_D values of 15nM for the $5HT_{1A}$ site without loss of the receptor subtype selectivity. High affinity for $5HT_{1A}$ site was also retained following n-propyliodophenyl substitution.

Therefore it has been shown that an iodine moiety can be incorporated into the 8-OH-DPAT structure without loss of affinity or specificity.

Acknowledgement: This work was supported by a grant from the Scottish Hospital Endowments Research Trust.

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36P REDUCTION OF PORCINE CAROTID ARTERIOVENOUS SHUNTING BY INDORENATE IS UNRELATED TO 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} OR 5-HT_{1D} RECEPTOR SUBTYPE

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Intracarotid infusion of 5-hydroxytryptamine (5-HT) diverts porcine carotid artery blood flow from its arteriovenous anastomotic (non-nutrient) fraction (AVAF) towards the arteriolar (nutrient) fraction (NF) by acting mainly on two sub-populations of 5-HT_1-like receptors (Saxena et al., 1989). Since 8-OH-DPAT and RU 24969, but not ipsapirone, mimic the effects of 5-HT on AVAF and the effect of RU 24969 is not antagonized by pindolol, the 5-HT_1-like receptor mediating AVA constriction is unrelated to 5-HT_{1A} or 5-HT_{1B} receptors (Bom et al., 1989; Saxena et al., 1989). Using the radioactive microsphere technique (Saxena et al., 1989), we analyzed the carotid haemodynamic effects of indorenate, a tryptamine derivative with high affinity for 5-HT_{1A} receptors (Dompert et al., 1985), in Yorkshire pigs (17-20 kg) during pentobarbital anaesthesia.

As shown in Table 1, administration of increasing doses of indorenate in saline-treated animals caused a dose-dependent decrease in CBF which was exclusively observed in the AVAF. The decrease in the AVAF was only slightly attenuated after pretreatment with ketanserin (0.5 mg kg^{-1}, i.a.); in contrast, the animals pretreated with methiothepin (1 mg kg^{-1}, i.a.) showed little response to indorenate. However, pretreatment with metergoline (1 mg kg^{-1}, i.a.) did not reduce the indorenate-induced changes in the carotid haemodynamics.

Table 1. Carotid vascular effects of indorenate in pigs.

Pretreatment		Baseline	Indorenate (μg kg^{-1} min^{-1}) 0.3	1.0	3.0	10.0
Saline	CBF	129 ±14	131 ±16	116 ±12	94 ±12*	63 ±12*
(n=6)	AVAF	99 ±12	103 ±16	87 ±16	63 ±12*	37 ± 8*
	NF	27 ± 3	25 ± 5	27 ± 4	31 ± 7	32 ± 8
Ketanserin	CBF	137 ± 8	139 ±10	131 ±10	118 ±12*	98 ±10*
(n=6)	AVAF	109 ±12	114 ±12	111 ±12	94 ±10*	67 ± 8*
	NF	25 ± 4	23 ± 2*	23 ± 2*	24 ± 2	29 ± 4*
Methiothepin	CBF	98 ±14	100 ±16	100 ±15	100 ±13	92 ±12*
(n=6)	AVAF	74 ±15	83 ±14*	80 ±15*	79 ±16*	75 ±14
	NF	27 ± 6	19 ± 2*	20 ± 1*	20 ± 1*	20 ± 1*
Metergoline	CBF	162 ±20	137 ±10*	132 ±15*	109 ±19*	87 ±19*
(n=6)	AVAF	93 ± 9	86 ± 8	80 ±11	59 ± 9*	42 ± 8*
	NF	68 ±15	51 ± 8*	52 ±10*	50 ±13*	45 ±12*

Data in ml min^{-1}; means ± s.e. mean; *, $p < 0.05$ vs. baseline.

We conclude that the reduction in carotid AVAF by indorenate is mainly mediated by 5-HT_1-like receptors which, however, do not seem to correlate with either the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} recognition sites identified from ligand binding studies, for which metergoline displays high affinity (Hoyer, 1988).

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Sumatriptan (GR43175), a selective agonist at the 5-HT_1-like receptor mediating contraction of the dog saphenous vein and the dog, primate and human basilar artery, is effective in the treatment of acute migraine attacks (see Humphrey et al., 1989; Saxena & Ferrari, 1989). Since 5-HT_1-like receptors also mediate the constriction of arteriovenous anastomoses (AVAs) as well as the dilatation of arterioles in the pig carotid circulation (Saxena et al., 1989), the present experiments were devoted to the study of the craniovascular effects of sumatriptan (10, 30, 100 and 300 $\mu g\ kg^{-1}$ i.v.) in pentobarbital-anaesthetized pigs (16-19 kg) using the radioactive microsphere technique (Saxena et al., 1989).

Sumatriptan decreased dose dependently total carotid blood flow by 39±9% and its AVA fraction by 66±10% at the highest dose used (Table 1). The decrease in the AVA fraction resulted in a rise in the difference in oxygen saturation between the arterial and jugular venous blood by 67±22% (not shown). Sumatriptan increased the over-all nutrient blood flow by 36±18%; this increase was noticed in the ears, head skin, tongue, fat and bones. The fraction of carotid blood flow delivered to the brain, eyes, muscles and salivary glands was not affected. Sumatriptan had no influence on blood pressure, but a small decrease in heart rate was observed during the experiments.

TABLE 1. Carotid and systemic haemodynamic variables (means± s.e.mean) in 8 pigs at baseline and 15 min after different i.v. doses of sumatrptan ($\mu g\ kg^{-1}$).

	Baseline	10	30	100	300
Carotid BF (ml min^{-1})	173 ±27	159 ±23	135 ± 25*	122 ±25*	97 ±21*
AVA BF (ml min^{-1})	136 ±26	119 ±22	97 ± 23*	72 ±21*	46 ±17*
Nutrient BF (ml min^{-1})	37 ±4	39 ±4	37 ± 4	49 ±7*	51 ±9*
Ear BF (ml min^{-1})	1.2 ±0.2	1.6± 0.3	1.8 ± 0.5	2.9 ±0.8*	3.1 ±1.0*
Skin BF (ml min^{-1})	1.2 ±0.2	1.6± 0.3	1.8 ± 0.4	2.8 ±0.8*	2.7 ±0.6*
Tongue BF (ml min^{-1})	1.5 ±0.2	1.9± 0.3	1.9 ± 0.3	2.4 ±0.4*	2.6 ±0.4*
Bone BF (ml min^{-1})	11 ±2	13± 2	13 ± 2	17 ±3*	18 ±4*
Fat BF (ml min^{-1})	0.7 ±0.2	0.7± 0.1	0.8 ± 0.1	1.1 ±0.2*	1.4 ±0.3*
MAP (mmHg)	85 ±4	86± 4	85 ± 4	83 ±6	76 ±8
Heart rate (beats min^{-1})	93 ±2	90± 2*	88 ± 2*	85 ±3*	82 ±3*

BF, Blood flow; MAP, Mean arterial blood pressure; *, $P<0.05$ vs baseline.

We conclude that the 5-HT_1-like agonist sumatriptan constricts cranial AVAs in the pig as has been reported in the cat (Perren et al., 1989). However, unlike in the cat, an increase in blood flow was noticed in some tissues.The constriction of arteriovenous anastomoses, also observed with several other anti-migraine drugs (Saxena et al., 1989), could be a factor in the relief of symptoms during acute attacks.

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38P EVIDENCE THAT THE CARDIOVASCULAR AND THE HYPOINSULINAEMIC EFFECT OF 8-OH-DPAT ARE MEDIATED THROUGH DIFFERENT MECHANISMS

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8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) reduces blood pressure (BP) and heart rate (HR) in the rat as a consequence of central 5-HT_{1A} receptor activation (Fozard et al., 1987) resulting in sympatho-inhibition and an increase in vagal tone. Since such autonomic changes would normally lead to hyperinsulinaemia and hypoglycaemia, it is therefore surprising that 8-OH-DPAT also inhibits plasma insulin levels and increases basal glycaemia (Chaouloff and Jeanrenaud, 1987; Bouhelal et al., 1989). Since 8-OH-DPAT increases both plasma corticosterone and adrenaline concentrations (Koenig et al., 1987; Bagdy et al., 1989), we have investigated the role of the adrenal gland in the metabolic vis-à-vis the cardiovascular effects of 8-OH-DPAT.

Adrenalectomized and sham-operated Sprague-Dawley rats (IFFA-CREDO, France) were implanted with catheters in the femoral artery and vein. Arterial BP and HR were recorded continuously from conscious animals as described previously (Fozard et al., 1987). Effects of i.v. injection of 8-OH-DPAT (150 µg/kg) or vehicle on basal and glucose-stimulated plasma glucose, insulin and corticosterone concentrations and BP and HR were determined simultaneously. Plasma insulin and corticosterone were determined using a radioimmunoassay.

Intravenous administration of 8-OH-DPAT (150 µg/kg) into conscious sham-operated animals caused significant increases (53 ± 6 %, n = 5) in basal glycaemia without any change in basal insulinaemia. These changes were associated with significant and sustained falls in BP (- 16 ± 2 mmHg, n = 5) and HR (- 94 ± 12 beats/min., n = 5) preceded by transient (< 10 min) increases only in BP (24 ± mmHg, n = 5). Glucose-stimulated plasma insulin levels were markedly inhibited (- 51 ± 7 %, n = 6) in 8-OH-DPAT-treated sham animals, without modification of the glucose tolerance. In adrenalectomized animals, 8-OH-DPAT-mediated initial vasoconstriction, hyperglycaemia and inhibition of plasma insulin were abolished. However, in these animals, 8-OH-DPAT produced falls in BP (- 18 ± 3 mmHg, n = 5) and HR (- 92 ± 12 mmHg, n = 5) that were comparable to those observed in sham animals. Plasma corticosterone was increased at 15 min in sham animals (+ 48 ± 10 %, n = 4) whereas it was undetectable in adrenalectomized animals.

The present data indicate that the 8-OH-DPAT mediated decrease in BP and HR on the one hand and initial increase in BP and its effects on the metabolic parameters on the other are produced through different mechanisms. The latter effects are clearly dependent on intact adrenals and may thus be a consequence of the release of adrenaline and/or corticosterone.

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39P ADRENALINE-RELEASING EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONISTS 8-OH-DPAT, BUSIPIRONE AND IPSAPIRONE IN THE CONSCIOUS RAT

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8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) causes hyperglycaemia and inhibits insulin release in conscious rats (Chaouloff and Jeanrenaud, 1987). The alpha 2-adrenoceptor antagonist idazoxan prevents the hyperglycaemia (Chaouloff and Jeanrenaud, 1987), thus suggesting catecholaminergic mediation. We have now further defined these relationships.

Male Wistar rats (300-350g) were anaesthetised with methohexital (40 mg/kg i.p.) and implanted with an indwelling heart catheter at least 6h before study. Plasma glucose and adrenaline (A) were respectively measured by a glucose analyzer and by HPLC-ECD after adsorption onto Al_2O_3 (Anton and Sayre, 1962).

Pretreatment with alpha-methyl-p-tyrosine (a catecholamine synthesis inhibitor) at a dose that decreased kidney noradrenaline (NA) but not adrenal A and NA (300 mg/kg i.p., 3h and 6h beforehand) did not affect 8-OH-DPAT-induced hyperglycaemia but it was decreased by the ganglionic blocker hexamethonium (15 mg/kg i.v. 15 min beforehand) and prevented by adrenalectomy. These results suggested that 8-OH-DPAT (0.3 mg/kg i.v.) caused hyperglycaemia by adrenal catecholamine release. In agreement, 8-OH-DPAT and two other 5-HT_{1A} receptor agonists (buspirone and ipsapirone) increased plasma A (Table 1).

Table 1. Effects of 8-OH-DPAT, buspirone and ipsapirone on plasma A

	Dose (mg/kg i.v.)	Plasma A (nM) 0 min	5 min	10 min	20 min
Saline		2.02 ± 0.27	1.84 ± 0.16	1.81 ± 0.15	1.52 ± 0.16
8-OH-DPAT	0.1	1.72 ± 0.17	5.21 ± 0.32 *	3.11 ± 0.12 *	2.51 ± 0.19
	0.3	1.92 ± 0.23	5.90 ± 1.02 *	4.77 ± 0.47 *	3.52 ± 0.28 *
	1.0	1.63 ± 0.17	10.62 ± 1.94 *	6.88 ± 1.08 *	5.03 ± 0.55 *
Buspirone	1.0	1.52 ± 0.11	2.68 ± 0.19 *	2.24 ± 0.27	2.08 ± 0.13
	3.0	1.43 ± 0.24	4.02 ± 0.91 *	3.93 ± 0.47 *	3.91 ± 0.95 *
	10.0	1.52 ± 0.31	5.97 ± 0.89 *	2.72 ± 0.49	4.28 ± 0.90 *
Ipsapirone	1.0	1.91 ± 0.31	3.52 ± 0.44 *	3.71 ± 1.00	2.10 ± 0.21
	3.0	1.54 ± 0.16	4.69 ± 1.57 *	3.32 ± 0.26	2.86 ± 0.52
	10.0	1.49 ± 0.47	5.58 ± 1.25 *	7.42 ± 1.32 *	9.65 ± 1.94 *

Values are means ± s.e.m. of 5-7 animals. * $P < 0.05$ (ANOVA followed by Newman-Keuls Q-test).

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40P PHARMACOLOGICAL CHARACTERIZATION OF THE ALDOSTERONE RESPONSE TO SEROTONIN IN RAT ZONA GLOMERULOSA CELLS

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The steroidogenic effects of serotonin (5HT) in adrenal zona glomerulosa (ZG) cells have previously been attributed to activation of $5HT_2$ receptors (Matsouka et al., 1985) based on the inhibitory effects of ketanserin. In this in vitro study pK_D values for a range of selective 5HT antagonists were estimated from their competitive inhibition of 5HT-induced aldosterone (aldo) and cyclic AMP (cAMP) production in a 90-95% pure preparation of rat ZG cells. Rat ZG cells obtained by collagenase digestion of adrenal capsular tissue were incubated at 37°C for one hour in medium 199 (0.5% BSA, 0.2% glucose) with increasing concentrations of 5HT (5×10^{-9} - 4×10^{-8}M) alone and in the presence of an antagonist. Aldo and cAMP outputs were measured by radioimmunoassay. Utilization of the Schild equation yielded pK_D values for the antagonists (Table 1) which correlated more closely with the $5HT_{1c}$ recognition site ($r = 0.93$ (aldo) and 0.80 (cAMP) than the $5HT_2$ site ($r = 0.63$ (aldo) and <0.5 (cAMP)) (data for comparison taken from Hoyer, 1988). We conclude that the aldosterone response to 5HT in rat zona glomerulosa cells is mediated by "$5HT_{1c}$-like" receptors which are coupled to adenylate cyclase in contrast to other tissues where $5HT_{1c}$ sites couple to phospholipase C.

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TABLE 1

ANTAGONIST	$5HT_{1a}$	$5HT_{1b}$	$5HT_{1c}$	$5HT_{1d}$	$5HT_2$	ZG (Aldo)	ZG (cAMP)
CYANOPINDOLOL	8.27	8.28	4.44	6.85	4.53	5.73	
KETANSERIN	5.86	5.86	7.01	6.00	8.86	6.01	6.13
MESULERGINE	6.23	4.88	8.79	5.20	8.42	7.63	7.13
METERGOLINE	8.10	7.39	9.19	9.09	9.03	7.42	7.32
METHIOTHEPIN	7.10	7.28	7.56	6.25	8.76	7.18	7.42
METHYSERGIDE	7.63	5.82	8.61	8.42	8.57	7.56	
MIANSERIN	6.03	5.21	8.00	6.37	8.08	7.39	7.19
PITZOTIFEN	6.15	5.51	8.08	5.56	7.81	7.19	6.95
RITANSERIN	5.37	4.88	8.64		9.25	7.06	7.36
SPIPERONE	7.18	5.27	5.94	5.27	8.76	6.00	6.18
CORRELATION WITH ZG (Aldo)	0.16	0.09	0.93	0.66	0.63		

41P THE EFFECT OF BRL 43694A (GRANISETRON) ON CAPSAICIN-INDUCED COUGH IN MAN

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The neurotransmitters involved in the control of the human cough reflex are unknown. Serotonin plays an important role as an inhibitor of the sensitivity of the cough reflex in the cat (Kamei et al., 1986). The activation of $5HT_3$ receptors has a facilitatory role in the closely related vomit reflex in both animal models and man. We have investigated the effect of the specific $5HT_3$ antagonist BRL 43694A on the sensitivity of the cough reflex and induced changes in airways resistance in normal males (n = 6). We have used inhaled capsaicin as a tussive agent which also causes a reflex increase in airways resistance (Fuller et al., 1988). Volunteers attended the laboratory on 2 occasions. Cough reflex sensitivity was measured by inhalation of single breaths of capsaicin at doses 0.4-100 nmol, or saline, in random order, recording the number of coughs with a microphone. The doses of capsaicin producing two or more coughs (D_2) and 5 or more coughs (D_5) were determined. Either vehicle or BRL 43694A 160 µg/kg was given by slow intravenous infusion in a double blind, randomised manner. Cough challenge with inhaled capsaicin was repeated at intervals after the infusion. Results were expressed as the mean ± standard error log dose of capsaicin causing 2 or more and 5 or more coughs (D_2 and D_5).

Time	D_2		D_5	
(min)	Placebo	Active	Placebo	Active
Pre	0.20 ± 0.10	0.10 ± 0.19	0.95 ± 0.10	0.85± 0.12
20	0.25 ± 0.07	0.10 ± 0.17	0.99 ± 0.23	1.1 ± 0.19
90	0.05 ± 0.14	0.15 ± 0.21	1.05 ± 0.14	1.0 ± 0.18
180	0.10 ± 0.08	0.20 ± 0.24	1.00 ± 0.12	1.05± 0.22

These results show that 160 µg/kg of BRL 43694A (which has proven to be effective in inhibiting the vomit reflex in man), has no effect on cough, caused by inhaled capsaicin. This suggests that $5HT_3$ receptors do not play an important role in controlling the normal human cough reflex, however, this mechanism may be important in disease and this requires further studies.

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42P NEUTROPHIL-ENDOTHELIAL CELL INTERACTIONS IN THE ACUTE INFLAMMATORY RESPONSE: DETECTION OF NOVEL ADHESIVE LIGANDS

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Neutrophil leukocyte (PMN) adhesion to endothelial cells (EC) is induced in vitro by the effect of chemotactic agents on PMN, by more prolonged treatment of EC with cytokines or endotoxin (LPS), or by brief stimulation of EC with thrombin. Two adhesive glycoproteins, CD11/18 on PMN and ELAM1 on cytokine-treated EC, have so far been identified as important ligands (Springer et al. 1984; Bevilacqua et al. 1987). We have developed a microwell assay using fluorimetric detection of elastase to quantify human PMN adhesion to human umbilical vein EC and employed monoclonal antibodies (MAb) directed against specific cell surface molecules to determine their involvement in PMN adhesion to EC, particularly in response to thrombin.

GMP140, not present at the surface of non-stimulated EC, but expressed on thrombin-stimulated EC, has a domain structure related to ELAM1 (Johnston et al. 1989). MAb 2.17 (anti-GMP140) blocked the increase in PMN adhesion to EC pretreated for 5 min with thrombin (0.5 U/ml) and reduced adhesion stimulated by LPS (1.5ng/ml for 4h) to 51.1±2.7% (means s.e., n=15). MAb 2.28 (anti-CD63, another thrombin-induced EC ligand) reduced adhesion to thrombin-stimulated EC to 43.4±4.0% (n=4) and LPS-stimulated adhesion to 61.4±4.1% (n=15). MAb 7E8, recognising a constitutively expressed EC surface glycoprotein, had no inhibitory effect. Cell surface ELISA demonstrated that MAb 1.2B6, a non-blocking anti-ELAM1, bound as expected to LPS-treated EC, but did not bind detectably to thrombin-treated EC. EC also express as a vitronectin receptor an integrin highly homologous with the thrombin-activated platelet glycoprotein IIb/IIIa. MAb C17, directed against the β chain, like MAb 2.17 blocked the increase in PMN adhesion to thrombin-stimulated EC and reduced adhesion to LPS-treated EC to 52.2±2.9% (n=6). Unlike MAb 2.17, MAb C17 also reduced adhesion to 10µM PAF-stimulated PMN to gelatin-coated plastic, to 50.0±1.9% (n=10). As previously reported, MAb 60.3 (anti-CD18) inhibited PMN adhesion to LPS-treated EC or to gelatin, and blocked adhesion to thrombin-stimulated EC. However, PMN from a patient known to be deficient in CD11/18, which failed to adhere to gelatin after PAF stimulation, adhered as well as control PMN to thrombin or LPS treated EC. We conclude that (1) PMN adhesion to thrombin-stimulated EC involves ligands including GMP140, but not ELAM1, on the EC; (2) these ligands additionally contribute to adhesion to LPS-treated EC; (3) the EC vitronectin receptor can contribute to PMN adhesion; (4) despite the important role of CD11/18 on the PMN, other ligands can be effective in binding to EC, but not to extracellular proteins, in CD11/18 deficient patients.

We thank Drs. Beatty & Harlan (60.3), Haskard (1.2B6), Van Mourik (C17) and Metzelaar & Niewenhuis (2.17, 2.28, 7E8) for gifts of MAb.

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43P NORADRENALINE-INDUCED CA^{2+} INFLUX IN THE ISOLATED GUINEA-PIG AORTA

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It has been reported that in the guinea-pig aorta gallopamil has hardly any effect on noradrenaline(NA)-induced contractions (Beckeringh et al.,1984). To investigate the possible role of a Ca^{++} influx, sensitive to Ca^{++} entry blockers (CEBs), we calculated the contribution of the NA-sensitive intracellular calcium pool to the contraction. We compared these data with effects of organic CEBs and inorganic cations. The effect of nifedipine on NA-induced $^{45}Ca^{++}$ uptake was investigated as well. Contractions and $^{45}Ca^{++}$ uptake induced by K^+ (80 mM) were studied for comparison.

Contractions were measured in helically cut strips of the aorta of albino guinea-pigs (350 g) under isometric conditions in Krebs-Henseleit solution, gassed with 95% O_2 and 5% CO_2 at 37°C to which no Ca^{++} was added (Ca^{++} "free") or which contained 1.25 mM Ca^{++}, or in a HEPES solution (1.25 mM Ca^{++}) gassed with 100% O_2 at 37°C, for the study of the cations. After exposing the strips to the Ca^{++}-"free" solution (t=0) K^+ (80 mM) and NA ($3x10^{-5}$ M) were added at varying time-points. Each preparation was exposed to K^+ or NA only once. In Ca^{++}-containing medium K^+- and NA-induced contractions were measured after pretreatment with nifedipine (10^{-6} M), diltiazem (10^{-5} M), flunarizine (10^{-5} M) and gallopamil (10^{-5} M) and 1 mM La^{+++}, Cd^{++}, Mn^{++}, Ni^{++} and Co^{++}. For the measurement of the $^{45}Ca^{++}$ movements we adapted the method described by Wermelskirchen et al. (1988) to the guinea-pig aorta. In brief, aortic strips were equilibrated for 60 min in Tyrode solution at 37°C, gassed with carbogen in the absence or presence of nifedipine (10^{-8}-10^{-6} M). Then the strips were labeled for 10 min in $^{45}Ca^{++}$ (1μCi)-containing solution in the absence or presence of the stimulus. Thereafter, the strips were washed for 45 min in Tyrode solution at 4°C and the residual $^{45}Ca^{++}$ was determined.

In Ca^{++}-"free" medium the K^+-induced Ca^{++} influx had disappeared after 10 min, indicating the absence of pharmacologically relevant extracellular Ca^{++}. The NA-induced contraction present after 10 min exposure to Ca^{++}-"free" medium was reduced but diminished only slightly after longer periods in the Ca^{++}-"free" medium. A monoexponential curve was obtained which revealed that 55% of the total NA-induced contraction can be attributed to intracellular Ca^{++}. Although CEBs depressed K^+-induced Ca^{++} influx completely, NA-induced Ca^{++} influx could not be antagonized at all. The cations inhibited both K^+- and NA-induced Ca^{++} influx. $^{45}Ca^{++}$ uptake could be increased by both K^+ and NA by 33 and 37%, respectively. However, whereas the K^+-induced $^{45}Ca^{++}$ uptake could be inhibited completely by nifedipine, that induced by NA not at all.

These data suggest that both K^+ an NA induce Ca^{++} influx. In contrast to the K^+-induced Ca^{++} influx which could be antagonized by both CEBs and cations, the NA-induced Ca^{++} influx can be inhibited by cations only.

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44P CYTOSOLIC FREE CALCIUM AND VASOCONSTRICTION IN THE NORMOTENSIVE RAT TAIL ARTERY

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Several recent reports have described techniques for the simultaneous measurement of agonist-induced changes in intracellular calcium levels $[Ca]_i$ and contractile activity in cultured cells or strips of aorta, a compliance vessel. We describe a method for the simultaneous recording of $[Ca]_i$ and vasoconstriction in a perfused caudal artery which largely retains its *in vivo* structure and is used as a model of resistance vessels. One cm segments of the caudal artery were dissected from male Wistar rats (12 months old, 574 ± 21 g, n = 6 to 8 for each experiment) and the endothelium was removed by gentle rubbing. The segment was mounted in a specially constructed spectrofluorimeter cuvette and perfused (1.5 ml/min) with oxygenated physiological saline solution (PSS) at 37°C. Fura 2/AM (Grynkiewicz *et al.*, 1985) was added (5 μM) to the perfusate for 90 min then vessels were washed with PSS for 30 min. Noradrenaline (NOR) or serotonin (5HT) (0.1 to 30 μM) were perfused for 1 min periods and increases in perfusion pressure (ΔP mm Hg) and relative $[Ca]_i$ (= the ratio of the fluorescence at the excitation wavelengths of 340 and 380 nm as a percentage of the ratio in pre-contracted state, ΔR%) measured.
Preliminary experiments showed that the ΔR% reflected changes in $[Ca]_i$: (1) changes at the isobestic point were small (- 1 to + 1 %), (2) the signal was abolished in a zero calcium perfusate and restored on addition of calcium, (3) ionomycin (10 μM) induced a 63 ± 4 % increase, and EGTA (10 mM) a 22 ± 3 % decrease in ΔR%, (4) no signal was obtained with an artery segment which had been previously heated at 100°C. Figure 1 shows that degree of vasoconstriction was a function of the increase in ΔR% and that 5HT produced a greater vasoconstrictor response for any given increase in ΔR%.

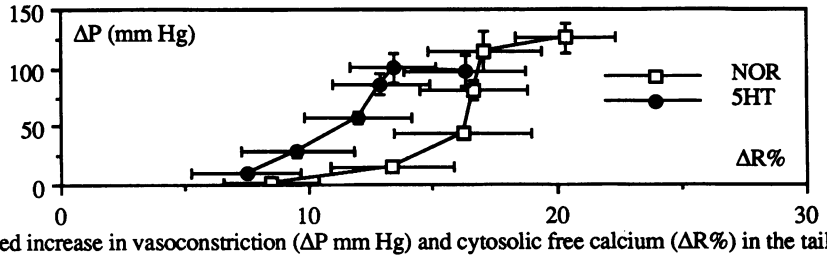

Figure 1 : Agonist-induced increase in vasoconstriction (ΔP mm Hg) and cytosolic free calcium (ΔR%) in the tail artery.

In conclusion, we describe a method for the simultaneous determination of vasoconstriction and $[Ca]_i$ in a perfused resistance artery preparation. Furthermore 5HT, when compared to NOR, appears to amplify the vasoconstrictor response to a given $[Ca]_i$.

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45P CALCIUM ANTAGONISTS DIFFERENTIALLY INHIBIT CAFFEINE-INDUCED CONTRACTIONS IN VASCULAR SMOOTH MUSCLE

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Calcium antagonists (CA's) can be divided into 3 subgroups upon pharmacological grounds: (1) 1.4-dihydropyridines such as nifedipine (N); (2) diltiazem (D) and verapamil (V); and (3) more lipophilic compounds with longer tissue equilibration times such as bepridil (B), lidoflazine (L), fendiline (FEN) and flunarizine (FLU); (Spedding, 1985). The effects of each of these CA's (10 μM, 90 min, incubation) was studied on caffeine-induced contractions of rabbit left renal artery rings, which were set up for isometric tension recording at optimal preload in both normal and Ca^{++}-free Krebs'solution containing EGTA (1 mM). Following each contraction and subsequent washing in Ca^{++}-free Krebs'solution, tissue Ca^{++} stores were replenished in normal Krebs'solution for 10 min before replacing the medium with Ca^{++}-free Krebs'solution. CA's or vehicle were in contact with the tissue throughout the experimental period.

Caffeine (1-60 mM) elicited concentration-dependent, transient phasic contractions which were unaffected by endothelium removal. Caffeine (30-60 mM) evoked maximum responses of 2.2 ± 0.1 g (n=29), which were slightly reduced in Ca^{++}-free Krebs'solution (1.9 ± 0.1 g, n=24: $p<0.001$, Student's t-test). Noradrenaline (1 μM), in contrast to caffeine, elicited both phasic and tonic contractile responses in normal Krebs' solution (maximum 3.4 ± 0.1 g, n=29).

N and D failed to affect the caffeine concentration-response curve; V only slightly depressed responses to high caffeine concentrations (>10 mM) in Ca^{++}-free medium; FLU and L exerted small inhibitory effects (20%) in both normal and Ca^{++}-free media, which remained unchanged even after 180 min incubation. In contrast, B and FEN markedly inhibited caffeine contractions in normal Krebs'solution, depressing maximal contractions by 15 and 50%, respectively. In Ca^{++}-free Krebs'solution, the inhibitory potency of FEN and especially B were enhanced, maximal responses to caffeine being reduced by 79 and 48% respectively.

In conclusion, transient caffeine-induced contractions of rabbit renal artery rings are mainly mediated by intracellular Ca^{++} mobilisation. Since the subgroup 1 and 2 calcium entry blockers, N, D, and V, failed to significantly affect caffeine-induced contractions, Ca^{++} influx via slow, L-type Ca^{++} channels is not involved in mediating the response. On the other hand, the subgroup 3 CA's generally tended to inhibit the caffeine-evoked responses. However, the piperazine derivatives, L and FLU, only mildly inhibited, whereas B and FEN markedly inhibited caffeine responses, particularly in Ca^{++}-free medium. Our data suggest that calcium entry blockers may be distinguished from intracellular CA's in the present experimental model, and that B and FEN possess additional or different intracellular sites of action to FLU or L.

Spedding, M. (1985) TIPS **6**, 109-114.

46P 12(S)-HYDROXYEICOSATETRAENOIC ACID INDUCED RELAXATION OF DOG ISOLATED CORONARY ARTERY

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Canine infarcted myocardium exhibits enhanced synthesis of 12 hydroxyeicosatetraenoic acid (12-HETE; Mullane et al, 1984). The cardiac actions of HETEs, however, are largely unexplored and in this study we have investigated the action of these agents on the tone of dog isolated coronary artery. Rings of the left anterior descending coronary artery (1-2mm i.d.) were mounted for isometric tension recording in Krebs at 37°C, using a method similar to that described by Edvinsson et al (1974). Resting tension was adjusted to 2.0g. Preparations were contracted with an EC_{50} concentration of the thromboxane A_2 mimetic, U46619 ($2.5x10^{-8}M$). Where used, methylene blue (MB, $10^{-5}M$) was added when tension had plateaued, whilst aspirin ($10^{-4}M$) was pre-incubated with the tissue for 30min prior to the addition of U46619. Cumulative concentration-effect curves to compounds shown in table 1 were then constructed. In some experiments, the endothelial cell (EC) lining was removed by rubbing with a stainless steel wire.

Table 1: Relaxant Activities of Acetylcholine (Ach), Isoprenaline (ISO), and HETEs (values are mean ±S.E.M.)

	Ach	Iso	HETES					
			15(S)	15(R)	12(S)	12(R)	5(S)	5(R)
$EC_{50}x10^{-8}M$	4.7±0.6	3.9±0.7	230±40	230±30	68±12	90±13	<20% relaxation at $10^{-6}M$	
n	35	15	4	4	16	4	2	2

The rank order of potency was 12(S) > 12(R) > 15(S) = 15(R), with 5-HETE showing litle activity (Table 1).

MB inhibited Ach (EC_{50}: 154±37x10^{-8}M, mean ± S.E.M. n=6) and 12(S) HETE (EC_{50} 332±170x10^{-8}M; mean ± S.E.M., n=4), but not Iso-induced relaxations. Aspirin had no effect on relaxation mediated by either acetylcholine or 12(S)-HETE. Rubbing of the intimal surface abolished acetylcholine-induced relaxation, but had no effect on responses induced by Iso or 12(S)-HETE. The results with MB suggest that both Ach and 12(S)-HETE induce an endothelium-dependent relaxation, but removal of the EC lining revealed that only Ach was truly EC-dependent. Thus 12 (S)-HETE induces an EC-independent relaxation of dog coronary artery which is not mediated by cyclo-oxygenase products.

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47P ENDOTOXIN DEPRESSES VASCULAR REACTIVITY TO VASODILATORS IN THE PITHED RAT

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Endotoxin depresses vascular responsiveness to several vasoconstrictors but not to endothelin in pithed rats (Guc et al. 1989). Re-examination of endothelin-induced changes in blood pressure suggested that the vasodepressor component was also impaired by endotoxin. We now report the effects of endotoxin on the vasodepressor responses to endothelin and to acetylcholine (ACh), 5-HT and sodium nitroprusside (SNP). Rats were pithed under ether anaesthesia and ventilated artificially (100% O_2; 53 strokes/min, 1ml/100g). Rectal temperature was maintained at 37 ± 0.5 °C. Cannulae were placed in one common carotid artery for measurement of arterial blood pressure, and in both jugular veins for drug injection. After 20 min equilibration, E. coli endotoxin (Difco 055:B5, 1.0 mg/kg, or 0.9% saline were infused i.v. over 4h. At 1h, dose-response curves were obtained to the above agents. Infusion of endotoxin produced a fall in mean arterial blood pressure which was maximal at 1h (control 59 ± 1 mmHg (n = 35; endotoxin 52 ± 1 mmHg, (n = 33); P<0.01). The initial depressor response to endothelin (0.01-3.0 nmol/kg), but not the subsequent pressor response was attenuated in endotoxin-treated rats (e.g. decrease in B.P., mmHg, to endothelin 0.3 nmol/kg was 16.7 ± 2.3 (n=17) in controls and 5.7 ± 0.8 in endotoxin treated rats (n = 13), P<0.05). The secondary depressor response, as well as the pressor response to low doses of 5-HT (0.1-1.0 μg/kg) was absent in endotoxin-treated animals and the responses to larger doses (3-100 μg/kg) were markedly attenuated. Similarly, responses to ACh (0.1-30 μg /kg) were diminished by endotoxin infusion. ACh did not affect heart rate but large doses (>10 μg/kg) produced an ischaemic ECG pattern accompanied by transient cardiac arrest. In endotoxin-treated rats this was seen with much lower doses of ACh (1.0 μg). To consider the possibility that impairment of depressor responses could be explained by the endotoxin-induced hypotension, effects of ACh were re-examined in rats in which the M.A.B.P. was elevated to 144 ± 6 mmHg using an infusion of vasopressin (0.07 U $kg^{-1}min^{-1}$). The depressor response to ACh was also impaired under these conditions (e.g. -45 ± 6.6 mmHg to 0.3 μg/kg in controls (n = 6); -22 ± 2.9 in endotoxin-treated (n = 6 , P<0.05). Animals receiving a bolus dose of vasopressin to elevate the M.A.B.P. to 134 ± 6.5 mmHg showed no impairment of responsiveness of SNP (0.3 - 1000 μg/kg). These results may be consistent with endotoxin-induced endothelial damage (Templeton et al, 1985). However, this cannot be the full explanation, since depressor responses to bradykinin (0.01-100 μg/kg) (endothelium-dependent) were not impaired. Moreover, salbutamol (5 ng - 500 μg/kg)-induced reductions in blood pressure were abolished.

M.O. Guc is supported by the Turkish High-Education Council and Hacettepe University. The work is supported by an EEC grant.

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48P EFFECT OF PERINDOPRIL ON CEREBRAL BLOOD FLOW IN AWAKE RENOVASCULAR HYPERTENSIVE AND NORMOTENSIVE RATS

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Chronic hypertension (HT) shifts the lower limit of cerebral blood flow (CBF) autoregulation (A) to a higher pressure level (Strandgaard *et al.*, 1973). Although acute administration of angiotensin converting enzyme inhibitors restores the lower limit of CBFA (Barry *et al.*, 1984), the chronic effects have not received much attention. We studied the effect of the angiotensin converting enzyme inhibitor, perindopril, on mean arterial pressure (MAP), basal CBF and CBFA in renovascular HT (2 kidneys, 1 clip model, n = 18) and normotensive (NT, n = 20), male Wistar rats. Seven weeks after renal artery clipping or sham operation, rats received daily i.p. injections of perindopril. The dose was increased from 1 to 8 mg/kg over the first 4 weeks until blood pressure was normalised. At 22 weeks of age MAP (aortic cannula), basal CBF (hydrogen clearance) and the lower limit of CBFA (hypotensive haemorrhage, Janian *et al.*, in press) were determined 16 to 20 h after the final injection.

Table 1. MAP, basal CBF and lower limit of CBFA in renovascular HT and NT treated with perindopril.

	Renovascular HT		NT		
	Perindopril	solvent	Perindopril	solvent	
n	12	6	8	12	* = P < 0.05
MAP (mm Hg)	113 ± 2	154 ± 6*	89 ± 3*	108 ± 2	with group
basal CBF (ml/100g/min)	85 ± 4	82 ± 3	104 ± 4*	86 ± 3	"NT-solvent"
lower limit CBFA (mm Hg)	90	150*	70	70	

Chronic (renovascular) HT caused a marked shift in the lower limit of CBFA but did not alter basal CBF. Treatment of HT rats with perindopril normalised blood pressure and restored CBFA. Chronic treatment of NT rats with perindopril increased basal CBF. In conclusion chronic treatment of renovascular HT rats with perindopril causes a shift in the lower limit of CBFA towards the value observed in NT rats.

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49P CLONIDINE RESTORES THE RENAL RESPONSE TO ATRIAL NATRIURETIC PEPTIDE IN RATS WITH CHRONIC ISCHAEMIC HEART FAILURE

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In human congestive heart failure (CHF) as well as in experimental CHF animal models, the kidney is relatively unresponsive, not only to endogenous circulating ANP but also to exogenous administration of the peptide (Cody et al. 1986; Scriven & Burnett 1985). We have recently shown that there is a functional antagonism between the renal sympathetic nervous system and the ANP system in the kidney (Pettersson et al. 1989). In CHF, not only is ANP increased, but there is also an increase in the renal sympathetic nerve activity (RSNA). In the present experiment, we aimed to investigate if pharmacological inhibition of the exaggerated sympathetic nerve activity could restore the diuretic and natriuretic response to exogenous ANP administration.

Left ventricular myocardial infarction was induced in Sprague-Dawley rats (n=16) by coronary artery ligature under pentobarbital anaesthesia. 30 days after this surgical procedure, the rats were implanted with carotid artery, jugular vein and urinary bladder catheters and placed in plexiglass cylinders. An isotonic saline (controls) or clonidine 5 μg/h infusion was given for 4 hours. Incremental doses of rat ANP (99-126) (0.25, 0.5 and 1.0 μg/kg/min) were then given to all animals. Urinary volume (UV), sodium ($U_{Na}V$), potassium ($U_{K}V$), mean arterial pressure (MAP) and heart rate (HR) were measured.

The continous clonidine infusion transiently increased UV but not $U_{Na}V$ or $U_{K}V$ compared to the saline controls. However after 4 hours of infusion, values were back to control levels. The graded ANP infusions significantly increased UV and $U_{Na}V$ at all ANP dose levels in the clonidine pretreated rats. In the control group there were only slight increases in UV and $U_{Na}V$ which were significantly below the responses seen in the clonidine pretreated group. After 0.5 μg/kg/min ANP infusion the following values were obtained in the two groups (mean ± s.e.mean):

	UV(μl/min)	$U_{Na}V$(μmol/min)	$U_{K}V$(μmol/min)	MAP(mmHg)	HR(beat/min)
Saline (n=8)	39.13 ± 12.45	4.26 ± 1.10	1.69 ± 0.26	110.3 ± 7.2	426.4 ± 20.3
Clonidine (n=8)	90.25 ± 13.69	8.81 ± 1.59	2.20 ± 0.39	99.1 ± 2.3	360.4 ± 10.2
P Value	<0.05	<0.05	n.s.	n.s.	<0.05

We conclude that the diuretic and natriuretic responses to exogenous ANP are significantly increased in CHF rats given low dose of clonidine, and that the relative unresponsiveness to ANP in CHF is due to an exaggerated RSNA.

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Scriven T.A. & Burnett J.C. Jr. (1985) Circulation 72, 892-897.

50P THE EFFECT OF FRUSEMIDE ON THE METABOLISM AND DETECTABILITY OF THEOPHYLLINE IN THE HORSE

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The high-ceiling diuretic frusemide is used in race horses for the treatment of oedema and prophylaxis of epistaxis. The increasing popularity of frusemide treatment in recent years has led to widespread concern over its effect on other drugs. Having investigated the effect of frusemide on the fate of an acidic drug, salicylic acid (D'Souza *et al.*, 1988) it was decided to study a basic drug. Theophylline was chosen in view of its therapeutic use and occasional occurrence as a drug of abuse.

Three horses received on separate occasions in a randomized cross-over design frusemide (1mg/kg; i.v) alone, [^{14}C]-theophylline (1mg/kg;100μCi/horse p.o.) alone and [^{14}C]-theophylline (1mg/kg;200μCi/horse p.o.) followed 4h later by frusemide (1mg/kg; i.v.). Serial venous blood samples were collected from an indwelling jugular cannula for 48h and urine was collected as voided for 96h.

Plasma theophylline concentrations were adequately described by a 2-compartment open model. Frusemide does not appear to have any effect on plasma concentration of theophylline. The elimination half-life of theophylline showed a degree of variability (14.9-20.5h) which is in agreement with previous studies. Only in one horse was the half-life decreased in the presence of frusemide. The concentration of radioactivity in urine showed a considerable dilution caused by the increased fluid output (8-9 litres of urine in 1 h), but the rate of excretion was unaffected, indicating that frusemide did not affect the renal handling of theophylline. Excretion was essentially complete in 96h, total radioactivity recovery being 64.0-76.5% (n=6), of the dose. Theophylline and its major metabolites were determined in urine by HPLC. 42.8% of the dose was excreted as 1,3-dimethyluric acid and 15.9% as unchanged theophylline. Two metabolites not previously reported in the horse were also detected, 8.9% as 1-methyluric acid and 1.6% as 3-methyluric acid. This pattern of elimination was very similar after frusemide treatment. Immediately after frusemide administration at 4h there was a transient increase in the excretion of unchanged theophylline which is in agreement with data in other species showing that theophylline elimination is urine flow dependent (Levy & Kaysooko, 1976). This effect is short lasting and elimination returns to normal after the massive diuresis.

These results indicate that frusemide, in contrast to its effects on salicylic acid where inconsistent drug detection may result, does not hinder the detection of the basic drug theophylline.

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51P COMPARISON OF OPIOID INHIBITION OF 5-HT_3 AND NK-3 EVOKED RELEASE OF [^{3}H]-ACH FROM THE GUINEA-PIG ILEUM MYENTERIC PLEXUS

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The neuronally located 5-HT_3 receptor of the guinea-pig myenteric plexus was originally described by Gaddum and Picarelli (1957) as "M" type in view of the inhibition of this part of the contractile response to 5-HT by morphine. However, it would seem apparent that this antagonism is not through a specific interaction with the 5-HT receptor but rather due to a functional depression of ACh output. The purpose of this study was to re-investigate the phenomenon in longitudinal muscle-myenteric plexus (LMMP) strips using the opioid receptor subtype selective ligands DAGO (μ), U50488 (k) and [D-Pen^{2,5}]-enkephalin (DPDPE) (δ). Contractility studies were combined with [^{3}H]-ACh studies to quantify the neuronal actions of 5-HT and the results were compared with those obtained from parallel experiments using the NK-3 receptor selective ligand senktide.

The methodology used has been previously described by us (Fox and Morton, 1989) where it was shown that under the conditions used the predominant contractile effect of 5-HT is mediated via 5-HT_3 receptors. [^{3}H]-ACh overflow from LMMP strips was evoked by approximately equiactive concentrations of 5-HT (10 M) and senktide (10nM) in the absence and then the presence of DAGO (1-100nM), U50488 (10nM-1μM) and DPDPE (10nM-1μM). In corresponding contractility studies concentration-response curves for 5-HT and senktide were established in the presence of the same range of concentrations of the opioid receptor agonists.

Both DAGO and U50488 produced a marked inhibition of 5-HT_3 and NK-3 mediated [^{3}H]-ACh release giving very similar IC_{50} estimates for each against 5-HT and senktide responses (18nM and 11nM respectively for DAGO, and 50nM and 18nM for U50488). Further, IC_{50} estimates of similar magnitude were obtained from the contractility studies. DPDPE had a lesser maximum effect at the highest concentration used and so IC_{50} values could not be estimated.

In conclusion, both the μ and the k agonists gave a similar degree of inhibitory modulation of 5-HT_3 as compared to NK-3 receptor mediated responses, which suggests that their two pathways of excitation converge at some level susceptible to opioid inhibition.

We acknowledge an SERC (CASE) award to AJF.

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52P GABA$_A$-RECEPTOR MEDIATED NON-ADRENERGIC NON-CHOLINERGIC RELAXATIONS IN THE CANINE ILEOCOLONIC JUNCTION

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In a previous study, we demonstrated the existence of an inhibitory non-adrenergic non-cholinergic (NANC) innervation in the canine terminal ileum and ileocolonic junction (ICJ) (Pelckmans et al., 1987). In the present study, we have attempted to evaluate the role of the putative neurotransmitter γ-aminobutyric acid (GABA) (Jessen et al., 1979) in this system and to characterise the receptors involved by means of specific agonists and antagonists.

After removal of the mucosa, circular muscle strips of the ileum and ICJ were prepared and mounted in organ baths filled with aerated Krebs-Ringer solution for isometric tension recording. Results are shown as the mean $\pm$ s.e.mean for the number of experiments indicated between brackets.

During a noradrenaline ($3x10^{-5}$M)-induced contraction, non-cumulative administration of GABA or the GABA agonist homotaurine ($3x10^{-6}$ - 10^{-3}M) evoked concentration-dependent relaxations in both ileum and ICJ, similar to the NANC relaxations obtained by electrical stimulation and acetylcholine ($3x10^{-5}$M). The GABA$_B$-agonist baclofen ($3x10^{-6}$ - $3x10^{-4}$M) did not induce relaxations. The maximal relaxations to GABA (10^{-3}M) and homotaurine (10^{-3}M), expressed as percent of the noradrenaline-induced contraction, were 73 $\pm$ 11 % (n = 6) and 60 $\pm$ 13 % (n = 5) respectively in the ileum, and 100 $\pm$ 11 % (n = 4) and 103 $\pm$ 12 % (n = 4) respectively in the ICJ. In both tissues, the GABA$_A$-receptor antagonist bicuculline (10^{-5}M) significantly shifted the concentration-response curves to GABA and homotaurine to the right.

Relaxations to GABA (10^{-4}M) were not blocked by atropine ($3x10^{-7}$M), timolol (10^{-5}M), guanethidine ($3x10^{-6}$M), domperidone (10^{-5}M), hexamethonium ($5x10^{-4}$M) and desensitisation to ATP or baclofen. In the ICJ, relaxations to GABA (10^{-4}M, 58 $\pm$ 2 %, n = 5) and homotaurine ($3x10^{-4}$M, 42 $\pm$ 8 %, n = 4) were completely blocked by tetrodotoxin ($6x10^{-7}$M) and desensitisation to GABA or homotaurine. Similar results were obtained in the ileum. Relaxations to electrical stimulation and acetylcholine were not affected by bicuculline or by desensitisation to GABA or homotaurine.

In summary, our results indicate the presence of GABA$_A$-receptors located on inhibitory NANC neurons in the canine terminal ileum and ICJ. Since bicuculline and desensitisation to GABA or homotaurine did not affect the relaxations to electrical stimulation and acetylcholine, it is unlikely that GABA is the final NANC neurotransmitter mediating these relaxations.

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53P EFFECTS OF PREJUNCTIONAL α-ADRENOCEPTOR ACTIVATION ON NEUROTRANSMISSION IN THE RABBIT SAPHENOUS ARTERY

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Contractile responses to nerve stimulation in the rabbit saphenous artery (RSA) are blocked by a combination of the P_{2x}-purinoceptor desensitising agent α,β-methylene ATP (mATP) and prazosin (Burnstock & Warland, 1987; Bulloch et al, 1988), suggesting purinergic and noradrenergic co-transmission (Burnstock, 1988). The present study examined the effect of pre-junctional α_2-adrenoceptor activation on the purinergic and noradrenergic components of neurotransmission in the rabbit saphenous artery.

Ring segments of rabbit proximal saphenous artery were placed in physiological salt solution maintained at 37°C and gassed with 95%O_2 :5%CO_2. Isometric contractile responses to electrical field stimulation (4-64 Hz, 25-40 V, 0.1 msec pulse width, for 1 sec) were recorded under a resting tension of 0.5-1 g wt.

The prazosin (1 μM)-resistant component of the contractile response of the RSA to nerve stimulation was virtually abolished by desensitisation with mATP (3 μM) confirming previous observations (Burnstock & Warland, 1987; Bulloch et al, 1988). However desensitisation with mATP (3 μM) produced variable effects on the whole contractile responses of the saphenous artery to nerve stimulation. Addition of prazosin (1 μM) after mATP virtually abolished responses in all situations.

Cocaine (10 μM) reduced contractile responses of the RSA to nerve stimulation at 4 Hz but potentiated responses at 16, 32 and 64 Hz. After desensitisation with mATP (3 μM), cocaine potentiated the contractile responses at all frequencies (4-64 Hz). After addition of prazosin (1 μM), cocaine reduced responses at 4 and 8 Hz, but had no effect on responses at 16-64 Hz. In the presence of cocaine, rauwolscine (0.3 μM) potentiated both components (mATP-resistant and prazosin-resistant) of the nerve response.

UK-14304 (0.001-0.1 μM) produced a similar concentration-dependent reduction of the contractile responses after desensitisation with mATP or after prazosin. These effects of UK-14304 were reversed by rauwolscine (0.3 and 1 μM).

The results show that the purinergic and noradrenergic components of neurotransmission in the rabbit saphenous artery are equally susceptible to α_2-adrenoceptor -mediated activation, exogenous and endogenous. Both components are influenced by neuronal uptake; removal of uptake reduces the purinergic component due to enhanced α_2-adrenoceptor mediated feedback whereas the noradrenergic component is potentiated due to enhanced post-junctional α_1-adrenoceptor activation. The results also show that mATP has an action in addition to desensitisation of P_{2x}-purinoceptors.

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54P STIMULATION-INDUCED OVERFLOW OF [^{3}H]-PHOSPHORYLCHOLINE AND [^{3}H]-ACETYLCHOLINE FROM THE ISOLATED GUINEA-PIG TRACHEA: INHIBITORY ROLE OF THE EPITHELIUM

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The parasympathetic nervous system plays a crucial role in regulating the airway diameter (see Gabella, 1987). In contrast to other organs little information has been obtained about mechanisms controlling the release of acetylcholine (ACh) from the trachea. Functional studies suggest the existence of presynaptic, inhibitory muscarinic receptors (Maclagan et al., 1989). The role and pharmacological properties of presynaptic receptors, however, should be evaluated also in release studies. The aim of the present study was, to measure the release of [^{3}H]ACh from the isolated trachea.

The guinea pig trachea was isolated, opened by dividing the muscle, cut transversely into parts of equal length and incubated (30 min) in a physiological salt solution that contained 5 μCi [^{3}H]choline. Field stimulation (biphasic pulses of 1 ms, 300 mA, 10 Hz) was applied during the labelling. After a subsequent washout period (60 min) and the addition of hemicholinium-3 tritium efflux was measured in 3 min fractions. The tissue was stimulated (S1, S2) electrically (3 Hz, 2 min) or chemically (veratridine, 30 μM, 6 min). When the radioactive overflow (choline, phosphorylcholine, ACh) was separated by HPLC, the incubation medium contained 10 μM neostigmine and 0.1 μM scopolamine. Epithelium was removed (controlled histologically in some experiments) by gently rubbing the luminal surface with a cotton-tipped applicator.

At the end of the washout period following amounts of the radioactive compounds were found in a single preparation: 34600 ± 9400 dpm ([^{3}H]ACh), 486000 ± 51000 dpm ([^{3}H]phosphorylcholine), 10000 ± 2000 dpm ([^{3}H]-choline, n = 5). Electrical field stimulation caused the outflow of [^{3}H]phosphorylcholine (3100 ± 1300 dpm, n = 6), whereas only small amounts of [^{3}H]ACh were released (550 ± 200 dpm). Likewise, electrical field stimulation of the tissue separated from the cartilaginous rings caused the dominant outflow of [^{3}H]phosphorylcholine. After removal of the epithelium, a condition without a marked change in the tissue contents of the radioactive compounds, field stimulation caused a marked release of [^{3}H]ACh in a calcium-dependent manner. The release of [^{3}H]ACh (8200 ± 900; n = 4) evoked by veratridine depended on extracellular calcium and was blocked by tetrodotoxin. Exposure to veratridine did not affect the outflow of radioactive choline or phosphorylcholine. Both the veratridine- and electrically-induced [^{3}H]ACh release were reduced by 1 μM oxotremorine, an agonist at muscarinic receptors.

In conclusion the present experiments show [^{3}H]ACh-synthesis in and [^{3}H]ACh-release from the isolated trachea. Release of [^{3}H]ACh is stimulated by veratridine or by electrical field stimulation, particularly after removal of the epithelium. The epithelium appears to exert a marked inhibitory tone on the release of ACh; a decline of this effect might contribute to bronchoconstriction under the condition of chronic bronchitis.

Supported by NMFZ University of Mainz and the Deutsche Forschungsgemeinschaft

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55P THE PRESYNAPTIC MUSCARINIC AUTORECEPTOR OF *TORPEDO* ELECTRIC ORGAN EXHIBITS THE M_1 SUBTYPE PHARMACOLOGY: EVIDENCE FROM DIRECT BINDING STUDIES

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Presynaptic muscarinic receptors in *Torpedo marmorata* electric organ (EO) have been partially characterised using radio-ligand binding (Dowdall *et al.*, 1983 and references therein). In rat brain, using functional assays, presynaptic muscarinic receptors have been subtyped as M2 (autoreceptor) or M1 (heteroreceptor) (Marchi & Raiteri, 1985). Recently, an M1 autoreceptor has been reported in chick heart (Jeck *et al.*, 1988). Using selective muscarinic antagonists (see table) we provide evidence that the *Torpedo* EO also has M1 autoreceptors.

Crude membrane preparations of EO and homogenates of *Torpedo* brain regions (for comparison) were used. Binding assays were carried out in a *Torpedo* ringer (280mM NaCl, 7mM KCl, 4.4mM CaCl2, 1.3mM MgCl2, 20mM Tris, pH 7.4) essentially as in Dowdall *et al.*, (1983). Data was analysed assuming either a one or two site model using an iterative curve fitting program.

Table 1 — Ki values (nM) of antagonist binding to *Torpedo* electric organ and forebrain (n>=3).

Antagonist	Electric organ		Forebrain	
Pirenzepine (PZ)	36±14	(0.89±0.19)	70±9 *	(0.80±0.04)†
AFDX-116	311±126	(1.00±0.04)	362±83	(0.91±0.09)
Methoctramine	290±40	(0.93±0.11)	267±25	(1.01±0.07)

Numbers in parentheses refer to the slope factors of the displacement curves. All values are mean ± standard deviation of the sample. *Corrected IC50 rather than Ki value. † PZ binding to *Torpedo* brain regions gave slope factors significantly less than 1. These data were best explained assuming a two site model with high affinity (Ki= 18±5nM) and low affinity (Ki= 307±110nM) sites for PZ .

It can be concluded that *Torpedo* EO contains a single class of muscarinic binding sites (slope factors not significantly different from 1) with a pharmacology most similar to that of the M1 muscarinic receptor subtype. The small but significant difference (P= 0.032) between the Ki values of PZ binding to EO and the *'Torpedo* M1' sites (high affinity component of PZ binding to brain tissues), may reflect differences in tissue preparation and/or subtle structural differences between the receptors.

A.C.G. is in receipt of an SERC studentship. PZ and AFDX-116 were donated by Karl Thomae GmbH and Boehringer Ingelheim Ltd.

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56P ANOMALOUS PA_2 VALUES FOR PARAFLUOROHEXAHYDROSILADIFENIDOL (PFHHSID) AT TRACHEAL MUSCARINIC RECEPTORS

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M_3 muscarinic receptors mediate contractions of guinea-pig ileal or tracheal smooth muscle (Eglen and Whiting, 1988). Lambrecht et al., (1988) propose that pFHHSiD is a M_3-selective antagonist at muscarinic receptors in the guinea-pig ileum (M_3; pA_2 = 7.8), in comparison to receptors in the left atria (M_2; pA_2 = 6.1) or rabbit vas deferens (M_1; pA_2 = 6.7). In the present study, we report the interaction of pFHHSiD at guinea-pig tracheal M_3 muscarinic receptors. Isolated tracheal strips were prepared using the method of Eglen and Whiting (1988) and pA_2 values were determined according to the method of Arunlakshana and Schild (1959). The pK_B value was obtained after imposing the unity constraint.

Agonist	Equilibration Period	pA_2	A-S slope	pK_B	N
Acetylcholine	60 min	7.13 ± 0.11	0.94 ± 0.10	7.19 ± 0.07	20
cis-Dioxolane	60 min	6.85 ± 0.20	1.43 ± 0.11	7.44 ± 0.09	14
cis-Dioxolane	120 min	7.26 ± 0.16	1.03 ± 0.03	7.23 ± 0.08	14
cis-Dioxolane	180 min	7.33 ± 0.32	0.97 ± 0.09	7.32 ± 0.07	15
cis-Dioxolane	60 min[a]	6.94 ± 0.17	1.40 ± 0.04	7.38 ± 0.10	19
cis-Dioxolane	60 min[b]	7.12 ± 0.11	1.03 ± 0.08	7.21 ± 0.09	18

Values are mean ± s.e. mean; pFHHSiD was studied at 0.3-10 μM. [a]-In the presence of 0.1 μM methoctramine (to exclude M_2 receptors). [b]-In the presence of 0.1 μM pirenzepine (to exclude M_1 receptors).

pFHHSiD, alone, did not elicit any contractile responses (1 nM-1 μM). The data in the table suggest that the pA_2 value for pFHHSiD was agonist independent and not due to an additional interaction with M_1 or M_2 receptors. Combination dose-ratio (DR) experiments, using 120 min equilibration periods, were conducted using atropine (0.1 μM; DR = 68.3) and pFHHSiD (3.2 μM; DR_2 = 75.3). The observed combination dose-ratio was 144, which was not significantly different from the expected combination (DR_1 + DR_2 - 1 = 143). These values suggest that pFHHSiD and atropine interact at the same receptor sites. The reason for the anomalously low pA_2 at tracheal M_3 muscarinic receptors remains to be determined although it is not concluded that it represents further muscarinic receptor heterogeneity.

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57P AIRWAY EPITHELIUM ACTIVELY METABOLISES ENDOTHELIN AND MODULATES ENDOTHELIN-INDUCED CONTRACTION IN GUINEA-PIG TRACHEA

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Airway epithelium, which modulates airway smooth muscle tone, contains enkephalinase, as shown by immunocytochemical methods (Sekizawa et al., 1987). A decrease in enkephalinase activity has been described following influenza infection, which may contribute to the observed hyperreactivity of bronchial smooth muscle to peptides (Jacoby et al., 1989). In the present study, we investigated the contractile activity of endothelins (ET_1, ET_2, and ET_3) and sarafotoxin S6b (S6b) in trachea and its modulation by the airway epithelium.

Guinea-pig tracheal strips with and without epithelium were prepared as previously described (Tschirhart et al., 1987). Briefly, 4 tracheal strips of 4 cartilaginous rings were cut from one trachea. Tracheal smooth muscle contractions were measured isometrically. Concentration-response curves (0.1 to 100nM) to the peptides (Peninsula Lab., U.S.A) were constructed cumulatively. Only one concentration-response curve was obtained from each tracheal preparation. The reactivities of adjacent strips, with and without epithelium, and with and without phosphoramidon (10μM, 30 min incubation), an enkephalinase inhibitor, were compared, allowing paired analysis of data.

Table 1 Effect of enkephalinase inhibition by phosphoramidon (Phospho; 10μM) on contractions induced by endothelins (ETs) and sarafotoxin (S6b) in guinea-pig tracheal preparations.

		+ Epithelium		-Epithelium	
Conditions	n	EC_{50} (nM)	E_{max} (mg)	EC_{50} (nM)	E_{max} (mg)
ET_1	6	25.21 ± 0.13	280 ± 90	6.71 ± 3.28*	730 ± 120*
ET_1+ Phospho	6	13.82 ± 4.51†	1040 ± 100†	3.87 ± 1.46*	770 ± 40
ET_2	6	35.54 ± 4.20	810 ± 270	5.84 ± 0.31*	1460 ± 60*
ET_2+ Phospho	6	13.62 ± 2.75†	1380 ± 120†	3.70 ± 0.35*	1390 ± 110
ET_3	6	22.31 ± 5.99	590 ± 150	11.51 ± 1.57	560 ± 30
ET_3+ Phospho	6	22.94 ± 2.88	660 ± 250	12.96 ± 1.12	530 ± 130
S6b	6	20.26 ± 7.91	1090 ± 140	2.97 ± 1.19*	870 ± 140
S6b+ Phospho	6	14.22 ± 2.14	940 ± 110	1.57 ± 0.37*	840 ± 90

* significantly different from intact tracheal strips ($P < 0.05$, Student's paired t-test). † significantly different from control, in the presence of phosphoramidon ($P < 0.05$, Student's paired t-test).

The results shown in Table 1 indicate that responses to ET_1 and ET_2 but not to ET_3 and S6b are enhanced in the presence of the enkephalinase inhibitor and that the former two peptides may be metabolized by enkephalinase present within the airway epithelium. Moreover, the presence of airway epithelium appears to be able to reduce tracheal smooth muscle responsiveness to ET_1, ET_2 and S6b perhaps by the release of an epithelium-derived relaxant factor (Tschirhart & Landry, 1986). Interestingly, ET_3, which is not sensitive to enkephalinase, seems not to be able to induce the release of such a relaxant factor. In conclusion, these results suggest that the airway epithelium is able to modulate guinea-pig tracheal smooth muscle responsiveness to some ETs, partly by affecting their metabolism and perhaps via the release of an epithelial relaxant factor.

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58P RELAXATIONS OF GUINEA-PIG TRACHEA INDUCED BY PLATELET-ACTIVATING FACTOR ARE EPITHELIUM-DEPENDENT AND ARE ANTAGONISED BY WEB 2086

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Platelet-activating factor (PAF) is a potent bronchoconstrictor *in vivo*, and causes a non-specific increase in airway hyperresponsiveness. However, conflicting data regarding its activity on guinea-pig trachea (GPT) *in vitro* were reported, with PAF being described as either a contractile or a relaxant agent (Malo *et al*, 1987, Prancan *et al*, 1982). In this study we have examined the effects of epithelial removal on PAF-induced responses of GPT, since the epithelium lining the GPT has been shown to influence the responses of various agonists (Hay *et al*, 1987).

Tracheae from male Dunkin-Hartley guinea pigs (300-500g) were used. Each GPT was halved, one half was kept intact, the other was stripped of epithelium. Zigzag strips were superfused with Tyrode's buffer (5ml/min). PAF was administered as a bolus injection. A combination of antagonists to histamine, ACh, 5-HT and α- and β-adrenoceptors, and indomethacin or WEB 2086 (Casals-Stenzel *et al*, 1987) were administered as a continuous infusion. Intact GPTs were also stimulated with PAF (1μM) in 10 ml organ baths containing Krebs-Henseleit buffer in the presence or absence of indomethacin and effluents assayed for prostaglandin (PG) release. In all cases, *n* ranged between 5 and 12.

PAF (10-300 pmol) induced dose-related relaxations of the basal tone of intact superfused GPT and was largely equiactive with salbutamol (3-300 pmol). Tachyphylaxis was not observed. The relaxations due to PAF were completely inhibited by indomethacin (1.4μM) but were unaffected by combined antagonists. WEB 2086, a PAF antagonist, at 1nM, attenuated the PAF-induced relaxations. Complete and specific antagonism was observed at 10 and 100nM. In contrast, PAF caused a single sustained contraction of epithelium-denuded GPT, even in the presence of indomethacin, FPL 55712 (leukotriene D_4/E_4 antagonist) or combined antagonists. In organ bath, PAF (1μM) relaxed intact GPT and stimulated the release of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (108±21.4, 36.9±9.2 and 17.5±4.4 pg/mg tissue respectively). Indomethacin (1.4μM) inhibited both the relaxations and PG release induced by PAF.
These results show that epithelium modulates the effects of PAF on GPT and that PAF exerts its action via specific receptors in this tissue. The PAF-induced relaxations of the basal tone are dependent on an intact epithelium and may be partially mediated by a cyclo-oxygenase product, possibly PGE_2, as recently reported by Brunelleschi *et al*, 1989, using histamine-precontracted GPT.

We thank the Frances and Augustus Newman Foundation for financial support.

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59P BAY U3405, A NOVEL THROMBOXANE RECEPTOR ANTAGONIST: ACTIVITY ON ISOLATED AIRWAY TISSUES

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We have previously described the effects of the thromboxane (TXA_2) receptor antagonists AH23848 and EP092 on prostanoid-induced contractions of guinea-pig and human airway smooth muscle preparations (McKenniff et al, 1988) and on U46619-induced contractions of the ferret tracheal ring (McKenniff et al., 1989). These results suggested that TXA_2 receptor subtypes might exist. Here we have evaluated the novel TXA_2 receptor antagonist BAY u3405 (Seuter et al; 1989), (9-(2-carboxyethyl)-3(R)-(4-fluorophenylsulphonamido)-1,2,3,4-tetrahydrocarbazole), against prostanoid-induced contractions of guinea-pig, human and ferret airway smooth muscle.

Airway preparations were set up as previously described (McKenniff et al., 1988). Tissues were equilibrated with BAY u3405 for 60min prior to addition of test agonist. All studies were performed using paired preparations.

Table 1. Antagonist potency of BAY u3405 against U46619-induced contractions.

Preparation	Number of preparations	pA_2	Slope
Guinea-pig tracheal ring	6	8.7 (8.5-8.9)	0.78 (0.51-1.05)
Human Bronchial muscle	4	8.8 (8.5-9.1)	1.15 (0.77-1.53)
Human Lung Strip	4	8.9 (8.6-9.2)	0.88 (0.75-1.01)
Ferret tracheal ring	4	8.1 (7.8-8.4)	1.09 (0.90-1.28)

95% confidence limits in parentheses. pA_2 values were calculated with the slope constrained to unity.

U46619-induced contractions were competitively antagonised by BAY u3405 (10^{-9}-10^{-6}M) on all four preparations (Table 1). 10^{-6}M BAY u3405 produced a substantial inhibition of prostaglandin(PG)D_2 and 9α 11β PGF_2-induced contractions of guinea-pig tracheal ring but had no effect on $PGF_{2\alpha}$ or 16, 16-dimethyl PGE_2-induced contractions of this preparation. On human lung strip 10^{-6}M BAY u3405 abolished the contractile response to all these prostanoids. The same concentration had no effect on contractile, or relaxant, responses to a selection of other agonists. (e.g histamine, isoprenaline, leukotriene D_4).

BAY u3405 is a potent, and selective, TXA_2 receptor antagonist. In contrast to other antagonists we have examined on the ferret tracheal ring, it is a competitive antagonist of U46619. It is equally potent on human bronchial muscle as a TXA_2 receptor antagonist suggesting it has no subtype selectivity.

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60P BAY U3405 ANTAGONISES U46619-INDUCED BRONCHOCONSTRICTION IN THE ANAESTHETISED GUINEA-PIG

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BAY u3405 has been identified as a potent thromboxane A_2 (TXA_2) antagonist on vascular smooth muscle and platelets (Seuter et al, 1989) and on guinea-pig and human airway smooth muscle (McKenniff et al, this meeting, Norel et al, this meeting). Prostaglandin D_2 (PGD_2) has been shown to act via the TXA_2 receptor in the airways (McKenniff et al, 1988), so BAY u3405 may prove useful in determining the role of TXA_2 and/or PGD_2 in diseases such as asthma. We have studied the airway activity of this compound in vivo using a modified Konzett & Roessler model (Gardiner, 1971).

Male Dunkin Hartley guinea-pigs (300-400g) were dosed with sodium pentobarbitone (120mg.kg^{-1} i.p.); sufficient to induce anaesthesia and abolish natural respiration. Following cannulation of the trachea, jugular vein and carotid artery each animal was ventilated and set up to record lung overflow volume and systemic blood pressure. A settling period of 20 minutes was allowed before the administration of the TXA_2 mimetic U46619. BAY u3405 was also evaluated against bronchoconstriction induced by leukotriene D_4 (LTD_4) (in the presence of indomethacin 10mg kg^{-1} i.v.) or histamine.

Since agonism can be a problem with some TXA_2 antagonists (Humphrey et al, 1986; Terres et al, 1987) we gave BAY u3405 intravenously over a wide dose range up to 10mg.kg^{-1} but we saw no bronchoconstriction, as reflected in overflow volume, and no change in mean systemic blood pressure.

BAY u3405 was administered i.v., p.o., or by the aerosol route at 20min, 1hr and 5min respectively prior to the intravenous administration of U46619 (0.01 - 100μg.kg^{-1}). It produced a dose-related inhibition of U46619-induced bronchoconstriction with the following ID_{50} values: 600μg.kg^{-1} i.v., 1.7mg.kg^{-1} p.o. and 0.1% w/v (given for 20 breaths) by aerosol. Studies on the duration of action of BAY u3405 demonstrate that a dose of 10mg kg^{-1} p.o. causes 66% inhibition of the U46619-induced bronchoconstriction at the ED_{50} dose one hour after treatment. After 4 hours approx 50% inhibition was observed and this level of inhibition was still present after 6 hours. BAY u3405 (1mg.kg^{-1} i.v.) had no effect against LTD_4 (0.1-10μg.kg^{-1} i.v.) or histamine (1-5μg.kg^{-1} i.v.) induced bronchoconstriction.

BAY u3405 is an orally active, potent and selective antagonist of TXA_2-induced bronchoconstriction in the guinea-pig in vivo. Such activity justifies the evaluation of this compound in airway diseases such as asthma

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61P AN EVALUATION OF THE INHIBITORY ACTIVITY OF BAY U3405 ON THE U46619 RESPONSE IN HUMAN ISOLATED BRONCHIAL MUSCLE PREPARATIONS

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We have evaluated a new TXA_2 antagonist BAY u3405 (Seuter et al, 1989, McKenniff et al, this meeting) on human isolated bronchial muscle and compared it with two structurally different thromboxane antagonists AH23848 and EP092 (McKenniff et al, 1988). Human bronchial muscle rings were placed in 10ml organ baths under a load of 2-3g and allowed to equilibrate for 90 min in Tyrode's solution at 37°C, gassed with 5% CO_2 in O_2. The maximal contraction (Emax) and pD_2 values ($-\log EC_{50}$ values) were determined from U46619 concentration-effect curves subsequent to a 30min incubation of bronchial rings in the absence (control) or presence of the different antagonists, 0.1μM, a concentration previously shown to induce a 10-100 fold rightward shift in the U46619 dose-contractile curve on guinea-pig airway tissue. (McKenniff et al, 1988 and 1989). The results are shown in the table below.

Antagonist	(N/P)	Histamine response (g)	U46619 E_{max} (g)	U46619 pD_2 value
Control	(4/4)	1.46 ± 0.40	1.77 ± 0.40	7.54 ± 0.21
BAY u3405	(4/4)	1.91 ± 1.01	2.82 ± 1.39	5.54 ± 0.24*
Control	(5/5)	1.73 ± 0.41	2.86 ± 0.79	7.36 ± 0.22
AH23848	(5/6)	2.09 ± 0.49	3.34 ± 0.91	7.34 ± 0.28
Control	(4/4)	1.97 ± 0.43	2.58 ± 0.75	7.34 ± 0.23
EP092	(4/4)	1.27 ± 0.35	2.74 ± 0.99	6.97 ± 0.11

Histamine (50μM) responses were obtained prior to incubation with the TXA_2 antagonists and were expressed as force in grams (g). Values are means ± s.e.m. and * indicates significantly different from controls ($p < 0.05$). N/P denotes the number of lung samples over number of preparations used.

In agreement with earlier observations by McKenniff et al, (1988), EP092 and AH23848 were weak thromboxane antagonists on this tissue whereas BAY u3405 is a potent thromboxane antagonist as has previously been reported.

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62P GR71251, A NOVEL, POTENT AND HIGHLY SELECTIVE ANTAGONIST AT NEUROKININ NK-1 RECEPTORS

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Receptors for substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) have been classified into three sub-groups (NK-1, NK-2 and NK-3), largely on the basis of the activities of selective agonists. Receptor classification would be greatly aided by selective neurokinin antagonists. Here, we describe the actions of GR71251 ([D-Pro^9[Spiro- -Lactam]Leu^{10},Trp^{11}]SP(1-11)), an analogue of SP incorporating a bicyclic conformational constraint, designed to eliminate NK-1 receptor-activating conformations.

At NK-1 receptors, GR71251 behaved as a potent reversible, competitive antagonist of SP methylester(SPOME)-induced contraction of the guinea-pig ileum longitudinal muscle (GPI) (mean pK_B ± s.e mean, 7.72 ± 0.06, n=16) and of SPOME-induced relaxation of the phenylephrine contracted rabbit aortic ring preparation (mean pK_B ± s.e. mean, 7.06 ± 0.07, n=23). In contrast, at 10μM, it was inactive against NKA-induced contractions of rat colon muscularis mucosae (NK-2 receptors) and NKB-induced contractions of rat everted portal vein (NK-3 receptors). Overall selectivity was confirmed by lack of activity against acetylcholine and histamine in the GPI and against contractions to cholecystokinin (CCK-8) and bombesin in guinea-pig gall bladder strips.

GR71251 was more potent than SP at releasing histamine from rat isolated peritoneal mast cells. At 1 μM, it released 33% of the total mast cell histamine content, compared to 10.5% released by the same concentration of SP. Therefore, when GR71251 was used in vivo, animals were pretreated with mepyramine to prevent the effects of released histamine. In anaesthetised rats, pretreated with Evans Blue (30mgkg^{-1} i.v.) to allow quantification of plasma protein extravasation and mepyramine (10mgkg^{-1} i.p.), GR71251 (0.1μmolkg^{-1} i.v.), significantly attenuated ($P<0.05$) the extravasation of dye into skin induced by intra-dermal injection of the selective NK-1 agonist GR73632 (Hagan et al., 1989): control (100pmol GR73632), 18.5 ± 2.2μg Evans Blue (n = 6); GR71251 treated, 9.6 ± 1.4μg Evans Blue (n = 4). In anaesthestised, artificially ventilated guinea-pigs, pretreated with mepyramine (5mgkg^{-1} i.v.), GR73632 (0.04-0.4nmolkg^{-1} i.v.) caused dose-related increases in insufflation pressure. This response was inhibited by GR71251 (3μmolkg^{-1} i.v.) given immediately before GR73632.

We conclude that GR71251, a potent and highly selective neurokinin NK-1 receptor antagonist, is a useful tool for characterising NK-1 receptor-mediated responses.

Hagan, R.M., Ireland, S.J., Jordan, C.C., Bailey, F., Stephens-Smith, M., Deal, M. & Ward, P. (1989) Br. J. Pharmacol. 98, 717P.

63P RECEPTORS MEDIATING NEUROKININ-INDUCED CONTRACTION OF THE GUINEA-PIG ISOLATED TRACHEA

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The guinea-pig isolated trachea (GPT) appears to contain both neurokinin NK-1 and NK-2 receptors since it is contracted by agonists selective for either type (Ireland et al., 1988). However, the lack of effect of certain neurokinin antagonists in GPT has been interpreted to indicate the presence of a single type of neurokinin receptor, distinct from NK-1 or NK-2 and termed NK-4 (see Maguire et al., 1989). In the present study, we have investigated the competence of these hypotheses.

GPT preparations were suspended in Tyrode's solution containing phosphoramidon (1μM) and bestatin (100μM; see Ireland et al., 1988). Both the NK-1-selective agonist substance P methylester (SPOMe; 1-300nM) and the NK-2 selective agonist [Lys^3,Gly^8-R-ɣ-lactam-Leu^9]neurokinin A(3-10) (GR64349; 0.3-100nM; Hagan et al., 1989) caused contraction of GPT. The NK-1-selective antagonist [D-Pro^9[spiro-ɣ-lactam]Leu^{10}-Trp^{11}] substance P(1-11) (GR71251; 0.1-30μM; Hagan et al., 1990) behaved as a reversible competitive antagonist of SPOMe-induced contraction (Schild plot slope 1.00 (95% confidence limits 0.85-1.16), mean pK_B (± s.e.mean) 7.05 ±0.06 (n=26)). In contrast, GR71251 (3μM) had little effect against GR64349-induced contraction (mean concentration-ratio (CR) 1.92±0.52 (n=4)). The NK-2-selective antagonist L659-877 (30μM; Maguire et al., 1989) did not affect SPOMe-induced contraction (mean CR 1.06±0.24 (n=4)). However, L659-877 (0.1-10μM) did cause parallel rightward displacement of the GR64349 concentration-response curve although the Schild plot had a slope of 0.37 (0.10-0.64). Mean concentration-ratios for L659-877, 0.3, 3.0 or 10.0μM, were 6.65±1.39, 24.21±9.58 and 9.14±1.15 respectively (n=4-6).

These results suggest that, in the guinea-pig trachea, neurokinins can act at NK-1 receptors for which the NK-1-selective antagonist GR71251 has a pK_B similar to that for NK-1 receptors in the guinea-pig ileum (7.72; Hagan et al., 1990). These receptors in the trachea are distinct from those activated by GR64349. The latter resemble NK-2 receptors. On present evidence, the hypothesis that a single, non NK-1/NK-2 receptor subtype mediates neurokinin-induced contraction of guinea-pig trachea is not tenable.

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64P THE EFFECT OF PEPTIDES ON TRACHEAL MUCUS VELOCITY (TMV) IN THE ANAESTHETISED FERRET

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Tracheal mucus velocity (TMV, mm/min) has been measured in the anaesthetised (sodium pentobarbitone, 40 mg/kg i.p.) ferret, by measuring the clearance rate of ^{32}P-labeled erythrocytes (Morley & Sanjar, 1983). Blood pressure and pulse rate were measured from a cannulated carotid artery and substances were injected intravenously via a cannulated jugular vein. TMV was measured at 10 minute intervals until at least 2 consistent values were obtained, after which test substances were injected intravenously and TMV measured 5, 15 and 30 minutes later. Student's t-test for paired observations was used to assess significance of differences, and # indicates at least $P<0.02$. With the exception of substance P (Sub P), none of the peptides tested increased TMV, despite pronounced effects on the cardiovascular system. Physalamine caused convulsions in anaesthetised ferrets, therefore, the capacity of this material to affect TMV could not be assessed. Sub P (1.0 ug/kg) dramatically increased TMV at all time points. In addition, there was profuse salivation immediately after injection (within 30 sec). These actions of Sub P were not mediated via a cholinergic mechanism since atropine, at a dose known to inhibit the effects of pilocarpine, was without effect. The lower dose of Sub P (0.1 ug/kg) did not stimulate TMV or cause salivation. Interestingly, Neurokinin A (NKA), a potent bronchoconstrictor agent (Uchida et al., 1987), which effected substantial cardiovascular changes in the ferret, did not increase TMV or provoke salivation. Although many peptides cause mucus secretion, increased TMV which requires stimulation of both mucus secretion and enhanced ciliary beat frequency, may only be the property of certain peptides.

Substance	Dose ug/kg	n	Basal TMV mm/min	TMV AFTER INJECTION (min) 5	15	30
Sub P	0.1	4	8.3±1.6	6.9±1.4	7.6±0.6	--
	1.0	5	7.9±1.0	17.0±3.3#	17.1±3.6#	18.0±3.0
Sub P + Atropine	1.0 / 50.0	5	8.3±0.9	17.8±1.6#	15.2±0.8#	18.3±6.0
NkA	1.0	4	7.9±0.7	6.1±0.9	7.8±1.1	--
Bombesin	10.0	4	7.6±0.9	6.7±0.9	7.7±1.2	--
VIP	10.0	5	6.2±0.4	7.8±0.8	8.3±0.7	--
CGRP	10.0	5	6.7±0.8	5.0±0.8	5.8±0.9	--

Morley, J. & Sanjar, S. (1983). J. Physiol. **340**, 16-17P.
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65P PERTUSSIS TOXIN INDUCES BRONCHOPULMONARY HYPERREACTIVITY TO HISTAMINE, 5-HT AND ANTIGEN IN GUINEA-PIGS, BUT SUPPRESSES RESPONSES TO FMLP

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Mice and rats inoculated with *Bordetella pertussis* vaccine show increased sensitivity to histamine, serotonin and anaphylaxis (Parfentjev and Goodline, 1948; Kind, 1958). This has been attributed to an acquired imbalance of two adrenergic effector systems, i.e., to a reduced functioning of the ß-adrenergic receptors or of some of the reactions between receptor activation and adrenergic end-response (Szentivanyi, 1968). We have shown that enhanced bronchoconstriction, BC (i.e., unspecific broncho-pulmonary hyperresponsiveness) follows the administration of a booster injection of antigen to actively sensitized guinea-pigs (Pretolani et al., 1988). This led us now to study the effects of pertussis toxin (PT), the active component of *B. pertussis* on broncho-pulmonary responsiveness. PT was administered i.v. to guinea-pigs at 0.8-20 µg/kg 6-72 h before they were stimulated, under pentobarbitone anesthesia, with i.v. histamine (0.5-16 µg/kg) or serotonin (0.5-8 µg/kg), at 10 min intervals. Bronchial resistance to inflation was evaluated by the method of Konzett-Rössler in cm H_2O. PT induced leukocytosis (lymphocytosis), and in 10 animals the number of circulating leukocytes increased from 5,700±800 to 38,900±3,700 at the dose of 20 µg/kg after 72 h. This effect was dose and time-dependent and started within 6 h. Initially no differences were observed between the bronchoconstrictor responses to histamine or to serotonin of control and PT-treated animals but, when propranolol was used (1 mg/kg i.v. and 3 mg/kg i.p.), BC was slightly increased only (% BC: 13.4±2.8 up to 19.6±3.5) in control, but was markedly increased (% BC; 8.9±2.8 to 70.5±4.4, $p<0.001$) in animals treated 72 h beforehand with PT at 20 µg/kg. Similar effects were observed with serotonin. In contrast, BC and the accompanying leukopenia induced by the i.v. administration of the secretagogue N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Boukili et al., 1986 and 1989) were antagonized by PT. Because of the contrasting effects on fMLP and on histamine and serotonin, isolated lungs provided by PT-treated animals where used . Under those conditions, BC and histamine and thromboxane A2 releases induced by the intra-pulmonary administration of fMLP were suppressed but the effects of OA (3 ng-100 µg injected to the lungs of guinea-pigs immunized with 10 µg ovalbumin (OA) in Al(0H)3 injected i.p. twice, at a 2-week interval) were enhanced. PT thus modifies negatively the signal transductions for cells involved in the lung responses to fMLP, but positively the effects of the direct constrictor agents histamine and serotonin and of antigen, which induces BC via these mediators. Our data suggest that PT prevents the effects of fMLP on a target other than the neutrophil, since it was effective on the isolated lungs (Boukili et al., 1989), possibly via its recognized effects on the Gi protein of other effector systems present in the lung. Hyperresponsiveness may result from an enhanced mediator release, possibly due to down regulation of a Gi protein, associated to a direct effect on smooth muscle, at a level which is under investigation.

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66P AN ANOMALOUS EFFECT OF SALBUTAMOL IN SENSITISED GUINEA-PIGS

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Eosinophils migrate to the intrapulmonary airways of sensitised guinea-pigs in response to inhaled allergen. Whilst assessing the capacity of anti-asthma drugs to inhibit this phenomenon, it was noted that animals pretreated with salbutamol (S) (1 mg/kg/day) by subcutaneous infusion invariably died on inhalation of allergen, in marked contrast to animals that were untreated or received other anti-asthma drugs. The contribution of altered airway smooth muscle function to this untoward effect has been investigated.

Guinea-pigs (450-600 gm) were sensitised by intraperitoneal injection (1 ml) of a suspension containing ovalbumin (OA, 10 ug/ml) and aluminium hydroxide (10 mg/ml) and separately with pertussis toxin (0.25 ml) on day 0, boosted on day 14 and implanted with either saline (C) or salbutamol (S) (1 mg/kg/day, Alzet minipump, s.c.) between day 21 and day 30. Six days later animals were anaesthetised with phenobarbitone (100 mg/kg i.p.) and pentobarbitone (30 mg/kg i.p.) paralysed with gallamine (10 mg/kg i.m.) and ventilated (1 hz, 8 ml/kg) via a tracheal cannula. Airway resistance (R_l cm H_20/l/sec) and compliance (C_{dyn} ml H_20/l/sec) were calculated from measurement of tracheal airflow and transpulmonary pressure (Digital electronic pulmonary monitoring system, Mumed Ltd., U.K.). Animals were challenged with aerosolised OA (10-1000 ug/ml for 10 min) and changes in R_l and C_{dyn} were monitored at each breath. Airway responses to inhaled OA or intravenous histamine (1.0 & 1.8 ug/kg) were expressed as the maximal increase in R_l (mean±sem).
Responses to histamine in naive animals (107±67, 198±77, n=4) were not dissimilar from C animals (109±48, 262±91, n=10). Prior treatment with S (1 mg/kg/day s.c.) resulted in a slight reduction of these responses (46±12, 139±42, n=10, NS). No response to inhaled OA (100 ug) was observed in naive animals, in contrast to C animals (132±38, n=10) which developed increased reactivity to histamine following antigen challenge (418±64, 799±76, n=10). In animals pretreated with S, the reaction to antigen (334±58, n=10) was significantly ($P<0.001$) increased, even though airway responses to histamine were slightly reduced (225±66, 613±106, n=10).

The present results demonstrate that pretreatment of sensitised guinea-pigs with S augments the response to antigen. Altered distribution or increased dosage of inhaled allergen, altered airway reactivity or hypoxic vasoconstriction are mechanisms that might contribute to this phenomenon.

67P IDENTIFICATION AND DISTRIBUTION OF N-METHYLADRENALINE IN THE CENTRAL NERVOUS SYSTEM OF THE RAT

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N-methyladrenaline (MA) has recently been identified in rat brain and retina by high performance liquid chromatography (HPLC) using isocratic coulometric detection and a variety of mobile phase conditions (Plummer et al., 1988). Confirmation of the identity of endogenous peaks was therefore sought by gas chomatography/mass spectroscopy (GCMS). Tissue was homogenised in 0.2M acetic acid and catechols were adsorbed to alumina at pH 8.6 and eluted with 0.2M acetic acid. Samples were dried under nitrogen at room temperature and derivatised with bis-trimethylsilyltrifluoroacetamide. GCMS was used to monitor selected ions(SIM). Fragments chosen for MA identification were 102, 355 and 398. Standard MA runs established the retention time at 10.143 minutes and a relative abundance ratio of the 355 and 398 ions of 46.6. A sample prepared from retinae of stressed rats was run on GCMS in SIM mode, and showed peaks at 102, 355 and 398 mass/charge values occurring at 10.143 minutes. Ion ratios for the two more massive ions 355 and 398 were 51.6, which seems close to the value obtained with standard MA in view of the small peak areas measured. Coincidence of the three ion peaks at the retention time for MA, and the close agreement between ion ratios is strong evidence for the identity of the endogenous compound at MA.

Unstressed male Wistar rats (250 - 300 g) housed at a constant 6 per cage until sacrifice in a quiet room were killed by stunning followed by decapitation at 2-4 pm and the concentration and distribution of catecholamines analysed by HPLC as previously described (Plummer et al., 1988), in samples from brain regions known to be rich in the adrenaline synthesising enzyme.

Table 1 Distribution of MA and other Catecholamines

	Retina	Thalamus*	Hypothalamus	Central Grey	C1	C2/3	Sp.Cord
Noradrenaline	0.53±0.06	616.3±23.2	1701±153	1377±54	929.6±44.6	901.2±87.3	366.4±16.0
Dopamine	11.70±1.27	140.1±7.18	544±82.0	256.6±41.1	75.6±7.2	95.6±6.0	43.1±4.2
Adrenaline	0.40±0.08	28.42±10.06	67.0±20.5	32.7±1.6	7.4±1.8	5.04±1.47	2.8±0.6
MA	1.21±0.28	18.14±3.10	10.5±4.3	53.8±7.8	0.17±0.01	1.58±0.85	1.03±0.33

Values for retina, ng/mg protein: other brain areas, pg/mg wet weight ±s.e. mean. *Midline nuclei.

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68P IMPORTANT METHODOLOGICAL CONSIDERATIONS IN REUPTAKE EXPERIMENTS USING [^{3}H]-MONOAMINES

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The methodology for [^{3}H]monoamine reuptake is well established. The accepted convention has been the routine inclusion of EDTA, ascorbic acid and a monoamine oxidase inhibitor, although there is little evidence that such precautions are necessary. Reuptake can be classified as either carrier-mediated (the difference between reuptake at 37°C and 0°C) or specific (defined with an uptake inhibitor). This may have important consequences for the results generated. In view of this, we have determined the effects of EDTA, ascorbic acid and pargyline on [^{3}H]monoamine reuptake into rat brain synaptosomes and have subsequently compared the kinetic parameters obtained for carrier-mediated and specific reuptake.

Crude synaptosomes were prepared from frontal cortex [for 5-HT and noradrenaline (NA)] or striatum [for dopamine (DA)] and incubated with unlabelled and [^{3}H]monoamine ([^{3}H]5-HT, 2.0nM; [^{3}H]NA, 20nM; [^{3}H]DA, 2.5nM) for 5 min at 37°C. Carrier-mediated reuptake was defined at 0°C and specific reuptake was defined with zimeldine 10^{-5}M (5-HT), desipramine 10^{-4}M (NA) or GBR 12909 10^{-5}M (DA). Reuptake was terminated by filtration. Effects of EDTA (0.13mM), ascorbic acid (1.1mM) and pargyline (50µM) were determined at a single concentration of unlabelled 5-HT (48nM), NA (130nM) or DA (197.5nM). Kinetic parameters were determined at 12 concentrations of unlabelled 5-HT (4-100nM), NA (20-750nM) or DA (20-700nM).

EDTA, ascorbic acid and pargyline had no beneficial effect on either carrier-mediated or specific reuptake of any monoamine. In fact, there was a non-significant 10-15% reduction in uptake in the presence of all three agents, whereas ascorbic acid alone reduced carrier-mediated and specific 5-HT reuptake by 21% ($p<0.01$) 22% ($P<0.05$) respectively. Comparison of kinetic parameters showed that Km and Vmax values for 5-HT and NA, but not for DA, were markedly lower for specific reuptake than for carrier-mediated reuptake (Table 1).

Table 1 Kinetic parameters for carrier-mediated and specific [^{3}H]monoamine reuptake

		5-HT	NA	DA
Km	Carrier-mediated	83 ± 4	261 ± 17	288 ± 24
	Specific	63 ± 3**	137 ± 5***	250 ± 20
Vmax	Carrier-mediated	5.23 ± 0.29	2.54 ± 0.20	98.6 ± 9.6
	Specific	3.80 ± 0.24**	1.44 ± 0.09***	89.6 ± 9.3

Km(nM), Vmax (pmol/mg protein/min). Values ± s.e. mean (n = 5-6) **p<0.01, ***p<0.001.

Hence, the results clearly illustrate that EDTA, ascorbic acid and pargyline do not improve monoamine reuptake into rat brain synaptosomes and this questions the motives behind their routine inclusion in these assays. In addition, it is unclear why there is a disparity between the kinetic parameters obtained using the carrier-mediated and specific reuptake approaches. However, the additional sites defined by carrier-mediated reuptake may be related to non-specific binding of the [^{3}H]neurotransmitters to the tissue.

69P SELECTIVE INHIBITION OF MAO-B IN RAT BRAIN BY RO 19-6327: CORRELATION WITH BINDING *IN VIVO*

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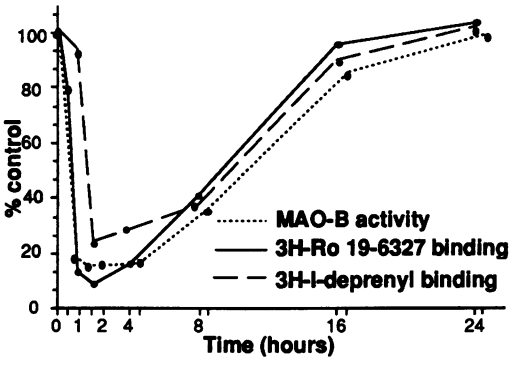
Ro 19-6327 [N-(2-aminoethyl)-5-chloro-2-pyridine carboxamide-HCl], a selective, reversible MAO-B inhibitor in rat brain and peripheral organs (Da Prada et al., 1987), is a potential antiparkinson agent. [^{3}H]Ro 19-6327 binds to human brain homogenates with high affinity, is saturable and specific (Cesura et al., 1989). Recently, the radioligand has been used to map the distribution and concentration of MAO-B in rat CNS, peripheral organs and human post-mortem brain by quantitative enzyme radioautography (Richards et al., 1988; Saura Marti et al., 1989).

We have now studied the time course of MAO-B inhibition and binding inhibition by Ro 19-6327 in rat brain in vivo. Male albino rats (~ 120 g) were administered Ro 19-6327 (0.71 mg/kg p.o.) at various times (30 min, 1, 2, 4, 8, 16, 24 h; n=3) prior to a tail vein injection of either [^{3}H] Ro 19-6327 or [^{3}H] l-deprenyl (s.a. 18.9 and 26.3 Ci/mmol resp.; 1 mCi/kg) and sacrificed after 30 min or 1 h respectively. Cryostat sections of brains from animals receiving the radiolabel were either untreated or briefly rinsed in buffer prior to exposure to LKB Ultrofilm® for 6 wks and analysed by quantitative radioautography. Brains of animals receiving non-radioactive Ro 19-6327 i.v. (12.5 µg/kg) and p.o. were used to measure MAO-B activity radiochemically (substrate phenylethylamine, 20 µmol/l).

As shown in the adjacent Figure, the time course of binding inhibition correlated extremely well with that of enzyme inhibition, although sections radiolabelled with [^{3}H]Ro 19-6327, but not with [^{3}H]l-deprenyl, required a brief rinse to remove loosely bound or unbound radioactivity.

We conclude that the kinetics of Ro 19-6327 binding *in vivo* are such that it could be used to estimate the degree and time course of enzyme inhibition in brains of healthy volunteers by PET analysis of [^{11}C]l-deprenyl binding.



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70P INHIBITION OF SYMPATHETIC NERVE ACTIVITY BY TRICYCLIC AND MONOAMINE OXIDASE-INHIBITOR ANTIDEPRESSANTS

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One of the frequent side effects of treatment with both tricyclic and monoamine oxidase (MAO) inhibitor antidepressant drugs is orthostatic hypotension. Theories which have been advanced to explain this phenomenon include both CNS and peripheral mechanisms. We have monitored sympathetic nerve activity by direct electrophysiological technique, in order to determine whether acute inhibition of neuronal NA uptake or MAO causes a reduction in sympathetic tone.

Male rats (230-260g) were anaesthetised with a mixture of chloral hydrate (60 mg/kg) and pentobarbitone (15 mg/kg). Electrical activity was monitored in the left renal nerve using fine Pt/Ir electrodes. Following amplification, the signal was filtered to accept frequencies between 100 and 2000 Hz, passed into a discriminator to eliminate low and high level activity, the discriminator output integrated, and the integrated signal displayed on a chart recorder.

Administration of both desipramine (0.3 mg/kg i.v.) and clorgyline (2 mg/kg i.p.) caused a parallel fall in blood pressure (BP) and in renal nerve activity (RNA). RNA fell by 51±9.6% and by 34±11.1% for desipramine and clorgyline respectively, and blood pressure fell by 16±5.9 and 31±5.4 mm Hg (30 min after injection, mean ±SEM, n=5). No significant changes occurred in either parameter following administration of maprotiline (1 mg/kg i.v.), which has less clinical hypotensive effect. Both parameters were also stable in control experiments over the period under study (90 min). The decrease in RNA and BP by both DMI and clorgyline was absent in rats pretreated with yohimbine (0.3 mg/kg). Yohimbine itself caused a brief fall followed by a rise (12±2.2 mm HG) in BP, and an increase (18±5.1%) in RNA. Pretreatment of the animals with prazosin (0.05 mg/kg i.v.) caused a reduction in BP (32±8.2 mm Hg) and an increase in RNA (11±11.6%). Subsequent injection of desipramine still caused a reduction in RNA by 22±4.4%.

The reduction in RNA by desipramine and clorgyline could be effected either by an action in the CNS or ganglia. A ganglionic effect is considered unlikely, since desipramine has also been shown to reduce preganglionic lumbar nerve activity in the rabbit (Cohen et al., 1989) and because of the ability of yohimbine to block this response. The data are therefore compatible with an action of desipramine and clorgyline to activate CNS alpha-2 adrenoceptors, resulting in a reduction in sympathetic tone, but the involvement of alpha-1 adrenoceptors in this effect cannot be ruled out.

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71P NIGULDIPINE DIFFERENTIATES BETWEEN α_1 ADRENOCEPTOR SUBTYPES LINKED TO INOSITOL PHOSPHOLIPID HYDROLYSIS AND POTENTIATION OF CYCLIC AMP FORMATION IN RAT BRAIN

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In rat cerebral cortex, $\alpha_1$adrenoceptors are linked to two signal transduction mechanisms, inositol phospholipid hydrolysis and potentiation of β-adrenoceptor stimulated cyclic AMP. The pharmacological profile suggests that different $\alpha_1$subtypes are involved (Robinson and Kendall, 1989). To date, there are no $\alpha_1$adrenoceptor antagonists which can specifically discriminate between $\alpha_1$mediated phospholipid hydrolysis and potentiation of cyclic AMP such that the cyclic AMP response persists in the absence of inositol phospholipid hydrolysis. Recently a binding study by Boer et al., (1989) has shown that the 1,4 dihydropyridine, niguldipine can discriminate between $\alpha_1$adrenoceptor subtypes in rat brain. Therefore, we have examined the effect of both isomers of niguldipine on noradrenaline stimulated cyclic AMP and inositol phospholipid hydrolysis and compared the results with the effect of a calcium channel inhibitor, PN200110 (Isopropyl 4-(2,1,3, benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxy-carbonyl-pyridine-3-carboxylate).

Total ^{3}H-inositol phosphate (^{3}H-IP) accumulation and ^{3}H-cyclic AMP formation in cerebral cortex slices from male Sprague Dawley rats were measured as previously described (Robinson and Kendall, 1989).

Noradrenaline (100μM) typically produced a 5 fold increase in ^{3}H-IP accumulation. (+) Niguldipine was a potent inhibitor of the response, with an IC_{50} of 81±21nM(n=3), Hill slope 0.80±0.1. The isomer (-) niguldipine was 100 fold less potent (IC_{50} 8.9±0.6μM) $nH_3$0.9±0.04). The calcium channel antagonist PN200110 had (up to 100μM) no effect on noradrenaline stimulated ^{3}H-IP accumulation. Isoprenaline (10μM) produced a 3 fold increase in ^{3}H-cyclic AMP formation. This was increased a further 3 fold by noradrenaline (100μM). The α-adrenoceptor component of the response was reduced by a maximum of 34% by (+) niguldipine (10μM). Higher concentrations of (+) niguldipine resulted in an increase in cyclic AMP levels. (-) Niguldipine reduced the α component of the response by 36% at 10μM. Higher concentrations (100μM) had no further effect. Neither isomer had any effect on isoprenaline stimulated cyclic AMP. PN200110 (up to 100μM) caused an increase (50±11%) in isoprenaline and noradrenaline stimulated cyclic AMP.

Niguldipine appears to be the first $\alpha_1$adrenoceptor antagonist to abolish noradrenaline stimulated inositol phosphate accumulation at concentrations which allow the cyclic AMP response to persist and provides further evidence that the $\alpha_1$adrenoceptors mediating inositol phosphate accumulation and cyclic AMP potentiation are different.

We wish to thank the SERC for financial support.

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72P INTERACTION BETWEEN ANTIDEPRESSANTS AND D-AMPHETAMINE ON OPERANT BEHAVIOUR

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d-Amphetamine can both facilitate and suppress operant behaviour, its effect on performance under variable-interval (VI) schedules of positive reinforcement being mainly suppressant (Morley et al., 1985). Tricyclic antidepressants can potentiate many of the pharmacological actions of d-amphetamine, for example locomotor activation (Dall'Olio et al., 1986). The present experiment examined whether two tricyclic antidepressants (imipramine, desipramine) and two "atypical" anditdepressants (fluvoxamine, trazodone) can potentiate the suppressant effect of d-amphetamine on VI performance.

Four experiments were carried out, each examining the effect of one antidepressant. In each experiment 12 female Wistar rats maintained at 80% of their free-feeding body weights were trained to press a lever under a VI 80-s schedule of sucrose (0.6M, 0.05 ml) reinforcement. At least 30 preliminary training sessions preceded the start of the drug treatment regimen. The effects of treatment with d-amphetamine sulphate (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/kg i.p.) or its vehicle (NaCl) were tested following pretreatment with the antidepressant (4, 8 mg/kg i.p.) or its vehicle (NaCl). Pretreatment injections were given 60 min before and treatment injections 10 min before the start of the 30-min session. The effects of d-amphetamine were expressed as percentage change in response rate compared with the response rate observed following treatment with the vehicle. The data were analyzed by two-factor analysis of variance (pretreatment, treatment) with repeated measures; the dose-response curves for d-amphetamine in the absence and presence of the antidepressants were compared using Dunnett's test (significance criterion, $P < 0.05$).

In all four experiments d-amphetamine had a dose-dependent suppressant effect on responding. Imipramine, desimpramine, and fluvoxamine significantly enhanced the suppressant effect of d-amphetamine, whereas trazodone had no significant effect.

d-Amphetamine is believed to act by releasing catecholamine neurotransmitters from presynaptic terminals, and potentiation of d-amphetamine's effects by tricyclic antidepressants may reflect the ability of these drugs to inhibit the catecholamine uptake (Dall'Olio et al., 1986). It is noteworthy that trazodone, which has little uptake-blocking action (Richelson & Pfenning, 1984), did not potentiate the effect of d-amphetamine. The fact that fluvoxamine, a selective blocker of 5-hydroxytryptamine (5-HT) uptake, did enhance the effect of d-amphetamine, may implicate 5-HT release in the behavioural actions of d-amphetamine.

This work was supported by Duphar B.V.

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73P REVERSAL BY INOSITOL OF THE PROCONVULSANT ACTION OF LITHIUM IN PILOCARPINE-TREATED MICE

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Lithium inhibits inositol-1-phosphatase activity in rat brain at concentrations close to those thought to be achieved during Li^+ therapy for manic depressive disorders in man (Sherman *et al*, 1986), leading to a decline in the availability of inositol for the phosphatidylinositol (Pi) second messenger system. Inositol depletion in the CNS may thus explain the ability of Li^+ to alter behavioural responsiveness to neurotransmitter receptor agonists. This has been investigated by examining the ability of inositol to reverse the proconvulsant effect of lithium on seizures induced by the cholinergic receptor agonist, pilocarpine.

Inositol or D-mannitol was co-administered intracerebroventricularly (icv) with either lithium chloride (LiCl) or 0.9% NaCl in a total volume of 5µl to male Swiss Webster mice (25-30g), 30 min before the administration of pilocarpine (100mg/kg i.p.). The latency for the induction of clonic seizures was then determined over the following 2 hours.

Neither LiCl (0.3-3µmol/mouse) nor pilocarpine induced seizures in mice when given alone. However, the latency to convulse was decreased dose-dependently by LiCl when given in conjunction with pilocarpine. Inositol co-administered with LiCl dose-dependently antagonised the proconvulsant effect of Li^+ when given centrally (Table 1), but high doses of inositol (up to 1g/kg) given intravenously were without effect. Inositol given icv (11µmol per mouse) also inhibited seizures when LiCl (10mmol/kg) was given subcutaneously. The ability of inositol to antagonise the proconvulsant effect of Li^+ was not mimicked by D-mannitol, a sugar that does not penetrate cell membranes. Furthermore, inositol (11µmol per mouse) did not antagonise seizures induced by pentylenetetrazol (120mg/kg, s.c.), indicating that inositol does not have anticonvulsant properties per se.

Table 1 MEAN LATENCY TO CONVULSE (min) ± s.e.mean (n = 8-32 mice per group)

LiCl	Inositol			D-mannitol	
	0	0.6	5.6	11	11
0	120.0	-	-	120.0	120.0
0.3	112.8 ± 7.3	120.0	120.0	120.0	-
1.0	102.7 ± 5.9*	103.8 ± 9.2	120.0	120.0	-
3.0	47.1 ± 2.8*	68.2 ± 11.5*	88.4 ± 5*	120.0	65.3 ± 6.6*

Doses are µmol per mouse, icv. *$p < 0.05$ vs non-lithium treated controls (ANOVA)

Thus, inositol selectively antagonises the proconvulsant action of Li^+ in pilocarpine-treated mice by a central mechanism, consistent with an involvement of the Pi second messenger system.

Sherman, W.R. *et al*, (1986) Fed. Proc. 45, 2639-2646.

74P EFFECTS OF ANTIDEPRESSANTS ON PRE- AND POST-SYNAPTIC α_2-ADRENOCEPTORS ASSESSED BY CLONIDINE HYPOACTIVITY AND MYDRIASIS

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The hypoactivity (sedation) and mydriasis (pupil dilatation) responses evoked by clonidine in conscious mice can be respectively used as functional indices of pre- and postsynaptic α_2-adrenoceptors in the CNS (Heal et al 1988a). However, adaptive changes in these adrenergic receptors, especially postsynaptic α_2-adrenoceptors, occurring after antidepressant administration are disputed. Consequently, we have used hypoactivity and mydriasis to simultaneously determine the effects of various antidepressants and electroconvulsive shock (ECS) on pre- and postsynaptic α_2-adrenoceptor function. In addition, sibutramine HCl (BTS 54 524), a novel antidepressant which rapidly down-regulates ß-adrenoceptors (Buckett et al 1988), was also examined for its effects on central α_2-adrenoceptors.

Male C57/Bl/6Ola mice (Olac, Bicester) weighing 20-30g were used. Drugs were dissolved in saline and injected i.p. (1 ml/100g). Antidepressants were injected daily for 14 days at the following doses (mg/kg):- sibutramine HCl (SIB, 3) dothiepin (DOTH, 50) amitriptyline (AMI, 10) desipramine (DMI, 10) zimeldine (10), tranylcypromine (TCP, 10) mianserin (10), clenbuterol (5). ECS (200V, 2s) was given to anaesthetised mice 5 times over 10 days. Responses to clonidine (0.1 mg/kg) were tested 24h after acute and chronic antidepressant treatment. Pupil diameter was measured before and 10 min after clonidine as described by Heal et al (1988b). Hypoactivity was then immediately rated 0-3 on 5 behavioural parameters at 10 min intervals for the following 50 min (modified from Drew et al 1979).

Clonidine produced a mean increase in pupil diameter of 0.17 ± 0.01 mm (n = 54) and an accumulated hypoactivity score of 26.3 ± 0.5 (n = 53). These responses were not affected by a single treatment with antidepressants or ECS. However, repeated administration of some antidepressants and ECS attenuated both hypoactivity and mydriasis (Table 1). Zimeldine, mianserin and clenbuterol were without effect.

TABLE 1 Reversal of clonidine responses by repeated treatment with various antidepressants and ECS

Response	SIB	DOTH	AMI	DMI	TCP	ECS
Hypoactivity	27 ± 5**	32 ± 7**	10 ± 2*	29 ± 8**	27 ± 3***	32 ± 4***
Mydriasis	32 ± 8*	42 ± 7***	29 ± 8*	35 ± 8*	38 ± 5***	60 ± 9***

Results are % reversal of control response ± s.e.mean. *P<0.05, **P<0.01, ***P<0.001 (n=7-9).

Overall, the results show that ECS and antidepressants which inhibit noradrenaline reuptake or monoamine oxidase attenuated pre- and postsynaptic α_2-adrenoceptor function after repeated administration. Selective 5-HT uptake inhibitors and atypical antidepressants did not affect either α_2-adrenoceptor population.

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75P CONTRASTING EFFECTS OF CHRONIC NICOTINE ON TYROSINE HYDROXYLASE ACTIVITY IN NORADRENERGIC AND DOPAMINERGIC NEURONES

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Chronic nicotine treatment increases the activity of tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine synthesis, in predominantly noradrenergically innervated areas but not in dopaminergic projection areas, within the time scale of 7 to 28 days (Smith & Joseph, 1989). We have now made a closer study of the effect of daily nicotine administration for 3, 5, 7 and 9 days, in noradrenergic cell bodies and terminals and in dopaminergic cell bodies in the substantia nigra (SN) and ventral tegmental area (VTA). In addition, nicotine was given for 7 days only, sufficient to elicit a response in the locus coeruleus (LC), then TH activity was assayed in the hypothalamus (HYP) and hippocampus (HIP) 14 days later. Dosing, tissue dissection and TH assays were as reported in Smith & Joseph, 1989.

Differences between saline injected and uninjected controls were not significant in any region studied although there was a trend for saline injection to increase TH activity in noradrenergic areas. Significant ($P<0.02$ or better) increases in TH activity were seen after 3 days' nicotine administration in the SN and VTA (170% and 173% of uninjected controls respectively), but declined to 100% (SN) and 120% (VTA) by 9 days despite continued nicotine administration. TH activity was increased to 150-180% of control in the LC from 3 to 9 days. An increase in TH activity in the cerebellum to 160% of control was observed only at 9 days. 7 days' nicotine treatment resulted in increases 14 days later in the HYP and HIP to 147% and 182% of saline controls respectively. This is closely similar to respective increases of 147% and 180% following continuous nicotine administration for 21 days (Smith & Joseph, 1989).

The early, transient increases in TH activity in dopaminergic neurones are consistent with activation of the enzyme (Zigmond, 1988). We have found no increases in TH activity in dopaminergic terminals at later times. By contrast, in noradrenergic neurones, a relatively early increase in enzyme activity at the cell bodies is followed by an increase in activity at the terminals even without continued administration of the drug. This is consistent with induction of the enzyme at noradrenergic cell bodies followed by axonal transport to the terminals, an effect analogous to that of single dose reserpine (Zigmond, 1988).

KMS is the holder of a Wellcome Trust Prize Studentship.

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76P INTERACTION OF CY 208-243 WITH D_1 DOPAMINE RECEPTORS: INFLUENCE OF GPP(NH)P

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CY 208-243 ((-)-4,5,6,6a,7,8,12b-hexahydro-7-methyl-indolo-(4,3-ab)-phenanthridine, CY) is a centrally active D-1 dopamine receptor partial agonist (Markstein et al., 1988). In the presence of CY the apparent dissociation constant (K_d) for the D-1-selective antagonist ligand 3H-SCH 23390 binding is increased in a concentration-dependent manner and there is an apparent decrease in the number of binding sites (B_{max}) (O'Boyle & Waddington, 1988). The present study investigates possible mechanisms underlying the CY-induced loss of D-1 receptors.

The binding of 3H-SCH 23390 to rat striatal membranes was carried out as previously described (O'Boyle & Waddington, 1987). Saturation curves were constructed from 6 concentrations of ligand in the presence and absence of 2 μM CY. A parrallel series of tubes contained 100 μM of the stable GTP analogue, Gpp(NH)p. Results are shown in the Table.

		K_d nM	B_{max} pm/g	n	
	control	0.34 ± 0.03	70.6 ± 5.8	4	
	CY 2μM	4.50 ± 0.64	48.7 ± 6.5*	4	
+Gpp(NH)p	control	0.33 ± 0.02	73.7 ± 6.1	4	means ± s.e.mean
	CY 2μM	3.71 ± 0.71	63.3 ± 6.4	4	*$p<0.05$ vs control

CY caused a significant reduction in B_{max} of 31% and a >10-fold increase in K_d. In the presence of 100 μM Gpp(NH)p, control binding values were unaltered but CY no longer caused a reduction in B_{max} while still causing an increase in K_d. In a separate series of experiments, the effects of pretreatment of membranes with CY on K_d and B_{max} were found to be readily reversed by washing the membranes.

These data suggest that CY may cause an apparent loss of D-1 receptors by displacing 3H-SCH 23390 from two sites for which 3H-SCH 23390 has equal affinity and for which CY has high and low affinity respectively. Since Gpp(NH)p reverses the the effect of CY on B_{max}, the CY-mediated loss of receptors may be explained as the masking of receptors with high affinity for CY.

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This work was supported by the Royal College of Surgeons in Ireland and the Health research Board.

77P PURPOSELESS CHEWING AND FACIAL TREMOR IN RATS: AN ELECTROMYOGRAPHICAL INVESTIGATION

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Administation of SKF 38393 to rats induces an increase in the frequency of purposeless chewing and facial tremor (Rosengarten et al, 1983), whilst only purposeless chewing has been reported to follow pilocarpine administration (Salamone et al, 1986). Using an electromyographical (EMG) detection system, combined with visual observations we have investigated the relationship between purposeless chewing and facial tremor in freely moving rats.

EMG signals were obtained from the right masseter muscle of male Wistar rats (300-325g at the time of surgery), using two stainless steel bipolar electrodes. These were implanted on to the outer surface of the masseter muscle sheath. After differential pre-amplification and filtration (Krohn Hite 3700, 150-300 Hz 48db/octave, with a 20db gain) the signal was stored on magnetic tape (Ampex PR 2230 16 channel FM data recorder). Rats were observed immediately following drug administration and were visually scored for the presence of purposeless chewing and facial tremor.

Acute administration of the D-1 agonist SKF 38393 (2.0-10.0mg/kg, sc), resulted in a dose dependent increase in the incidence of facial tremor and purposeless chewing. Acute administration of the anticholinesterase physostigmine (0.1-0.4mg/kg,ip) also resulted in a dose dependent increase in the incidence of facial tremor and purposeless chewing (Table 1.).
Purposeless chewing did not produce a consistent change in the basal EMG signal and was scored as present or absent on the basis of visual observations. Facial tremor, involving the lower jaw and masseter muscles produced a characteristic EMG signal with a duration between 1.0 and 1.5 seconds. The signal consisted of several distinct groups of high amplitude EMG spikes, separated by periods of EMG inactivity. The EMG signal had a periodicity of between 7 and 10 Hz. Facial tremor was often observed in bursts, coinciding with increased purposeless chewing frequency.

Table 1. Effect of drug treatment on facial tremor and purposeless chewing incidence in rats.

Treatment	Facial Tremor	Purposeless Chews	Treatment	Facial Tremor	Purposeless Chews
Control	2.3 ± 1.7	69.7 ± 26.9	Control	3.0 ± 2.3	93.3 ± 25.6
SKF 38393 2mg/kg	17.0 ± 5.6	214.5 ± 31.7	physo 0.1mg/kg	5.2 ± 3.3	473.2 ± 100.0
SKF 38393 5mg/kg	30.5 ± 5.2	247.8 ± 42.7	physo 0.2mg/kg	11.2 ± 3.7	436.5 ± 33.5
SKF 38393 10mg/kg	22.3 ± 4.9	296.2 ± 39.4	physo 0.4mg/kg	20.0 ± 5.0	644.3 ± 68.7
	H = 12.2*	F = 8.7Δ		H = 8.5*	F = 12.8Δ

All results expressed as mean ± SEM, Facial tremor analysed using Kruskal-Wallis ANOVA * $P < 0.05$, chews, one-way ANOVA Δ $P < 0.01$
Although identical purposeless chewing and facial tremors are induced following administration of SKF 38393 and physostigmine, the preferential induction of one of the behaviours by each drug suggests that facial tremor and purposeless chewing are separate behaviours. This electromyographical detection system is ideally suited to further investigate the relationship between these behaviours.

78P BLOCKADE OF D_1 DOPAMINE RECEPTORS BY SCH 23390 LEADS TO ENHANCEMENT OF REM SLEEP IN THE RAT

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The D-1 dopamine receptor antagonist SCH 23390 (SCH) induces several behavioral effects similar to those of nonselective neuroleptics. A specific response associated with D-1 receptors blockade only has not emerged yet. While studying the sedative potential of SCH by recording the sleep-waking cycle in the rat, we observed that the drug markedly enhanced rapid eye movement (REM) sleep. Except for chlorpromazine (Kafi & Gaillard, 1978), most neuroleptics have little or no effect on REM. Thus, we designed a study to assess the effects of SCH on REM patterns, as compared with haloperidol (HAL).

Male Sprague-Dawley rats were implanted with electrodes for recording of electroencephalographic (EEG) activity. Recordings (6 h) were scored visually and stages of wakefulness (W), non-REM sleep (NREM) and REM sleep (REM) were classified. REM was evaluated as duration, number of episodes and latency of the first episode after onset of NREM. Since the analysis revealed that drugs effects were significant in the first 3-h only, we refer to this part of the recordings. HAL (0.1-1 mg/kg po) did not change REM patterns. SCH (0.003-0.03 mg/kg sc) increased both REM duration and number of episodes, and was fully effective even at the lowest dose used (0.003 mg/kg). Unlike cholinergic agents (Gillin and Borbély, 1985) SCH did not modify significantly REM latency after NREM onset. The Table shows data related to 3-h recording period.

Drug	Dose (mg/kg)	Duration (min)	Episodes (No.)	Latency (min)
C	--	4.5 ± 1.1	2.2 ± 0.4	55.4 ± 8.7
HAL	0.1	3.2 ± 2.0	1.5 ± 0.8	44.3 ± 15.5
	0.3	5.5 ± 2.0	2.3 ± 0.8	78.7 ± 15.5
	1	5.8 ± 2.0	2.8 ± 0.8	61.3 ± 15.5
SCH	0.003	10.1 ± 1.9 *	4.7 ± 0.7 *	39.1 ± 14.4
	0.01	10.3 ± 1.4 **	4.2 ± 0.5 *	53.5 ± 10.6
	0.03	9.4 ± 1.4 *	3.8 ± 0.5 *	50.1 ± 10.6

* $p<0.05$; ** $p<0.01$ compared with controls (t-LSD test).

Considering the very low doses of SCH used it seems that D-1 receptor blockade specifically promotes the generation of REM. Moreover, these findings are relevant in view of the significance of REM as a marker of affective disorders (Gillin & Borbély, 1985) and the limited number of REM-enhancing drugs available.

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79P *IN VIVO* BINDING OF THE REPORTEDLY SELECTIVE D_2 AGONIST N-0437 IS NOT DISPLACED BY DOPAMINERGIC LIGANDS

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The non-catechol aminotetralin N-0437 exhibits an apparently selective dopaminergic D2 agonistic profile in certain *in vitro* and *in vivo* preparations (own observations and van der Weide et al. 1986, 1988). In order to relate *in vitro* observations with behavioural data we have investigated the binding properties of 3H-N-0437 *in vivo* in rat brain.
For the determination of total binding, 15µCi 3H-N-0437 (73.6Ci/mmol,Amersham) was administered via the tail vein to male rats (100-150g). After 50 min animals were decapitated, brains were removed and striata, dissected on ice, were homogenised in 9ml phosphate buffer (25mM, pH7.5). Aliquots of homogenate (3ml) were immediately filtered under vacuum, filters were washed (3x5ml ice cold buffer) and radioactivity estimated by scintillation spectrometry. Amount of ligand bound was expressed as cpm/g tissue. Estimations were made in triplicate using each of 5 separate brains per condition. For comparison, the *in vivo* binding of 3H-spiroperidol (71.5 Ci/mmol) was examined, employing the same protocol. In attempts to determine the nonspecific binding of the respective ligands, a range of compounds shown *in vitro* to be potent displacers in striatal membrane preparations were administered intraperitoneally 10 min before tracer infusion.
Under these conditions, total binding of 3H-N-0437 in striatal membranes represented 147804 ± 29943 cpm/g tissue (mean ± S.D.,n=20). However, administration of either the D2 antagonist spiroperidol (0.002-10 mg/kg) or the potent D2 agonists lisuride (5mg/kg), PHNO (0.25 mg/kg) and apomorphine (50 mg/kg) failed to reduce the total binding of 3H-N-0437.Only unlabelled N-0437 displaced the tritiated compound, reducing binding to 3.5 ± 0.8% (mean ± S.D.,n=5) of total level following 10 mg/kg i.p. In contrast, in the same conditions, these compounds were effective displacers of *in vivo* 3H-spiroperidol binding in striatum (e.g. lisuride, 5mg/kg, displaced 100%).
These observations can be interpreted in several ways. First, a tritiated metabolite of N-0437 may be binding to non-dopaminergic sites which then mask the D2 binding of the unchanged compound. Second, the bound label may represent unchanged N-0437 bound to non-dopaminergic sites, while its pharmacological D2 activity is mediated by an unlabelled metabolite. Third, the binding may represent that of a metabolite which exerts a functional D2 agonism not directly mediated by dopaminergic receptors.

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80P CARBIDOPA PRETREATMENT PREVENTS THE ALTERATIONS IN L-DOPA PHARMACOKINETICS AFTER CHRONIC TRREATMENT OF RATS WITH L-DOPA PLUS CARBIDOPA

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Chronic treatment of Parkinson's disease with L-DOPA plus a peripheral dopa decarboxylase inhibitor leads to response fluctuations. The reasons for these fluctuations remain unknown, but may relate to alterations in the pharmacokinetic handling of L-DOPA during long-term therapy. Previously we reported that 12 months administration of L-DOPA plus carbidopa to rats resulted in an altered pharmacokinetic profile following an acute oral (po) or intra-aortic (ia) bolus of L-DOPA alone (Rose *et al.* 1989). We now report the effect of chronic administration of L-DOPA plus carbidopa on the pharmacokinetic profile and peripheral metabolism of an acute bolus of L-DOPA to rats pretreated with carbidopa.

Male Wistar rats (125 ± 2g) received L-DOPA (191-220 mg/kg/day) plus carbidopa (24-26 mg/kg/day), or carbidopa (24-26 mg/kg/day) via their daily drinking water for 12 months. Control rats received normal tap water. Animals were withdrawn from drug treatment for 4 days and fasted for 16 hours prior to testing. Two days prior to drug administration, rats were cannulated in the descending aorta. Animals were treated with carbidopa (25mg/kg ip) 1 hour prior to administration of L-DOPA (50 mg/kg po or ia). Blood samples (200µl) were collected from the aortic cannula 15 minutes prior to, and at intervals up to 6 hours after L-DOPA administration. Plasma concentrations of L-DOPA, 3-OMD, dopamine, DOPAC and HVA were measured using HPLC with electrochemical detection.

Chronic administration of L-DOPA plus carbidopa had no effect on the t1/2, Vd, Clp or AUC of L-DOPA after acute oral or intra-aortic administration to rats pretreated with carbidopa (Table 1). The area under the plasma concentration-time curve for 3-OMD, dopamine, DOPAC and HVA after acute administration of L-DOPA to carbidopa-pretreated rats were also unaffected by chronic drug administration.

Table 1 L-DOPA pharmacokinetics in rats pretreated with carbidopa

		CONTROL		CARBIDOPA		L-DOPA plus CARBIDOPA	
		po	ia	po	ia	po	ia
t1/2	(h)	1.1 ± 0.1	1.0 ± 0.1	2.3 ± 0.3	1.0 ± 0.2	1.6 ± 0.5	0.8 ± 0.0
Vd	(L/kg)	1.2 ± 0.5	1.2 ± 0.2	3.4 ± 1.0	1.2 ± 0.2	2.5 ± 1.1	1.1 ± 0.2
Clp	(L/kg/h)	0.6 ± 0.2	0.8 ± 0.1	1.0 ± 0.3	0.8 ± 0.1	1.1 ± 0.4	1.0 ± 0.2
AUC	(mg.h/L/kg)	131 ± 9	254 ± 12	100 ± 18	258 ± 38	110 ± 18	255 ± 23

The peripheral pharmacokinetics and metabolism of acutely administered L-DOPA to rats pretreated with carbidopa was unaltered by chronic L-DOPA plus carbidopa administration. This is in contrast to the altered pharmacokinetics of L-DOPA when administered alone where the Vd and Clp were increased after chronic L-DOPA plus carbidopa administration (Rose *et al.* 1989). This suggests that alterations in the metabolism of L-DOPA after chronic administration of the drug may be implicated in the onset of fluctuations in Parkinson's disease, but only if used alone.

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81P ALTERATIONS IN BRAIN IRON AND FERRITIN LEVELS IN MULTIPLE SYSTEM ATROPHY AND HUNTINGDON'S CHOREA

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Neuronal loss in Parkinson's disease (PD) and progressive supranuclear palsy (PSP) is associated with increased total iron levels (Sofic et al, 1988; Dexter et al, 1989). However in PD, but not in PSP, ferritin levels were reduced, suggesting altered iron handling may occur in PD. Zinc levels were also selectively increased in PD brain. We now report on two other basal ganglia neurodegenerative diseases namely, multiple system atrophy (MSA) (cell loss in nigra, putamen and caudate) and Huntington's chorea (HC) (cell loss in putamen and caudate.

Post-mortem brain tissue was obtained from 8 MSA patients (mean age 63.4 years ± 3.0) and 13 control patients (mean age 66.0 years ± 3.9). HC tissue was obtained from 4 patients (mean age 67.0 years ± 5.9) and from 25 control patients (mean age 73.1 years ± 3.5). Patients were matched for other post-mortem parameters. Brain tissue was solubilised, and the iron and zinc content measured by inductively coupled plasma (ICP) spectroscopy. Brain ferritin levels was measured by a RIA technique using an antibody raised against human spleen ferritin.

In patients with MSA no differences in toatal iron levels were observed in the cerebral cortex, globus pallidus or cerebellum, but increased iron levels were found in substantia nigra, putamen and caudate when compared to control tissue (Table 1). Ferritin levels were increased in the substantia nigra and putamen of MSA patients when compared to controls, but not in other brain regions. In HC total iron levels were increased in the putamen and caudate, but not in the substantia nigra, cerebellum or cerebral cortex when compared to control tissue (Table 1). No difference was observed in ferritin levels in all areas of the HC brain tested compared to control tissue. No changes in zinc levels were found in MSA or HC.

Table 1 Total iron and levels in MSA, HC and control brains

BRAIN AREA	TOTAL IRON (nmoles/g dry weight)			
	CONTROL	MSA	CONTROL	HC
Caudate nucleus	8167 ± 467	10689 ± 566*	11174 ± 999	17409 ± 2019*
Putamen	(L)12388± 2158	(L)19690 ± 3152	(T)14012 ± 1423	(T)20177 ± 6877*
	(M)1118 ± 1415	(M)18622 ± 2482*		
Substantia nigra	(T)10436± 1191	(T)16606 ± 1202*	(C)13266 ± 1515	(C)12901 ± 1367

Values represent mean ± SEM. * P< 0.05 compared to controls (Student's t test). T= Total, C= Compacta, L= Lateral, M= Medial

Altered iron levels are not specific to PD and are associated with neurodegeneration in a variety of basal ganglia disorders. In PSP, MSA and HC, but not in PD, increased iron levels are associated with increased or normal ferritin levels. Only in PD does the normal physiological response to increased iron i.e stimulation of ferritin synthesis, not occur.

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82P GENERATION IN THE PAF-INJECTED RAT PLEURAL CAVITY OF EOSINOPHILIN, A CHEMOATTRACTANT FOR EOSINOPHILS

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The role of PAF in acute anaphylaxis is now recognized. PAF is also a potent in vitro chemoattractant agent for human, rat and guinea-pig eosinophils. Since it is difficult to reconcile the rapid degradation of PAF in biological fluids with a putative role as a trigger for in vivo eosinophilia, we now studied its ability to induce pleurisy in the rat, and demonstrated that the late (after 6 h) and prolonged (up to 24 h) eosinophilia is accounted for by the formation of a chemoattractant specific for eosinophils, which was named eosinophilin. In a first set of experiments, PAF (1ug) in 0.1 ml of Na Cl 0.9%-BSA 0.01% was injected into the pleural cavity of unaesthetized male Wistar rats of approximately 250g. Exudation and unaccompained by pleural leukocyte content alterations were noted 1 h later. Six hours after challenge, exudation was reduced and the pleural washing contained a mixture of mononuclear cells, neutrophils and eosinophils. After 24 h and up to 72 h later, only eosinohils were noted. The administration of PAF and its antagonist BN 52021 (5-20 ug) of dexamethasone (10-100 ug) or of nedocromil sodium (10-60 ug) suppressed the acute effects as well as eosinophilia evaluated after 24 hours. The transfer of the pleural washings of PAF-injected animals to the pleural cavity of recipient rats, induced eosinophilia after 18-24 h which was not noted if BN 52021, dexamethasone or nedocromil sodium were administered to the donor animals. These inhibitors were ineffective when they were administered to the recipient animals before the pleural washings of the PAF-injected animals, suggesting that an eosinophilic principle was formed in the pleural cavity of the donor animals, which was named eosinophilin. In confirmation, these washings induced a marked and concentration-dependent eosinophil migration when tested in vitro, with a modified micro- Boyden chamber. It is noteworthy that generation of the eosinophil chemoattractant occured from 6 h after the intra-pleural administration of PAF. The mixed cyclooxigenase inhibitor BW 755C (25 mg/kg, i.p. 1h before PAF), did not block the formation of eosinophilin but the co-administration of PAF with the protein synthesis inhibitor cycloheximide or the transcription inhibitor actinomycin D (100 or 30 n moles/cavity), suppressed altogether eosinophil attraction by PAF in recipient animals and formation of eosinophilin detected in vivo. Since eosinophilin failed to attract neutrophils, it is distinct from IL-8 and should be a novel activity with a potentially important role in diseases where eosinophils are involved.

83P PAF-INDUCED INTRATHORACIC NEUTROPHIL ACCUMULATION IN ANAESTHETISED GUINEA-PIGS

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Intracutaneous injection of PAF causes sequential accumulation of both platelets and neutrophils, whilst intravenous injection of PAF leads to formation of thrombi which include neutrophils in the pulmonary microcirculation (Dewar et al., 1984). Recently, it has been possible to define the kinetics of intrapulmonary accumulation and dispersal of radiolabelled platelets in response to PAF (Smith et al., 1989). This method has been adapted to study the interaction of neutrophils and platelets in response to PAF in vivo.
Neutrophils were isolated from peripheral blood by sedimentation in methylcellulose/hypaque followed by centrifugation on discontinuous Percoll density gradients. Neutrophils (>90% pure, >95% viable) were incubated with ^{111}Indium-oxine (25 µCi/3 X 10^6 cells). The labelled cells (10 X 10^6/ animal) were injected intravenously into spontaneously breathing animals anaesthetised with a combination of xylazine (3 mg/kg, i.m.) and ketamine (30 mg/kg, s.c. and 15mg/kg/h, i.v.). Radioactivity over the thorax was measured using NaI crystal scintillation detectors (Thorn EMI, UK) interfaced with an AIMSplus isotope monitoring system (Mumed Ltd., UK).
Immediately after injection of labelled neutrophils, thoracic count rates were high but fell rapidly to a slowly declining plateau (% decrease: 0-30 min, 32%; 0-60 min, 41%). PAF infusions (90 ng/kg, i.v., over 5 min) caused reproducible increases in thoracic counts (response area: 1st infusion, 22.9; 2nd, 22.2) Blood volume, measured as changes in thoracic count rate of ^{111}In-oxine labelled erythrocytes in separate experiments, fell sharply in response to PAF (response area: 1st infusion, -17.6; 2nd, -13.5). Pretreatment of recipient animals with a polyclonal rabbit anti-guinea-pig platelet serum 24 h before injection of labelled neutrophils, reduced the platelet count to below detectable levels. However, the magnitude of neutrophil accumulation in response to PAF (90 ng/kg, i.v.) was not diminished in thrombocytopaenic animals compared to normal rabbit serum treated controls. Iloprost (10 ng/kg/min, i.v.) did not reduce the magnitude of increase in thoracic count rate in response to PAF (90 ng/kg, i.v.) yet abolished the corresponding response of platelets.
It is concluded that thoracic accumulation of neutrophils in response to intravenous PAF is independent of platelet activation.

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84P INTERFERENCE OF A NOVEL PAF ANTAGONIST COMPOUND, PCA 4248, WITH ACTIVE ANAPHYLACTIC SHOCK *IN VIVO* AND HYPERRESPONSIVENESS TO PAF IN PERFUSED GUINEA-PIG LUNGS

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A novel PAF antagonist, PCA 4248, was recently shown to block endotoxin-induced hypotension in the rat (Ortega *et al.*, this meeting). This led us to study its effects against active anaphylaxis in guinea-pigs sensitized with low amounts of ovalbumin (OA) [10 µg in Al (OH)3] injected i.p. twice at two weeks interval and challenged seven days later by the intratracheal (i.t.) instillation of 100 µg of OA under a volume of 100 µl. In preliminary experiments, PCA 4248 administered i.v. at 10 mg/kg, blocked significantly ($P<0.01$) bronchoconstriction by the i.t. administration of 1µg of PAF to non-sensitized guinea-pigs. Accordingly, PCA 4248 was administered to actively sensitized and boosted guinea-pigs at 30 mg/kg, i.v., 6 min before OA. As in case of other PAF antagonists described, bronchoconstriction of active shock in our model was not reduced by PCA 4248.
Since Desquand *et al.* (submitted) reported that the booster injection during immunization is critical for determining whether the anaphylactic bronchoconstriction becomes or not refractory to the PAF antagonists BN 52021 and WEB 2086, we performed experiments with guinea-pigs actively sensitized as above but not boosted. Under those conditions, PCA 4248 at 30 mg/kg reduced bronchoconstriction by the i.t. administration of OA (5-100 µg) ($P<0.05$). Bronchoconstriction was blocked more intensively when the compound was administered i.t. at 10 mg under a volume of 10 µl, 15 min before OA ($P<0.01$). Thus, PCA 4248 is indeed anti-anaphylactic in actively sensitized and non-boosted guinea-pigs.
Using the perfused lung, Pretolani *et al.*, (1988) demonstrated that active sensitization with a booster injection leads to bronchopulmonary hyperresponsiveness to PAF and to failure of the PAF antagonists BN 52021 and WEB 2086 to inhibit PAF itself *in vitro* (Pretolani *et al.*, 1989). This led us to further investigate the effects of PCA 4248 on PAF-induced bronchoconstriction, thromboxane B2 formation and histamine release using isolated lungs. In those from non-sensitized guinea-pigs, PCA 4248 (10^{-6} - 10^{-5} M) dose-dependently blocked bronchoconstriction by 100 and 1000 ng of PAF, as well as thromboxane B2 formation induced by 1000 ng of PAF. Surprisingly, in lungs from actively sensitized and boosted guinea-pigs, used at 10^{-5} M, PCA 4248 also blocked bronchoconstriction by 100 and 1000 ng of PAF, as well as thromboxane B2 formation and histamine liberation induced induced by 1000 ng of PAF, despite the negative results with the other antagonists refered to above.
In conclusion, the novel PAF antagonist PCA 4248, which inhibits anaphylactic bronchoconstriction of actively sensitized and non-boosted guinea-pigs, possesses additional original properties observed on perfused lungs, where it maintains its ability to antagonize PAF, irrespective of the immunitary status of the animal.

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85P WEB 2086 INHIBITS PAF-INDUCED PLATELET AGGREGATION AND PLASMA PROTEIN EXTRAVASATION IN THE HORSE

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Allergic conditions of the skin and lungs are commonly encountered in the horse. Since PAF has been implicated in the pathogenesis of such conditions in other species, and PAF antagonists are being used increasingly to evaluate the role of PAF in various physiological and pathophysiological processes, the inhibitory effect of WEB 2086 on PAF-induced responses has now been examined, initially in healthy horses.

WEB 2086 was administered intravenously (i.v.) at a dose of 3 mg/kg to a group of 4 New Forest ponies. Plasma protein extravasation was monitored in the skin by calculating wheal volumes from measurements of skin fold thickness and wheal diameter made 32 min after intradermal (i.d.) injection of 25 or 100 ng/site PAF when responses were maximal. Measurements were carried out before, and at 30 min, 1.5, 3, 6 and 24 h after administration of WEB 2086. The percentage inhibition of wheal volume caused by WEB 2086 was then determined at each time point. Blood samples were also taken prior to and at 30 min, 6 and 24 h after injection of WEB 2086 for measurement of ex vivo platelet aggregation.

Equine platelets aggregated to PAF over the dose range 10^{-10} - 2 x 10^{-8} M. Following administration of WEB 2086 there was a rightward shift in the dose response curve to PAF which was maximal at 30 min when the amount of PAF required to produce a half maximal response was increased 290-750 fold in the 4 animals tested. At 24 h responses had returned to pretreatment values. PAF induced plasma protein extravasation in the skin was also inhibited after treatment with WEB 2086 (Table).

PAF (ng/site)		% inhibition of wheal volume 30 min	1.5 h	3 h	6 h	24 h
25	(n = 3)	80 ± 6**	88 ± 16***	99 ± 9**	73 ± 8***	47 ± 16
100	(n = 4)	89 ± 9*	95 ± 11*	97 ± 11*	84 ± 9*	22 ± 25

Results are expressed as mean ± s.e. mean; * $p < 0.005$;** $p < 0.01$; *** $p < 0.05$ versus pretreatment values; Students t test. i.d. injection of 25 or 100 ng PAF/site prior to administration of WEB 2086 produced wheal volumes of 154 ± 30 ul and 257 ± 98 ul respectively.

Thus intravenous administration of WEB 2086 at a dose of 3 mg/kg to healthy ponies is sufficient to not only inhibit PAF-induced platelet responses ex vivo, but also PAF-induced increases in vascular permeability in the skin. The effect of WEB 2086 on PAF induced cellular accumulation is now being evaluated.

We are grateful to Boehringer Ingelheim Vetmedica GmbH for financial support and WEB 2086. APF is a Horserace Betting Levy Board Training Scholar.

86P THE INFLAMMATORY EFFECTS OF PAF IN EQUINE SKIN

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Platelet activating factor (PAF) has been implicated as a mediator of inflammation in a number of species (Braquet et al, 1987). In the present study the inflammatory effects of PAF have been investigated in the skin of clinically normal ponies.

Six normal ponies received intradermal injections of vehicle (phosphate buffered saline containing 0.25% equine serum albumin (PBS/ESA); 50µl volume), lyso-PAF (10µg per site), PAF (0.001-10µg per site) and histamine (0.22µg free-base per site), as a positive control. Wheal volumes were calculated from measurements of skinfold thickness and wheal diameter carried out between 2 and 64 minutes after intradermal injection. In a separate study six mm punch biopsies were taken at 1, 2 and 4h after administration of vehicle (PBS) and PAF (1µg per site). Paraffin sections were cut, stained with Giemsa, and the inflammatory cell infiltrates quantitated histologically.

PAF at doses of 0.1µg and greater significantly increased vascular permeability when compared with the vehicle, peak responses occurring at 32 min (Table 1). Lyso-PAF (10µg per site) did not significantly increase vascular permeability.

Table 1

	vehicle	PAF(µg per site) 0.001	0.01	0.1	0.33	1.0
Peak wheal volume (µl)	35 ± 13	70 ± 29	126 ± 37	220 ± 47**	249 ± 53*	315 ± 100*

Results are expressed as mean ± s.e.mean; * $p<0.05$, ** $p<0.01$ vs vehicle; Student's t test.

In contrast to observations made in other species (Humphrey et al,1984), responses to equimolar doses of histamine (0.22µg free-base per site) and PAF (1µg per site) were similar, 519 ± 60µl and 315 ± 100µl respectively at 32 minutes.

In addition to causing increases in vascular permeability intradermal injection of PAF also induced neutrophil accumulation in the dermis between 1 and 4h when compared to the vehicle, the peak response occurring at 2h (Table 2). Eosinophil, mononuclear and mast cell numbers remained unchanged throughout the study.

Table 2

	Number of neutrophils per 4mm^2 of dermis 1h	2h	4h
vehicle	6 ± 3	171 ± 55	80 ± 34
PAF (1µg per site)	123 ± 37*	469 ± 120*	339 ± 78*

Results are expressed as mean ± s.e.mean; * $p<0.05$ vs vehicle; Student's t test.

The ability of PAF to increase cutaneous vascular permeability and cause neutrophil accumulation in the skin of clinically normal ponies is consistent with a possible role for PAF as a mediator of inflammatory conditions in this species.

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87P PAF ANTAGONISTS INHIBIT ANGIOGENESIS IN A RAT SPONGE MODEL

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Angiogenesis, the formation of blood vessels, is normally under stringent control. Persistent angiogenesis can lead to pathological conditions such as atherosclerosis, diabetic retinopathy and rheumatoid arthritis. Recent studies have established the role of several polypeptide growth factors in angiogenesis (Folkman & Klagsbrun, 1987). However, the role of small molecular weight inflammatory mediators is less well characterised. Platelet activating factor (PAF) has a unique repertoire of biological activities and target cells. It increases vascular permeability, induces platelet aggregation and promotes leucocyte chemotaxis. To investigate its role in angiogenesis, we have examined the effects of (1) PAF and (2) three structurally unrelated PAF antagonists, L 659,989 (Hwang et al, 1988), WEB 2086 (Casals-Stenzel et al, 1987) and CV-6209 (Terashita et al, 1987) on the time course of neovascularisation in a rat sponge model.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in female Wistar rats (150-200g) and vascularisation of the implants was monitored using a ^{133}Xe clearance method as previously described (Andrade et al, 1987). Under standard conditions, the ^{133}Xe clearance from control sponges is initially due to passive diffusion (13.6±2.5% in 6 min) but increases from day 6 after implantation as the sponges become vascularised until it approaches that of normal skin (68±4% in 6 min) by day 14. Daily administration of 10^{-10} mole PAF, but not lyso-PAF, into the sponges enhanced their neovascularisation as shown by increased ^{133}Xe clearance compared to controls. Conversely, the PAF antagonists produced a dose related (1-100µg per day) decrease in ^{133}Xe clearance. For example the clearance value of the control group on day 11 after implantation was 53.3±2.8% (n=26), while 10µg daily doses of the PAF antagonists L 659,989, WEB 2086 and CV-6209 reduced it to 31.5±2.5%, 29.9±3.6% and 40.0±2.8% respectively (n=13-18). On day 14 after implantation the control clearance value was 53.9±3.2% (n=17) but the corresponding values for L 659,989 and WEB 2086 remained low at 25.6±2.3% and 29.6±3.9% (n=6-7), indicating a prolonged inhibition of the standard angiogenic response. In addition, preliminary histological studies have shown the vascularity and cellularity of the implanted sponges were modulated by the compounds tested. These observations suggest a role for PAF in the modulation of angiogenesis.

We thank the British Heart Foundation and Boehringer Mannheim GmbH for financial support and Drs Chabala, Meade, Heuer and Oka for the supply of L 659,989, WEB 2086 and CV-6209, respectively. RLS is an MRC scholar.

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88P EVALUATION OF CALCIUM-MOBILISING PROPERTIES OF PHOSPHOROTHIOATE-CONTAINING ANALOGUES OF INOSITOL (1,4,5) TRISPHOSPHATE IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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The recent development of synthetic analogues of the second messenger D-myo-inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) has facilitated investigation of the $Ins(1,4,5)P_3$ receptor and its linkage to Ca^{2+} release from intracellular sites (Nahorski and Potter, 1989). We describe here the properties of two phosphorothioate-containing derivatives of $Ins(1,4,5)P_3$, DL-myo-inositol 1,4-bisphosphate 5-phosphorothioate (DL-IP_3-5S) and DL-myo-inositol 1,4,5-trisphosphorothioate (DL-IP_3S_3), in permeabilized SH-SY5Y cells. Suspensions of SH-SY5Y cells were 'electroporated' in a cytosol-like medium containing 5mM ATP by exposure to 12 discharges of a 3µF capacitor (field strength, 3.75kV/cm; time constant, 0.1 msec). Experiments investigating calcium release from $Ins(1,4,5)P_3$-sensitive pools were performed by loading ^{45}Ca into the cells for 15 minutes at 20°C before challenge with $Ins(1,4,5)P_3$ or its analogues. In 2 minute incubations $Ins(1,4,5)P_3$ and its analogues were equally efficacious in their ability to release sequestered ^{45}Ca (50-60% maximal release). However, $Ins(1,4,5)P_3$ was a more potent stimulus (EC_{50}=0.11±0.04µM, $\bar{x}$± S.E.M. n=6) than DL-IP_3-5S (EC_{50}=0.8±0.17µM,n=6) and DL-IP_3S_3 (EC_{50}=2.5±0.29µM,n=6). After 15 minute incubations with the cells, the dose response curves for $Ins(1,4,5)P_3$ and its analogues were shifted to the right. This shift was more marked for $Ins(1,4,5)P_3$ (3.1 fold,p=0.001) than for DL-IP_3-5S (1.75 fold,p=0.006). The shift for DL-IP_3S_3 (0.3 fold) was not significant (p=0.17). These data suggest that both $Ins(1,4,5)P_3$ and DL-IP_3-5S are metabolised during the 15 minute incubations, but that DL-IP_3S_3 is not. These results were confirmed by monitoring Ca^{2+} release continously with a Ca^{2+}-sensitive electrode. Consistent with the resistance of DL-IP_3S_3 to metabolism, this analogue produced a sustained release of Ca^{2+}. In contrast, stimulation of Ca^{2+} release by $Ins(1,4,5)P_3$ was reversed rapidly and that caused by DL-IP_3-5S was reversed only slowly. In some experiments, added $Ins(1,4,5)P_3$ was 'spiked' with [^{3}H]-$Ins(1,4,5)P_3$ and resulting metabolites were analysed. [^{3}H]-$Ins(1,4,5)P_3$ destruction, predominantly by dephosphorylation, was found to parallel ^{45}Ca reuptake. In contrast, earlier findings suggest that DL-IP_3-5S and DL-IP_3S_3 are both 5-phosphatase resistant (Nahorski and Potter, 1989). Thus, the apparent metabolism of DL-IP_3-5S in the experiments described, suggests that it may be phosphorylated by $Ins(1,4,5)P_3$ 3-kinase. Indeed, treatment of the phosphorothioate-containing analogues with a crude kinase preparation from brain for 30 minutes revealed a dramatic loss of ^{45}Ca mobilizing activity due to DL-IP_3-5S (4.3 fold shift), but not DL-IP_3S_3. Under similar conditions, $Ins(1,4,5)P_3$ was inactivated in 10 minutes. These data suggest that these agents may be particularly useful in determining the significance of phosphorylation and dephosphorylation of $Ins(1,4,5)P_3$ in Ca^{2+} homeostasis.

This work was supported by The Wellcome Trust and The European Social Fund.

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89P [CA^{2+}] ELEVATIONS DIFFERENTIALLY AFFECT RECEPTOR-STIMULATED INOSITOL PHOSPHATES ; ACCUMULATION IN MOUSE AND RAT CORTEX

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Initial attempts to resolve the role of calcium ions in the inositol phospholipid second messenger system prompted two main opposing views. Firstly that a raised intracellular calcium ion concentration was the trigger for breakdown of inositol phospholipid, or that elevated intracellular calcium was a consequence of inositol phospholipid turnover. Both events may occur for certain receptor-linked systems, that is, initial turnover of phospholipid giving rise to a raised intracellular calcium ion concentration sustaining the breakdown of phospholipid (Eberhard & Holz, 1989). We have previously observed that an increase in extracellular calcium ion concentration leads to an increase in histamine-induced inositol phosphates accumulation in mouse cerebral cortical slices (Alexander et al., 1989), in apparent contrast to the reduced response to carbachol seen in rat cerebral cortical slices (Baird et al, 1989). We have investigated, therefore, whether these inconsistencies result from differences in receptors or species differences.

Accumulation of [^{3}H]inositol phosphates ([^{3}H]InsPx) in either mouse (C57bl) or rat (Wistar) cerebral cortical slices was carried out in the presence of 5 mM LiCl and 1 U/ml adenosine deaminase as previously described (Alexander et al, 1989). [^{3}H]Inositol incorporation was carried out in the absence of added calcium ions, prior to addition of agonist with $CaCl_2$ to a final concentration of 1.3 or 4 mM.

Assay of the accumulations of [^{3}H]InsPx at 1.3 and 4 mM $CaCl_2$ indicated that for the majority of the agents studied (i.e. glutamate 3 mM; KCl 25 mM; 5-hydroxytryptamine (5HT) 1 mM; A23187 33 µM; carbachol 1 mM), the rise in ambient calcium ion concentration was without effect in either mouse or rat cerebral cortical slices. However, the responses to noradrenaline (NA, 0.1 mM) and histamine (HA, 1 mM) were enhanced in both species (% of control; n=3/4: mouse NA 219±35; HA 282±3; rat NA 188±33; HA 262±73).

In medium containing 1.3 mM $CaCl_2$, the presence of the calcium ionophore A23187 in combination with the various agents tested above also resulted in heterogeneity in the [^{3}H]InsPx responses in mouse cerebral cortical slices. The responses to glutamate and KCl were both virtually abolished, while the responses to 5HT, NA and HA were all enhanced by the presence of A23187 (5HT 175±7; NA 171±29; HA 144±19; n=3/4). The [^{3}H]InsPx accumulation generated by carbachol was unaffected by the presence of A23187 (110±24).

These results suggest heterogeneity in the mechanism of inositol phospholipid breakdown induced by various agents.

We thank the Wellcome Trust for financial support.

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90P 2-AMINO-4-PHOSPHONOBUTYRATE (AP4) HAS NON-SPECIFIC STIMULATORY EFFECTS ON GUINEA-PIG BRAIN PHOSPHOINOSITIDE TURNOVER

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The glutamate analogue 2-amino-4-phosphonobutyrate (AP4), has been reported to influence phosphoinositide (PI) turnover in rat brain slices (Schoepp & Johnson, 1988). We have studied the effect of AP4 on inositol monophosphate formation in 300µm slices from guinea pig cortex and hippocampus, in view of the potent inhibitory effect of L but not D-AP4 on guinea pig hippocampal mossy fibre synaptic transmission (Lanthorn et al 1984).

As described previously, (Gibbons et al. 1989) L-AP4 potently inhibited responses to ibotenate (IC_{50} = 620±30µM for hippocampus). At higher concentrations it had direct (TTX-insensitive) stimulatory effects in both hippocampal (EC_{50} = 1.77±0.04mM; maximal stimulation = 186±2%) and cortical slices. The inhibition was non-competitive and incomplete (max 60% in hippocampus, 67% in cortex). Unlike the inhibitory effect, the stimulatory action of L-AP4 was not stereospecific and was seen with a number of analogues (Table 1).

Table 1 Drug	Conc (mM)	Stimulation (% basal)	% Inhibition (v. 1mM ibo)
L-serine O phosphate	0.1		20
	1.0	148 ± 7	37
DL 2-amino-4-arsenobutyrate	1.0	101 ± 3	50
2-amino-4-ω-carboxymethyl-	1.0		0
phosphinobutyrate	5.0	134 ± 8	0
2-amino-4-ω-methylphosphino	1.0		5
butyrate	5.0	132 ± 3	8

The pharmacological profile of the inhibitory effects on PI turnover was not the same as that for the inhibition of synaptic transmission (Fagg & Lanthorn 1985), suggesting that the two processes are distinct. The stimulatory effects were non-specific.

The phosphino derivatives of AP4 were a kind gift of Dr G.E. Fagg. SJG is a SERC CASE student with MSD.

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91P EVIDENCE THAT BRADYKININ STIMULATION OF PHOSPHATIDIC ACID PRODUCTION IN ADRENAL CHROMAFFIN CELL CULTURES MAY BE BY DIRECT ACTIVATION OF PHOSPHOLIPASE D

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Bradykinin stimulates release of catecholamines from cultured bovine adrenal chromaffin cells (Owen et al., 1989). Bradykinin also stimulates the formation of inositol phosphates in these cells (Plevin and Boarder, 1989); it is not known whether the increased turnover of phosphoinositides which this indicates is involved in the stimulation of release. To further understanding of the relationship between phospholipid hydrolysis and bradykinin elicited secretion we have studied the effects of bradykinin on diacylglycerol accumulation and phosphatidic acid production.

Bovine adrenal chromaffin cells were prepared by collagenase digestion, purified by centrifugation and differential plating and cultured on 'Primaria' multiwell plates. After 3-6 days in culture cells were loaded with either 0.037 MBq/ml of [^{3}H]-arachidonic acid overnight, 0.37 MBq/ml of [^{3}H]-glycerol for 36-40 hours, or 0.25 MBq/ml of [^{32}P]orthophosphate for 2.5 hours. Arachidonic acid and glycerol labelling were used for measurement of diacylglycerol, while phosphate labelling was used for phosphatidic acid. After stimulation lipids were extracted prior to separation by thin layer chromatography.

Initial experiments to measure diacylglycerol showed that we could not detect a bradykinin induced increase in [^{3}H]-diacylglycerol levels with either [^{3}H]-arachidonic acid or [^{3}H]-glycerol labelling. Inclusion of diacylglycerol kinase inhibitor, R 59022 (Janssen), or diacylglycerol lipase inhibitor, RG 80267 (Revlon), failed to reveal agonist induced increases in [^{3}H]-diacylglycerol levels. However, using cells labelled with [^{32}P]orthophosphate we found that bradykinin caused an increase in formation of [^{32}P]-phosphatidic acid, a phosphorylated product of diacylglycerol metabolism. Bradykinin stimulates phosphatidic acid production in these cell cultures with an EC_{50} of 2-5nM. Fifty percent of the bradykinin stimulated phosphatidic acid production was independent of extracellular calcium. The protein kinase C activating phorbol ester tetradecanoyl phorbol acetate (TPA) alone produced only a very small enhancement of [^{32}P]-phosphatidic acid production while 10 minute pretreatment of cells with TPA failed to affect the response to bradykinin; control, 329±8; TPA(100nM), 507±7; bradykinin (10nM), 2108±93; bradykinin plus TPA, 2191±234; figures are d.p.m. of [^{32}P]-phosphatidic acid, mean±S.E.M. (n=3).

These results suggest that bradykinin stimulation of phosphatidic acid production is not a consequence of either phospholipase C or protein kinase C activation. One possible explanation is that bradykinin acts by a more direct activation of phospholipase D.

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92P PROPERTIES OF INOSITOL (1,4,5) TRISPHOSPHATE RECEPTORS IN LIVER AND CEREBELLUM

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Inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) binding sites have been demonstrated in a wide range of cell types, but only the cerebellar Ins(1,4,5)P_3-binding protein has been purified (Supattapone *et al.*, 1988). It has been suggested, however, that the cerebellar sites may differ from peripheral binding-sites (Willcocks & Nahorski, 1989). We have compared liver and cerebellar Ins(1,4,5)P_3-binding sites and show that their molecular target sizes (estimated by radiation inactivation) are identical - 260kDa. Furthermore, the potencies of a range of agonists and antagonists (Ins(1,4,5)P_3, Ins(2,4,5)P_3, ATP, L-α-glycerophospho-Ins(4,5)P_2, 2,3-bisphosphoglycerate) in competing with specific Ins(1,4,[^{32}P]5)P_3 binding are very similar in the two tissues.

Ins(1,4,[^{32}P]5)P_3 bound to a single class of high affinity binding sites in freeze-dried, saponin-permeabilized hepatocytes and in a cerebellar membrane preparation (liver (n=4), K_D=7.8±1.1nM, B_{max}=1.2±0.3pmols/mg protein, Hill coefficient=1.02±0.09; cerebellum (n=3), K_D=6.5±2.4nM, B_{max}=15.8±0.8pmols/mg protein, Hill coefficient=1.01±0.01). The rank order of potency of a range of Ins(1,4,5)P_3 analogues (D-Ins(1,4,5)P_3, DL-Ins(1,4,5)P_3, DL-Ins(1,4,5)P_3[S]$_3$ and L-Ins(1,4,5)P_3) in competing with specific Ins(1,4,[^{32}P]5)P_3 binding in the hepatocyte preparation was identical to the order of potency in mobilizing intracellular Ca^{2+} stores of freshly prepared saponin-permeabilized hepatocytes. When measured under identical conditions in fresh cells, binding and release curves were superimposable, providing evidence that in liver, the Ins(1,4,5)P_3 binding site is the receptor that mediates the effects of Ins(1,4,5)P_3 on intracellular Ca^{2+} stores.

Our data, therefore, are consistent with the view that the high affinity Ins(1,4,5)P_3-binding site in liver is the receptor that mediates Ca^{2+} release, and that this site is indistinguishable from the cerebellar Ins(1,4,5)P_3 receptor.

Supported by the MRC, the Nuffield Trust and an MRC studentship.

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93P STIMULUS-DEPENDENT INHIBITION OF HUMAN POLYMORPHONUCLEAR LEUKOCTYE (PMN) ACTIVATION BY THE PGD_2 MIMETIC ZK 110.841

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The recently synthesized metabolically stable PGD_2 mimetic ZK 110.841 has been shown to be a potent inhibitor of platelet function (Thierauch et al., 1988) and f-Met-Leu-Phe (FMLP)-stimulated superoxide anion (O_2^-) generation as well as lysosomal enzyme release from human polymorphonuclear leukocytes (PMN) (Ney, 1989). The present study investigates the effect of ZK 110.841 on PMN activation induced by platelet activating factor (PAF) and the Ca^{++} ionophore calimycin (A23187) in comparison to PGD_2 and PGE_1.

Washed human PMN ($5x10^6$/ml) were incubated for 10 min at 37°C with ZK 110.841, PGD_2, PGE_1 or vehicle prior to stimulation with PAF (3 µM) or A23187 (10 µM) for another 10 min at 37°C. The O_2^- generation was measured as superoxide dismutase-inhibitable reduction of cytochrome C. β-Glucuronidase release was determined as a parameter of PMN degranulation. Cyclic AMP levels of stimulated PMN were measured by radioimmunoassay.

The O_2^- generation from PMN was dose-dependently stimulated by PAF. At a concentration of 3 µM this agonist induced the release of 11.1±2.1 nmoles $O_2^-/5x10^6$ cells (n=5). Treatment with ZK 110.841 resulted in a dose-dependent inhibition of this reaction (IC_{50}:30 nM), which appeared to be stronger than those seen with PGD_2 or PGE_1 (IC_{50}:0.1-0.3 µM). None of the prostaglandins inhibited A23187 (10 µM)-stimulated O_2^- release. ZK 110.841, PGD_2 and PGE_1 (10 µM) significantly antagonized the PAF-induced β-glucuronidase release by 26±2%, 26±4% and 27±6% of control, respectively. Again, ZK 110.841 was unable to block the ionophore-induced degranulation. The ZK 110.841-induced inhibition of PMN function was associated with a dose-dependent increase in intracellular cAMP levels from 5.9±1.0 pmoles (control) to 39.9±2.5 pmoles (10 µM ZK 110.841, n=5) per $5x10^6$ PMN. Although no inhibition of A23187-stimulated PMN function was observed, all prostaglandins induced a 7-9fold increase in intracellular cAMP levels in A23187-activated cells.

These data demonstrate a potent inhibition of PAF-stimulated, i.e. receptor-mediated activation of human PMN by the PGD_2 mimetic ZK 110.841. This effect is comparable to PGD_2 and PGE_1 and probably mediated by stimulation of intracellular cAMP. Recently, it has been reported that different inhibitory receptors for PGE_2 and PGD_2 exist on PMN cell surface membranes (Rossi & O'Flaherty, 1989). The present results suggest that ZK 110.841 binds to the PGD_2 receptor and activation of adenylate cyclase occurs. Nevertheless, activation of cAMP levels is not sufficient to block PMN activation as demonstrated by the lack of inhibition after challenge with the Ca^{++} ionophore A23187. Alternatively, ZK 110.841 may inhibit receptor mediated Ca^{++} fluxes.

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94P OEDEMA FORMATION INDUCED BY IL-1 *IN VIVO*: INTERACTIONS WITH THE NEUROPEPTIDE, CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN RABBIT SKIN

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Chemoattractants such as the complement factor C5a act synergistically with vasodilator prostaglandins to induce oedema formation (Wedmore & Williams, 1981). However, interleukin-1 (IL-1), a potent mediator of neutrophil accumulation in vivo, is a weak mediator of oedema formation when injected intradermally (id) with PGE_2 in rabbit skin (Watson et al, 1989). This study shows that in the presence of the long lasting vasodilator CGRP, the potent activity of IL-1 as a mediator of increased microvascular permeability is revealed.

Oedema formation in response to id-injected agents was measured in the rabbit skin by the accumulation of intravenously (iv)-injected ^{125}I-albumin (Brain & Williams, 1985). IL-1($1.4.10^{-14}$mol/site) was pre-injected into rabbit skin before id CGRP (10^{-11}mol/site) as it has previously been shown that IL-1 acts via a time and protein synthesis dependent mechanism (Rampart & Williams, 1988). Oedema was measured for 1h after id CGRP or saline. Maximal oedema was observed at sites which received IL-1 1h before CGRP (Oedema µl/site: IL-1 13.0±2.7µl; IL-1 + CGRP 67.0±12.5µl; CGRP 18.6±2.2µl; saline 7.1±0.5µl, mean±s.e.mean n=5 rabbits). Oedema induced by IL-1 + CGRP was neutrophil dependent. In rabbits treated with anti-neutrophil antiserum (0.5ml/kg iv), oedema was abolished (without antiserum IL-1 + CGRP 76.9±12.1µl: after antiserum IL-1 + CGRP 14.3±1.9µl/site mean±s.e.mean n=4 paired rabbits).

In conclusion, IL-1 can induce oedema formation via a neutrophil dependent mechanism in the presence of a long-lasting vasodilator in the rabbit skin.

TLB is an MRC scholar. We thank Celltech for human αCGRP and Roche Products Ltd for human IL-1α.

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95P GLYCINE SITE ANTAGONISTS PROTECT AGAINST HYPOXIA-INDUCED INJURY TO RAT CORTICAL CELL CULTURES

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Hypoxia-induced injury to neuronal cell cultures has been used as a method to assess the potential neuroprotective actions of compounds at the cellular level in the absence of complicating factors encountered in vivo such as metabolism and cardiovascular effects. Competitive and non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists have been shown to be highly effective in protecting against this sort of damage (Goldberg *et al*, 1987). We have used this model to investigate the neuroprotective actions of HA-966 and 7-chlorokynurenic acid (7-ClKYNA), NMDA antagonists which act at the glycine modulatory site of NMDA receptor complex. Their effects were compared to those produced by the non-competitive NMDA antagonist, MK-801.

Primary cultures of rat cortical neurones were prepared from 17 day gestation foetal rats essentially as described by Priestley *et al*, (1989). After 3 weeks in culture, cells were bathed in a serum-free medium and representative fields were marked and photographed using phase-contrast microscopy. Culture dishes were then divided into three groups with at least six cultures per group. A control group received no further treatment prior to the anoxic challenge. Test cells received one of the NMDA antagonists immediately prior to the anoxia. A third group were returned to an 02/C02 incubator thus providing an indication of on-going, basal, mortality. The control and test cells were placed in a 95% N2/5% C02 flushed incubator for 2.5-5 hours, after which they rejoined the basal group in the 02/C02 incubator and were left for a further 18 hours. At the end of this period the same fields as before were rephotographed. The degree of protection was quantified by measuring the amount of the cytosolic enzyme lactate dehydrogenase (LDH) released into the culture medium. A qualitative assessment of any neuroprotective action was also provided by the photographic evidence. In control dishes anoxia resulted in extensive neuronal degeneration assessed visually and by a marked increase in LDH release into the culture medium compared to basal LDH levels. MK-801, HA-966 and 7-ClKYNA produced a complete protection of the neuronal injury when assessed visually and reduced LDH levels comparable to or below those of the basal group. The neuroprotective effect of these three compounds was concentration-dependent with the following approximate EC_{50} values: MK-801, 30nM; 7-ClKYNA, 30μM and HA-966, 200μM.

These results indicate a neuroprotective effect of compounds acting at the glycine site on the NMDA receptor complex. Interestingly, HA-966 produced full neuroprotection even though this compound does not completely block NMDA responses (Kemp *et al*, 1988).

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96P EVIDENCE THAT THE HIPPOCAMPAL DAMAGE PRODUCED BY TETANUS TOXIN CAN BE PREVENTED BY THE NMDA RECEPTOR ANTAGONIST, MK 801

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Excitatory aminoacid receptors have been implicated in the mediation of neuronal damage produced by transient ischaemia (Simon et al., 1984). In normal brain neuronal activity is maintained by the integrated actions of excitatory and inhibitory afferents. In many brain regions, such as the hippocampus, glutamic acid and GABA are the respective neurotransmitters which probably subserve these functions.During transient ischaemia the extracellular concentration of glutamate increases and this produces neurodegeneration which can be prevented by NMDA receptor antagonists (Beneviste et al., 1984; Simon et al.; 1984). Excitation and perhaps degeneration could also arise if GABA-mediated inhibition is disrupted. We have tested this hypothesis by using tetanus toxin (TT) which is known to produce a long lasting impairment of inhibitory transmission in the CNS. TT was injected into the hippocampal CA1 region (coordinates: AP=-4mm; L=2mm; V=2.4mm) of male Wistar rats (250-280 g) under chloral hydrate (400 $mgKg^{-1}$ i.p.) anaesthesia. Behavioural activity of the rats was studied twice daily and the animals were sacrificed 24 hours, 7 or 10 days after TT injection for histological analysis. A single dose of TT (1000 mouse minimum lethal doses, MLDs; n=3 rats) failed to produce any neuropathological effect 24 hours after the treatment. By contrast, a statistically significant ($p<0.05$) reduction in the number of cells in the CA1 region was observed after 7 (21.5% reduction) and 10 (29.5% reduction) days (n=3 rats in each case). A lower dose (500 MLDs; n=3 rats) of TT did not produce any reduction in the cell number 7 days after the injection whereas 2000 MLDs(4 days afterTT) reduced the cell number by 37.5%. Neutralized TT (1000 MLDs;n=3 rats) did not produce any neuropathological effects. Dose and time-dependent behavioural stimulation was also induced by TT (500-2000 MLDs; n=10 per each dose) and this culminated in generalized convulsions in all rats receiving 1000-2000 MLDs.Treatment with the NMDA antagonist MK 801 (0.3 $mgKg^{-1}$ i.p., given 1h before and after TT injection then once daily for 4 or 7 days; n=10 per each group), abolished the neuropathological and convulsive effects evoked by 1000 and 2000 MLDs TT. In addition, MK 801 protected the animals from the lethal effects of TT. By contrast, diazepam (3 $mgKg^{-1}$, using the same schedule as for MK 801) failed to prevent any of the adverse effects of TT. In conclusion, our results have shown that the focal injection of TT produces neuronal loss in the rat hippocampus wich may be mediated through an unopposed action of excitatory amino acid.

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97P THE GLUTAMATE RECEPTOR SYSTEM IN SCHIZOPHRENIC HIPPOCAMPAL FORMATION: A POST-MORTEM RECEPTOR AUTORADIOGRAPHIC STUDY

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Neuropathological changes are recognised in the hippocampal formation in schizophrenic brain (Kerwin, 1989). In addition the role of glutamate receptors in the pathogenesis of schizophrenia is becoming increasingly apparent (Meldrum and Kerwin, 1988). Using quantitative receptor autoradiography we have performed an analysis of the glutamate receptor system in the hippocampal formation in normal and schizophrenic postmortem.

Hippocampi from 7 schizophrenic and 9 control brains, were sectioned at 20 uM intervals and receptor autoradiography performed using 100 nM ^{3}H-glutamate (total glutamate receptors), 100 nM ^{3}H-glutamate + 10 µM kainic acid + 2.6 µM quisqualic acid (NMDA receptor); 40 nM ^{3}H-kainate (KA receptor) or 50 nM ^{3}H-CNQX (quisqualate receptor) non-specific binding was determined by 1,000 fold excess. Quantification was performed on an IBAS II.

The main finding was a loss of kainate receptors from the CA_4/CA_3 zone on the left and losses in the dentate and parahippocampal gyri (table 1).

Table 1

Area	Dentate	CA_4	CA_3	CA_2	CA_1	Parahippocampal gyrus	
Control							
Right	28±4.0	57.2± 7.6	88± 6.7	33±6.1	27.9± 5.5	41.7±6.4	**P<0.002
Left	14.2±2.7	54.2±12.7	66.6±13.7	26.4±5.6	23.9± 8.6	50.8±9.7	*P<0.04
Schizophrenic							Pmoles/g
Right	7.9±2.9*	29± 8.2*	27.7± 4.0*	38.4±1.25	25.3±11.0	25.3±3.1*	
Left	9.5±1.1*	18.3± 2.1	17.9 4.3**	10.9±3.0**	9.0± 1.2*	27.7±7.9*	

There was also loss of the quisqualate receptor in CA_4/CA_3 regions and there was complete preservation of NMDA receptors in schizophrenic hippocampi.

No differences could be found in total glutamate receptors.
These findings reflect the possible importance of glutamate in the pathophysiology of schizophrenia.
Brains were supplied by Dr Gavin Reynolds (Nottingham) and Dr Clive Bruton (MRC, Runwell).

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98P THE GERBIL HIPPOCAMPAL SLICE: AN *IN VITRO* MODEL OF ISCHAEMIA?

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Bilateral transient occlusion of the common carotid arteries of the Mongolian gerbil evokes a characteristic pattern of neuronal loss in the hippocampus (Kirino, 1982). Although the mechanisms responsible for this ischaemia-induced neuronal death are not fully understood, evidence suggests that the excitatory amino acids (EAAs), glutamate and aspartate, play an important role. The aim of the present study was to develop a model of ischaemia in vitro to investigate the primary events that occur when neurones are exposed to anoxic/hypoglycaemic conditions.

Transverse hippocampal slices (500µm) were prepared from the brains of freshly-killed, male, Mongolian gerbils (65-85g). Individual slices were placed on an inclined plane and superfused at 20 or 30°C with artificial cerebrospinal fluid (aCSF), composition (mM): NaCl 124, KCl 1.9, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, glucose 11, equilibrated with 95% O_2/5% CO_2. $MgSO_4$ (2mM) was present in the aCSF only during the dissection. Anoxic solutions were obtained by equilibrating aCSF with a 95% N_2/5% CO_2 gas mixture. Hypoglycaemic aCSF contained a reduced glucose concentration (2mM). Data was collected from the CA1 pyramidal cell layer of slices that had a response KCl to (10mM) of at least 0.5mV, using the grease gap method described by Blake et al. (1988). Depolarizing potential changes were recorded when the slice was superfused with NMDA (3-30µM; E_{max}>0.28 mV, n=10-13), AMPA (1-30µM; E_{max}>0.42mV, n=3-4), kainate (3-100µM; E_{max}>0.32mV, n=5) or L-glutamate (1-10mM; E_{max}>0.32mV, n=5). In response to superfusion with anoxic aCSF solutions at 30°C, a transient depolarization (<0.15 mV, n=12) was observed with brief exposure (2 and 5 min) which was followed by a marked hyperpolarization (>1mV, n=12) when the exposure time was increased (20 and 50 min). Under these conditions, a further hyperpolarization occurred upon reoxygenation of the slice. All potential changes were completely reversed within 15 min of reoxygenation. Superfusion with hypoglycaemic aCSF produced transient depolarizations (E_{max}<0.2 mV, n=12). When a combination of anoxic and hypoglycaemic aCSF was used, the initial transient depolarisation was potentiated, whereas the hyperpolarising response was unaffected. Preliminary experiments suggest that the hyperpolarization is due to the activation of a potassium conductance. The responses to anoxia and hypoglycaemia were much greater at 30°C than at 20°C. NMDA responses were reduced after exposure of tissues to anoxia and hypoglycaemia. The extent of this depression was found to depend on three variables: a) the duration of the period of anoxia, b) the glucose concentration of the aCSF, and c) the temperature of the slice. The response to AMPA was also depressed following a 20 min period of anoxia/hypoglycaemia, however, the responses to kainate or GABA were not. In summary, the results suggest that the gerbil hippocampal slice may be used to study the primary events that occur when neurones are exposed to anoxia and hypoglycaemia.

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99P A NEW METHOD TO COMPARE THE RESPONSES OF THE ARTERIAL AND VENOUS VESSELS WITHIN THE ISOLATED PERFUSED SUPERIOR MESENTERIC VASCULAR BED OF THE RAT

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The *in vitro* study of the venous vasculature has always been more difficult than that of the arterial vasculature. This abstract describes a retrogradely perfused venous preparation that allows direct comparison between effects of drugs on the veins and arteries within the isolated perfused superior mesenteric vascular bed of the rat.

Male albino Wistar rats (200-350g) were killed by a blow to the head and exsanguinated. The abdomen was opened and the ileocolic and colic branches of the superior mesenteric artery and vein were tied. The superior mesenteric artery (McGregor, 1965) and vein were cannulated with plastic cannulae (internal diam. 0.61 mm) and the vascular bed perfused via the artery for 5 min at 2 ml/min with Krebs' buffer containing heparin (100 U/ml). At the end of this period the intestine was separated from the mesentery and the preparation supported on a Petri dish while the arteries (2-5 ml/min) and veins (2 ml/min) were perfused independently with warmed (37.5°C) and gassed (95%O_2:5%CO_2) Krebs' buffer containing indomethacin (5 μM). Changes in perfusion pressures were measured by means of pressure transducers. In some preparations U46619 (0.3-3 μM) was infused intra-arterially and intravenously to produce an increase in arterial and venous perfusion pressures of 17.8±2.5 mmHg (n=8) and 6.3±1 mmHg (n=8), respectively. Vasodilatations were recorded in response to bolus doses of drugs (1 μl) admininstered into both sides of the preparation and gossypol (3 μM, Forstermann et al., 1986) used to discriminate between endothelium-dependent and independent vasodilators. In other experiments, the pressor responses of the entire perfused mesentery and intestine were compared to the separately perfused systems to assess the relative contribution of the arterial and venous portions of the mesentery to the responses of the whole vascular bed.

Arginine-vasopressin (3-100 pmol, n=5), noradrenaline (0.3-10 nmol, n=5), 5-hydroxytryptamine (0.3-10 nmol, n=5) and KCl (1-60 μmol, n=4) were all more active as constrictors of the arterial than venous vessels; whereas, angiotensin II (3-100 pmol, n=5) and U46619 (10-300 pmol, n=5) were more active as constrictors of veins than arteries, on both the separately perfused and entire perfused preparations. Endothelin-1 (3-100 pmol, n=5) constricted both the arterial and venous vessels but it's effect was significantly longer on the venous vessels. In preconstricted preparations acetylcholine (0.01-100 nmol, n=7) and bradykinin (0.001-1 nmol n=9) caused endothelium-dependent dilatations of both sides of the circulation but ADP (0.01-100 nmol, n=5) only of the arterial vessels. Sodium nitroprusside (0.01-100 nmol, n=7) caused endothelium-independent vasodilatations of both portions of the circulation.

Thus this is a useful preparation for comparing the arterial and venous vasculatures. It also demonstrates that veins as well as arteries, display endothelium-dependent dilatations.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Ltd.

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100P ENDOTHELIN, EDRF AND PROSTACYCLIN ARE RELEASED FROM BOVINE VENOUS AS WELL AS ARTERIAL ENDOTHELIAL CELLS

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Endothelin (ET) and endothelium-derived relaxing factor (EDRF) are released from arterial endothelial cells (EC) by various receptor-mediated stimuli (1,2). Few studies have assessed the release of EDRF from venous blood vessels (3). Here, we have measured the stimulated release of EDRF and prostacyclin (PGI_2) from venous (bovine vena cava) and arterial (bovine aorta) EC grown to confluence on cytodex beads. Two ml of beads ($10\text{-}15 \times 10^6$ cells) were packed in a jacketed column and perfused (5 ml min^{-1}, 37 °C) with gassed (95% O_2, 5% CO_2) Krebs' solution containing SOD (10 units ml^{-1}). To measure EDRF release, the effluent of columns of EC was superfused over four spirally cut rabbit aortic strips denuded of their endothelial layer. The effluent was subsequently collected for 6-keto $PGF_{1\alpha}$ determination as a measure of PGI_2 release from the EC. To study the release of ET, venous EC and arterial EC were grown to confluence as previously described (2) in 10 cm petri dishes . Endothelin-like immunoreactivity was quantified by a double antibody assay for ET-1 and ET-2 (Amersham, kit # RPA-535).

Venous EC released as much EDRF as arterial ones when stimulated with BK or ADP. Characteristically, the threshold concentration of BK required to release detectable amounts of EDRF was 0.05 pmol for venous EC and 1 pmol for arterial EC. An inhibitor of EDRF formation, NG-monomethyl-L-Arginine (3×10^{-5}M), reduced in a similar fashion the release of EDRF induced by BK (10 pmol) in venous and arterial EC (n=4). PGI_2 was released from venous EC when stimulated with BK (1, 10 or 30 pmol) in a concentration dependent fashion (0.5±0.17, 0.97±0.15 and 1.41±0.32 ng ml^{-1} respectively, n=4). ET was detected in smaller quantities from venous EC than from arterial EC. After a 4 hr incubation period with thrombin (1 IU ml^{-1}), the amount of ET released from 7×10^6 cells was 0.40±0.08 ng ml^{-1} for venous EC and 1.37±0.09 ng ml^{-1} for arterial EC, an increase of over 70% compared to basal release of ET after 4 hr incubation ($p<0.05$, n=6).

Veins are more sensitive to ET-1 than arteries (4,5,6). The finding that functional venous endothelial cells also release ET further supports a vasomodulatory role for this peptide in the venous circulation.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Ltd. PDJ is a Fellow of the Canadian Heart Foundation.

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101P RELATIONSHIP BETWEEN L-ARGININE CONCENTRATION AND EDRF RELEASE IN BOVINE AORTIC ENDOTHELIAL CELLS

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L-Arginine (L-Arg) is considered to be the endogenous precursor of endothelium-derived relaxing factor (EDRF; Palmer *et al.*, 1988a). Normal cell culture medium contains about 600 μM L-Arg. Interestingly, L-Arg has little effect on the release of EDRF from endothelial cells (EC) grown on microcarrier beads in normal medium and then superfused in a column with Krebs' solution. L-Arg also has no direct relaxing effect on rabbit or rat aorta up to millimolar concentrations (Thomas *et al.*, 1989). However, when EC are cultured for 24 h without L-Arg, addition of L-Arg (10-30 μM) to the Krebs' buffer somewhat potentiates the release of EDRF induced by bradykinin (BK; Palmer *et al.*, 1988a), indicating a depletion of the L-Arg pool in these cells so that the intracellular concentration of L-Arg becomes rate-limiting for EDRF formation.

We have compared the intracellular concentration of L-Arg (measured by reversed phase HPLC/fluoresecence detection) in bovine cultured aortic EC and the ability of these cells to release EDRF (measured by the cascade bioassay technique) following stimulation with BK or ADP. L-Arg levels were determined in EC grown in normal culture medium (Arg(+) cells) with or without stimulation of EDRF release and in EC cultured for 24 h without L-Arg (Arg(-) cells) in the presence or absence of N^G-monomethyl-L-arginine (L-MA), an inhibitor of EDRF synthesis (Palmer *et al.*, 1988b).

The intracellular concentrations of L-Arg and L-Citrulline (L-Cit) in Arg(+) cells were 106.9±9.4 μM (±SEM; n=11) and 28.0±7.1 μM (n=7), respectively. In Arg(-) cells, L-Arg decreased to 5.4±0.1 μM (n=3), whereas L-Cit showed no significant change. These cells, however, still release EDRF to a similar extent as Arg(+) cells (n=14). When L-MA (500 μM) was added to the L-Arg free medium, L-Arg remained at control levels (120.8±5.1 μM; n=3) but L-Cit increased 5 to 6-fold (n=3), presumably due to a metabolism of L-MA via the EDRF biosynthesis pathway. Arg(+) cells were also stimulated by ADP infusions for up to 2 h. There was a considerable and sustained release of EDRF, but only a minor decrease in the level of L-Arg (maximum of -25%; n=3) and no change in L-Cit. In contrast, various other amino acids, such as L-aspartate, L-glutamate or L-glutamine decreased by 75-97% after stimulation with ADP, suggesting that the intracellular concentration of L-Arg is somehow maintained during EDRF release.

Thus, our findings indicate that after stimulation of EDRF release or L-Arg depletion, EC can either generate L-Arg from some other, as yet unknown source, or that another L-Arg-containing compound, such as a peptide, can serve as an alternative precursor for EDRF formation.

The William Harvey Research Institute is supported by a grant from Glaxo Research Group Ltd.

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102P BRADYKININ-INDUCED ELEVATIONS OF CYTOSOLIC CALCIUM IN BOVINE AORTIC ENDOTHELIAL CELLS: ROLE OF INTRACELLULAR AND EXTRACELLULAR CALCIUM

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Elevation of cytosolic calcium plays an important role in the regulation of several endothelial functions. We examined bradykinin-induced rises in intracellular calcium $[Ca^{2+}]_i$ in bovine aortic endothelial cells (BAEC) and determined the contributions of intracellular and extracellular calcium pools in the response.

BAEC were seeded on to sterile glass coverslips and loaded with fura-2 (2 μM) for 45 min. Coverslips were then suspended across the diagonal of a quartz cuvette containing HEPES (10 mM) buffered Krebs at 37°C. Excitation was alternated between 340 and 380 nm every 3 seconds and emission at 509 nm determined. $[Ca^{2+}]_i$ was determined using the equation of Grynkiewicz et al. (1985). In the presence of 1.8 mM calcium, bradykinin (10 nM) increased $[Ca^{2+}]_i$ from a resting level of 86±4 nM to a peak of 826±84 nm (n=5) within 30 s, which subsequently decayed to a plateau which was 174±19 nM (n=5) after 5 min. In the absence of calcium (0.5 mM EGTA added), bradykinin induced a transient rise in $[Ca^{2+}]_i$ reaching a peak of 370±40 nM (n=5) and the plateau phase was abolished. Subsequent addition of calcium (2.3 mM) resulted in a biphasic response; a rapid rise to a peak of 414±92 nM within 30 s which then decayed to a plateau of 194±20 nM after 5 min (n=5). In other experiments, cells were bathed in 1 mM calcium, and 2 mM EGTA was added 1 min before bradykinin which then induced a transient rise in $[Ca^{2+}]_i$ reaching a peak of 330±10 nM (n=3), but the plateau phase was abolished. Nickel (Ni^{2+}), which blocks calcium entry into cells (Hallam et al., 1988), was used to determine whether the reduction in peak $[Ca^{2+}]_i$ in the absence of extracellular calcium or the presence of EGTA was a consequence of rapid depletion of an intracellular store, or the loss of a rapid calcium influx component. In the presence of Ni^{2+} (4 mM) and 1.8 mM calcium, bradykinin induced a transient rise in $[Ca^{2+}]_i$ reaching a peak of 790±47 nM (n=5) within 30 s; this was not significantly different from the peak observed in control cells, but the plateau phase was abolished.

In conclusion, the results with Ni^{2+} suggest that the transient rise in $[Ca^{2+}]_i$ results from discharge of an intracellular store and the plateau phase results from calcium influx, findings similar to those described for human umbilical vein endothelial cells (Hallam et al., 1988). The intracellular store of calcium appears to comprise two pools only one of which is rapidly depleted in absence of extracellular calcium or the presence of EGTA.

This work was supported by the British Heart Foundation.

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103P HAEMODYNAMIC EFFECTS OF PROLONGED INFUSIONS OF N^G-MONOMETHYL-L-ARGININE (L-NMMA) IN CONSCIOUS RATS

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We investigated the effects of sustained inhibition of nitric oxide production in male Long Evans rats by giving infusions of L-NMMA (see Moncada et al., 1989). Animals were anaesthetized (sodium methohexitone 60 mg kg^{-1} i.p., supplemented) and had renal, superior mesenteric and hindquarters, pulsed Doppler probes implanted. At least 7 days later rats were re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) for implantation of intravascular catheters. Experiments began the following day when mean blood pressure (MBP), instantaneous heart rate (HR) and renal, mesenteric and hindquarters Doppler shift signals were recorded continuously. Changes in vascular conductance were calculated from the latter and MBP. L-NMMA was infused at 30 mg $kg^{-1}h^{-1}$ for 6h and measurements were made throughout this period and for 3h after infusion; a separate group of animals received a saline infusion.

Table 1 Cardiovascular changes during and after infusion of L-NMMA 30 mg $kg^{-1}h^{-1}$ (n=8) or saline (n=4) in conscious Long Evans rats. Values are mean ± s.e.m. * P<0.05 versus baseline (Friedman's test)

	During infusion				After infusion			
	1h		6h		1h		3h	
	L-NMMA	Saline	L-NMMA	Saline	L-NMMA	Saline	L-NMMA	Saline
MBP (mm Hg)	26±4*	4±3	32±4*	-2±3	18±5*	0±4	6±2	-3±2
HR (b min^{-1})	1±7	23±16	-29±9*	25±13	-27±11	0±3	-9±13	5±3
Conductance (%)								
Renal	-12±5*	0±5	-23±5*	-2±1	-11±6	-7±1	-7±3	-2±2
Mesenteric	-32±4*	0±6	-41±6*	0±4	-29±7*	-5±6	-21±6*	-7±4
Hindquarters	-31±4*	0±6	-51±4*	3±8	-35±6*	-8±12	-33±6*	-8±7

The results in the Table indicate that L-NMMA causes hypertension and reductions in renal, mesenteric and hindquarters conductances that are sustained during a 6h infusion. Furthermore the mesenteric and hindquarters vasoconstrictions persist for at least 3h after infusion, although MBP does not remain elevated, indicating cardiac output is reduced at this time.

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104P CALCITONIN GENE-RELATED PEPTIDE (CGRP) ENDOTHELIUM-DEPENDENT RELAXATION IN RAT AORTA IS INHIBITED BY L-NMMA

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N^G-monomethyl L-arginine (L-NMMA) inhibits the formation of nitric oxide, the endothelium-derived relaxing factor (EDRF) released in some preparations by acetylcholine (Rees et al., 1989). This inhibition can be reversed by the L- but not by the D- isomer of the amino acid arginine. Calcitonin gene-related peptide (CGRP) is also capable of releasing an EDRF in some preparations. The reported differences in second messenger accumulation associated with the transduction of nitric oxide (cGMP) (Griffith et al., 1985) and the CGRP-EDRF (cAMP) (Grace et al., 1987), has given rise to speculation that CGRP-EDRF may not be nitric oxide, but a novel compound. We investigated this possibility utilising the known properties of L-NMMA on acetylcholine induced relaxation and comparing these with the effects on endothelium-dependent relaxation to CGRP.

Rat thoracic aortic rings of approximately 4mm in length were suspended on tungsten wires (diameter 0.125mm) and equilibrated for 75 minutes in Krebs solution bubbled with 95% O_2/5% CO_2 at 37°C. Noradrenaline (10^{-7}M) was added and the contraction assessed for stability over a period of 15 minutes. Any tissues not exhibiting 80% relaxation of the NA-induced tone to acetylcholine (10^{-6}M) were discarded as having damaged endothelium. L-NMMA, L-arg, D-arg or combinations of these were added and 15 minutes allowed for equilibration before, successively, dose/response curves to acetylcholine (0.3-10μM), CGRP (3-300nM) and sodium nitroprusside (SNP) (3-1000nM) were constructed, each curve being separated by at least 20 minutes. Acetylcholine (IC_{50} 100nM), was approximately 10 fold less potent than either CGRP or SNP. The maximum relaxations evoked were 100 percent for acetylcholine and SNP with CGRP giving a relaxation of 60 percent of the NA induced tone. L-NMMA (30μM - 100μM) caused dose-related inhibition of both the acetylcholine curve and CGRP curve with a non-parallel, rightward shift of the dose/response curve and depression of the maximum response to 70 and 10 percent of the NA evoked tone respectively. The SNP response was not reduced by L-NMMA (30-100μM). Acetylcholine and CGRP responses in the presence of 100μM L-NMMA were partially restored with 100μM L- but not D-arginine. D-arg and L-arg (100μM) potentiated the effects of both acetylcholine and CGRP indicating that perhaps the basicity of the compounds exerts a non-specific effect.

It would appear from these results that the endothelium-dependent relaxation induced by CGRP is affected by L-NMMA in a similar fashion to acetylcholine. There are a number of explanations for this including the possibility that L-NMMA is not specific in its action on L-arginine uptake or that the CGRP-EDRF, although different from nitric oxide, shares L-arginine as a common precursor. Finally, both CGRP and acetylcholine could release nitric oxide with cAMP and cGMP accumulation being causally unrelated to the relaxant response.

We thank Dr S. Moncada for the gift of L-NMMA and Celltech Ltd for financial support. D.W.G. is an MRC student.

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105P ENDOTHELIN-1 MRNA IN THE RAT KIDNEY

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Endothelin-1 (ET-1) is a potent vasoconstrictor with mitogenic properties isolated from aortic endothelial cell supernatants. The cDNA sequences for porcine and human pre-pro-endothelin-1 are known. Two other endothelins have been described from analysis of rat (rat ET=ET-3) and human (ET-2 and ET-3) genomic DNA. However, in the rat little is known of the transcriptional expression of these endothelins. As it appears that ET-1 peptide is not stored within endothelial cells, we have attempted to determine and characterize ET-1 mRNA expression in the rat using cDNA amplification with the polymerase chain reaction (PCR) (Saiki et al 1989) as the first stage in the investigation of the role of this peptide in rat models of cardiovascular disease.

RNA was extracted from rat kidneys. cDNA was generated using 10-50mg of total RNA, cloned murine Maloney reverse transcriptase and oligo-dT or a specific ET-1 antisense primer (derived from known pig or human cDNA sequences). PCR was performed using Taq DNA polymerase and 2 primers (one was that used for reverse transcription (RT); the other was identical to part of the ET-1 sequence) designed to span a large intron and yield a 400bp cDNA amplification fragment. After 40 PCR cycles (93°C,30s; 60°C,30s; 72°C,2min; Final extension:72°C,30min) the products were electrophoresed and blotted onto nylon membranes. The band specificity was determined by i) Southern analysis with a 5'[^{35}S] probe complementary to a sequence within the primers, ii) restriction endonuclease digestion with PvuII and HindIII and iii) dideoxy sequencing of the DNA. The distribution of these transcripts in the rat kidney was studied by in situ hybridization with a 36mer [^{35}S] labelled antisense probe (Nunez et al 1989).

After PCR, a band of the predicted size (~400bp) was seen in all the samples studied when oligo-dT or the pig antisense primer was used for RT and PCR, but not when human primers were utilised. The presence of this 400bp band was dependent on RT. Southern analysis showed that the [^{35}S]-probe hybridized selectively to this band. Restriction fragment analysis revealed that the 400bp band contains a PvuII restriction site (like pig ET-1 cDNA), but no HindIII site (unlike pig and human ET-1 cDNAs). Dideoxy sequencing confirmed that it was similar, but not identical to pig or human ET-1 sequences. In situ hybridization localised ET-1 mRNA selectively to larger blood vessels, but not capillaries or glomeruli.

We have demonstrated that ET-1 mRNA is expressed in the rat kidney. Changes in this mRNA can now be studied in rat models using specific rat primers and probes.

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106P EARLY ATHEROSCLEROSIS INDUCED BY A PERIVASCULAR COLLAR IMPAIRS MUSCARINIC ENDOTHELIUM-DEPENDENT RELAXATION IN THE RABBIT CAROTID ARTERY

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Previous studies with hypercholesterolemic animals demonstrated that fatty streaks impair endothelium-dependent vascular relaxation to acetylcholine (ACh). We applied a new model in which perivascular manipulation results in the generation of a neo-intima, the first step in the development of atherosclerosis, within 7 days (Booth et al., 1989). The aim of this study was to determine whether the neo-intima affected the release of, and the vascular responses to endothelium-derived relaxing factor.

A non-occlusive, flexible silastic collar was placed around the left carotid artery of rabbits (2.0 - 3.5 kg). The sham operated contralateral artery was used as a control. Seven days later two duplicate rings (cranial (A), caudal (B), 3-4 mm long) were cut from the control and the left (collar mid region) carotid artery, mounted in organ chambers, and isometrically contracted with phenylephrine (0.35 μM). Subsequently, cumulative dose-relaxation curves were made. Concentrations producing half maximum relaxation (EC_{50}) were calculated. They are expressed as mean [95 % confidence interval] for six rabbits and were analysed with a 2 x 2 analysis of variance with collar (2) and duplicate (2) as within rabbit factors.

ACh induced 90 to 100 % relaxation of control and collar-treated segments. A statistically significant shift (effect collar, $p = 0.031$) of the dose-response curve was seen when a neo-intima was present (EC_{50} 0.05 [0.02-0.10] and 0.06 [0.02-0.19] μM for control segments A and B, and 0.12 [0.06-0.25] and 0.15 [0.06-0.37] μM for collar-segments A and B respectively). There were no differences between cranial or distal segments of either control or proliferating carotid arteries, and interaction between collar and duplicate was absent. SIN-1 and nitroglycerin evoked complete relaxation of segments with a neo-intima and their controls. The presence of intimal lesions did not affect the EC_{50} of these agonists which liberate nitric oxide via different mechanisms.

These results indicate that the neo-intima did not affect the capacity of the underlying smooth muscle cells to relax in response to guanylate cyclase stimulation by exogenous nitric oxide. However, the relaxation elicited by stimulation of the endothelial muscarinic receptors was suppressed, suggesting that release or diffusion of endogenous nitric oxide was impaired by the neo-intima.

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[1] Research Assistant of the Belgian National Fund for Scientific Research.

107P N^G MONOMETHYL-L-ARGININE RESTORES PRESSOR RESPONSES TO NORADRENALINE IN ENDOTOXIN-TREATED RATS

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A reduced vascular responsiveness to a variety of vasoconstrictor substances occurs in clinical sepsis and in animal models of sepsis and endotoxaemia (Parratt, 1989). The mechanism of this reduced responsiveness is unclear but recent evidence obtained from aortic ring preparations removed from rats treated with lipopolysaccharide (LPS) suggests that it is due to the excessive production of nitric oxide (Fleming et al., 1990). The present studies were designed to explore whether N^G-monomethyl-L-arginine (L-NMMA, a specific inhibitor of nitric oxide production) could restore the depressed vascular responsiveness resulting from LPS administration in vivo.
Rats were anaesthetized with pentobarbitone and cannulae placed for blood pressure measurement and drug administration. E. coli LPS (5 mg kg^{-1} h^{-1}) or saline (0.4 ml h^{-1}) were infused intravenously and L-NMMA (30 mg kg^{-1}) or its solvent (saline) were administered intravenously 10 min prior to the end of the LPS or saline infusions. Pressor responses to noradrenaline (NA) were obtained before, and after 60 mins of infusion in the presence or absence of L-NMMA. Table 1 shows that the L-NMMA restored the reduced NA pressor responses towards normal. This effect of L-NMMA was reversed by L- but not D-arginine.

Table 1 Changes in diastolic systemic arterial pressure (mmHg) induced by NA in anaesthetized rats administered saline or lipopolysaccharide (LPS) in the presence and absence of L-NMMA. Results are shown as the mean ± SEM of 5-6 experiments. **P<0.02 vs saline control (student's t-test).

Infusion	100	300	1000 ng kg^{-1} noradrenaline
Saline	14±3	28±4	48±4
LPS	3±2**	5±4**	13±5**
Saline + L-NMMA	14±5	19±5	26±5
LPS + L-NNMA	10±3 ns	15±3 ns	29±4 ns

These results provide further support for the concept that LPS-induced loss of vascular reactivity is due to the excessive production of nitric oxide from L-arginine.

Supported by a grant from the Commission of the European Communities (ST2J-0457-6).

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108P GLOMERULI SYNTHESISE NITRITE IN EXPERIMENTAL NEPHROTOXIC NEPHRITIS

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Vascular endothelial cells (Palmer et al., 1988) and immunologically-activated macrophages (Marletta et al., 1988) synthesize nitric oxide (NO) from L-arginine. The major stable end-product in culture is NO_2^-. Activated macrophages accumulate in glomeruli (gl) (Cook et al., 1989) and are responsible for injury in experimental immune complex glomerulonephritis (GN). We examined the production of NO_2^- by isolated gl and of urinary NO_2^- in accelerated nephrotoxic nephritis in the rat. In this model, GN (inflammatory cell infiltration, glomerular injury and proteinuria) is induced in preimmunized rats by intravenous injection of rabbit antibodies against rat glomerular basement membrane antigen (Unanue and Dixon, 1965). Kidneys from normal or nephritic rats (4 h, 1, 4 and 21 days after induction of GN) were perfused with saline and gl were isolated by sieving. 2000 gl/ml were incubated in 16 mm wells for 48 hr at 37°C under 4% CO_2. The incubation medium was Eagle's MEM without phenol red, supplemented with sodium pyruvate, glucose, HEPES, 10% foetal calf serum and antibiotics. NO_2^- was measured in culture supernatants and in urine by adding an equal volume of Griess reagent (sulphamethazine 1%, naphthyl ethylenediamine dihydrochloride 0.1%, orthophosphoric acid 2.5%). Absorbance was measured at 550 nm; the background value from medium incubated alone was subtracted. Normal gl did not produce NO_2^- (< 1.5 nmol/2000 gl/48 hr) spontaneously or when stimulated with lipopolysaccharide (LPS; 1 µg/ml) or A23187 (2 µg/ml). Nephritic gl spontaneously produced NO_2^-, which was maximal from gl isolated 24 hr after induction of GN (158.4 ± 8.4 nmol/2000 gl/48 hr, n=4). NO_2^- production was inhibited 75 - 100% by N^G-monomethyl-L-arginine (300 µM), strongly suggesting its production from L-arginine via NO. NO_2^- production was increased at 21 days but not at other times by LPS (1 µg/ml; 29.7 ± 10.2 nmol/2000 gl/48hr compared with 11.4 ± 5.5 in non-LPS- treated gl at this time, $p < 0.05$). NO_2^- was undetectable (< 1.5 nmol/ml) in normal rat urine. In GN rat urine however, NO_2^- was observed; the greatest quantity (5231 ± 2900 nmol/24 hr, n=4) was produced 1-2 days after induction of GN, falling to 1850 ± 735 nmol/24 hr (n=4) by 6-7 days after induction. Macrophages are the most likely source of NO_2^- in nephritic gl since the peak production at 24 hrs corresponds to the major influx of macrophages. However, the possibility that some NO_2^- is derived from endothelium cannot be excluded. The production of NO in nephritic gl may have implications for both the mechanism of glomerular injury and glomerular haemodynamics.

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109P EFFECT OF ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASODILATORS ON RENAL FUNCTION IN THE ANAESTHETISED RABBIT

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A wide variety of vasoactive factors affect intrarenal vascular tone and glomerular filtration rate (GFR). We have compared the renal effects of endothelium-independent and -dependent vasodilators. The following parameters were measured in NZW rabbits anaesthetised with pentobarbitone(15 mg/kg bolus) and althesin 0.5 mg/kg/min; systemic mean arterial pressure (MAP), renal blood flow (RBF) with an electromagnetic flow probe and GFR by clearance of ^{51}Cr-EDTA. All vasodilators were administered as a continuous infusion into the aorta 1 cm above the origin of the renal arteries with measurements 10 minutes after starting infusion. Baseline values were determined prior to infusion of each vasodilator and 15 minutes after the infusion was stopped and results expressed as percentage change from baseline.

Prostacyclin (PGI_2) and nitroprusside (NP), both endothelium-independent vasodilators, produced dose dependent falls in MAP, GFR and RBF(Table-approximately equipotent (MAP) dose shown). In contrast acetylcholine (ACh) and Substance P (SP) produced similar falls in MAP but increases in GFR.

Results: Mean ± SEM (n = 5 - 8).

Dose nmols/kg/min		MAP	GFR	RBF
PGI_2	0.4	-18.5 ± 3.6	-37.7 ± 13.3	-32.3 ± 6.8
NP	30	-29.7 ± 3.1	-67.0 ± 2.4	-40.7 ± 2.4
ACh	10	-15.1 ± 2.0	+34.6 ± 11.2	+33.5 ± 9.5
SP	30	-17.4 ± 1.9	+45.5 ± 23.1	-21.8 ± 10.8

Indomethacin (2 $mgkg^{-1}$), methylene blue (0.8 mg kg^{-1} hr^{-1}; Hogan et al, 1988) and NG-monomethyl-L-arginine (10 mg kg^{-1} hr^{-1}; Rees et al, 1989), inhibitors of cyclooxygenase and EDRF respectively, attenuated the fall in MAP seen with ACh. The effect on GFR was even greater with the combination of indomethacin and methylene blue changing the 34.6% ± 11.2 increase in GFR to a 22.8% ± 10.5 fall. This indicates that the effect of acetylcholine is the result of the release of EDRF and PGI_2.

Our results indicate that endothelium-independent vasodilators may affect both pre and post glomerular vessels, resulting in a fall in transglomerular basement membrane pressure (P) and therefore a fall in GFR. Conversely, endothelium-dependent vasodilators appear to have a heterogeneous effect, probably acting predominantly on pre-glomerular vessels and leading to an increase in P and GFR.

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110P VASOPRESSIN INCREASES CYCLIC GMP IN LLC-PK_1 KIDNEY EPITHELIAL CELLS *via* THE L-ARGININE/NITRIC OXIDE PATHWAY

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Vasopressin has recently been shown to increase cyclic GMP (cGMP) in a pig kidney epithelial cell line (LLC-PK_1) by activating soluble guanylate cyclase (Leitman et al., 1988). In the light of recent studies (Garthwaite et al., 1989; Knowles et al., 1989) the L-arginine/nitric oxide (NO) pathway appears to be a transduction mechanism for the activation of soluble guanylate cyclase. The present study investigates the role of L-arginine and its conversion into NO for cGMP stimulation in LLC-PK_1 cells.

LLC-PK_1 cells (passage 201-205) were obtained from the American Type Culture Collection and cultured in 35 mm culture dishes as described previously (Schröder & Schrör, 1989). Confluent cells were exposed for 10 min to N^G-monomethyl-L-arginine (L-NMMA), the arginine enantiomers, haemoglobin or superoxide dismutase in a balanced salt solution containing 0.5 mM isobutylmethylxanthine. Vasopressin and other agonists were added and incubation was continued for another 10 min. cGMP levels were determined by radioimmunoassay.

Vasopressin at 1 nM to 1 µM induced a concentration-dependent increase in cGMP. Preincubation of cells with 1 µM, 10 µM and 100 µM L-NMMA reduced cGMP stimulation at all vasopressin concentrations investigated. At 1 µM vasopressin, the 7-fold cGMP stimulation was inhibited by 25%, 71% and 90%, respectively. This inhibition by L-NMMA was antagonized by L-arginine (2 mM) but not D-arginine (2 mM). L-NMMA did not affect cGMP stimulation by sodium nitroprusside thus precluding a direct inhibitory effect of L-NMMA on soluble guanylate cyclase. In cells that were cultured for 24 h in L-arginine-free medium, L-arginine (2 mM) but not D-arginine (2 mM) potentiated vasopressin-induced cGMP elevation by up to 130%. The potentiating effect of L-arginine on cGMP accumulation was not inhibited by cycloheximide (1-100 µM) suggesting free L-arginine rather than L-arginine containing peptides as immediate precursor of NO. cGMP elevation at 1 µM vasopressin was increased by 54% in the presence of 15 U/ml superoxide dismutase and decreased by 50% in the presence of 1 µM haemoglobin. cGMP stimulation in LLC-PK_1 cells by the calcium ionophore A23187 was also found to be L-arginine-dependent. In contrast to that, cGMP stimulation by atrial natriuretic peptide was independent of L-arginine demonstrating that activation of particulate guanylate cyclase is not linked to the L-arginine/NO pathway.

These results demonstrate that, in kidney epithelial cells, vasopressin increases cGMP via the L-arginine/NO pathway. This is in line with the hypothesis that NO formation from L-arginine is a widespread mechanism for coupling membrane receptors to the activation of soluble guanylate cyclase.

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111P ENDOTOXIN-INDUCED INTESTINAL DAMAGE IN THE RAT IS ENHANCED BY L-NMMA, AN INHIBITOR OF NITRIC OXIDE FORMATION

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The hypotension, increased vascular permeability and gastro-intestinal damage produced by endotoxin may result from its direct action on the vascular endothelium or as a consequence of secondary mediator release. Recent studies in the rabbit, showing that inhibition of endothelium-derived nitric oxide (NO) synthesis by N^G-monomethyl-L-arginine (L-NMMA) increases systemic arterial blood pressure, suggest that NO may have a regulatory role in the vasculature in-vivo (Rees et al, 1989). We have now investigated the effects of L-NMMA, on endotoxin-induced intestinal damage in the rat.

Fasted male Wistar rats (225-275) were anaesthetised (pentobarbitone, 60mg/kg i.p.) and the carotid artery cannulated for measurement of systemic arterial blood pressure (BP). E.coli lipopolysaccharide (LPS., 0111:84, 50mg/kg) was injected via the tail vein and 15 min later the jejunal damage scored on a scale of 0-3 (Boughton-Smith et al, 1989). Changes in vascular permeability were measured as the intestinal leakage of ^{125}I-human serum albumen (10μCi) injected i.v. 30 min prior to LPS. Haematocrit (HCT), erythrocyte (RBC) and leucocyte (WBC) concentrations in arterial blood (100μl), taken from the carotid artery, were measured on a Clay Adams Haematological Analyser.

LPS (50mg/kg, i.v.) produced a low level of damage in the jejunum (score, 1.3 ± 0.2, n=15) and induced plasma leakage (236 ± 44μl plasma/g jejunum; n=10, P<0.01). Prior administration of L-NMMA (10-50 mg/kg. i.v.), 5 min before LPS, dose-dependently enhanced the jejunal damage (score, 2.5 ± 0.3 and 3.0 ± 0, at 20 and 50mg/kg respectively, n=4; P<0.01). Furthermore, L-NMMA (50mg/kg) markedly augmented the plasma leakage produced by LPS (70 ± 4% increase, n=13, P<0.01). D-NMMA (50mg/kg, i.v.) had no effect on either jejunal damage or plasma leakage. L-arginine (300 mg/kg iv), administered immediately after L-NMMA, inhibited the enhanced jejunal damage (by 81 ± 22%, P<0.05) and plasma leakage (by 85 ± 25%, P<0.05), whilst D-arginine (300 mg/kg iv) had no effect. L-NMMA (10-50mg/kg iv) increased BP by 21-30 mmHg, but did not affect the fall in BP induced by LPS. Small increases in RBC (12 ± 2% increase, n=6, P<0.01) and HCT (7 ± 2% increase, P<0.05) determined 15 min after LPS, were significantly enhanced by L-NMMA (30 ± 3% and 21 ± 5% increase for RBC and HCT respectively; n=8, P<0.001), whilst WBC was unchanged. L-NMMA alone did not produce intestinal damage or plasma leakage.

These results with L-NMMA suggest that the formation of NO from L-arginine by endothelial cells or possibly activated leucocytes following endotoxin challenge may contribute to the maintenance of integrity of the intestinal vasculature, and thereby reduce tissue damage.

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112P EFFECTS OF GUANYLATE CYCLASE ACTIVATION AND TYPE I PHOSPHODIESTERASE INHIBITION UPON HUMAN PLATELET AGGREGATION

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Nitric oxide (NO) released as EDRF from the vascular endothelium or from nitro-dilators can stimulate platelet soluble guanylate cyclase (SGC) leading to a rise in cGMP and inhibition of platelet aggregation. However, in blood, the effect of this liberated NO is most probably limited by the presence of haemoglobin (Evans et al., 1989). Additionally, the generated cGMP will also be reduced due to breakdown by selective cGMP (type I) phosphodiesterase (PDE). The purpose of the present investigation was therefore to assess the influence of these various factors on the platelet inhibitory profile of the nitro-dilator compounds, sodium nitroprusside (SNP) and SIN-1 (3-morpholino-sydnonimine), by examining their anti-aggregatory effect in the presence and absence of red blood cells and the type I PDE inhibitor, M&B 22,948.

Platelet aggregation was determined in human whole blood (WB), platelet-rich plasma (PRP) and in gel-filtered platelets (GFP) utilising a platelet counting technique (Lumley & Humphrey, 1981). The inhibitory effect of the agents tested was assessed by dividing the concentration of aggregatory agonist required to evoke 50% platelet aggregation in the presence of inhibitor by that in its absence and expressing the result as a concentration ratio (CR). In WB, SNP at concentrations of 10,100 and 1000μM produced mean (n=4) ADP CR values of 1.4, 2.4 and 4.6 respectively. In similar experiments using ADP in PRP and GFP, the potency of SNP was increased approximately 15 and 50-fold. Similarly, SIN-1 at concentrations of 10,100 and 300μM resulted in mean (n=4) ADP CR values of 1.5, 3.5 and 9.9 respectively in WB; the inhibitory effect of SIN-1 was increased by 25 and 90-fold in PRP and GFP. In WB, SNP (100μM) also inhibited aggregation induced by U-46619 and collagen to the same extent as ADP. M&B 22,948 (100μM) was without significant effect upon ADP-induced aggregation in WB, PRP or GFP. Whilst the platelet inhibitory effects of SNP, SIN-1 or M&B 22,948 alone in WB were either modest or absent, when SNP or SIN-1 (100μM) was combined with M&B 22,948 (100μM) a marked potentiation of their inhibitory effects was observed. For example, in WB mean (n=4) ADP CR values of 24 and 60 were obtained for M&B 22,948 with SNP and SIN-1 respectively. This apparent synergy between SGC activators and a type I PDE inhibitor was observed against all agonists tested (ADP, collagen and U-46619). In contrast, M&B 22,948 (100μM) was without effect on the inhibition of ADP-induced aggregation produced by PGI_2 (1 and 3nM), demonstrating the selectivity of this inhibitor.

In conclusion, the potency of SGC activators is lowest in WB and highest in GFP where red blood cells are absent. The basal activity of the human platelet SGC appears to be low since M&B 22,948 showed little inhibitory action in its own right. However, the activity of the human platelet type I PDE appears to be high since the effectiveness of SGC activation is markedly enhanced in the presence of type I PDE inhibition.

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113P REDUCTION IN THE NUMBER OF THROMBOXANE P-RECEPTORS (B_{max}) ON HUMAN PLATELETS AFTER EXPOSURE TO GR 32191

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Repeat dosing with the thromboxane (TP)-receptor antagonist GR 32191 produces a cumulative antagonism of U-46619 -induced platelet aggregation *ex vivo* which is not accompanied by a parallel increase in plasma levels of the drug. Binding of [^{3}H] GR 32191 to intact human platelets *in vitro* is characterised by an exceptionally slow dissociation from the platelet TP-receptor (Armstrong et al, 1989) which may in part explain the cumulative effect of the drug. In the present study we have examined the effects of exposure of platelets either *in vitro* or *in vivo* to GR 32191 upon the number (B_{max}) of platelet TP-receptors.

In the *in vitro* study platelets were pre-incubated with saline or unlabelled GR 32191 at 37°C for 30 or 60 min. This was followed by incubation with a 100 fold volume of assay buffer for 3 h at 37°C to ensure complete dissociation of the drug from the TP-receptor. Samples were centrifuged at 450g for 20 min and the platelet pellets were resuspended in assay buffer (2.5 ml). Inhibition curves were determined by incubating increasing unlabelled GR 32191 in the presence of 2 nM [^{3}H] GR 32191 for 30 min at 37°C (Armstrong et al, 1989). Pre-incubation with 10 or 100 nM GR 32191 for 30 min resulted in a reduction in maximal [^{3}H] GR 32191 binding to 81 ± 9% and 53 ± 8% respectively; a 60 min pre-incubation time gave a further reduction in maximal [^{3}H] GR 32191 binding to 65 ± 6% and 47% (values n=3-4). No effect on K_d (2-3 nM) was observed. This effect is peculiar to GR 32191 as pre-incubation with other TP antagonists BM 13.177 (0.5 mM) or SQ 29,548 (5 μM) was without effect on [^{3}H] GR 32191 binding. Similar results were obtained by determination of B_{max} by Scatchard analysis using 1-30 nM [^{3}H] SQ 29,548 as the ligand.

Three volunteers received 20 mg GR 32191 12-hourly for 7 days. Blood samples (day 1) were taken prior to ingestion of GR 32191 and compared with samples 12 h after the first dose of GR 32191 (day 2), 12 h after the final dose of GR 32191 (day 8) and 84 h after the final dose of GR 32191 (day 11). Each platelet pellet was divided in 2, one part for "control" Scatchard analysis using [^{3}H] SQ 29,548 and the other for Scatchard analysis after dilution /dissociation. Results are shown in Table 1.

Table 1 Effect of ingestion of GR 32191 on the number of platelet TP receptors (B_{max}).

"Control"	K_d (nM)	B_{max} (fmol /10^8platelets)	Diln/ Dissoc	K_d (nM)	B_{max} (fmol / 10^8platelets)
Day 1	3.3	57.6	Day 1	10.0	21.4
Day 2	6.3	43.3	Day 2	8.8	19.1
Day 8	5.8	27.8	Day 8	10.9	13.1
Day 11	5.1	57.9	Day 11	12.9	15.3

In conclusion, exposure of human platelets *in vitro* to GR 32191 results in a fall in the TP-receptor B_{max}. Following oral dosing with GR 32191 to man a similar profile was seen. In both studies the reduction in B_{max} was observed under conditions where bound drug would be expected to be removed by dilution from the receptor. This effect of GR 32191 upon TP-receptor number may contribute to the fall in response of human platelets to TP-agonists even when GR 32191 is cleared from the circulation.

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114P COMPARISON OF BINDING SITES FOR [^{125}I]-ENDOTHELIN-1, 2, 3, BIG ENDOTHELIN, SARAFOTOXIN AND MOUSE VASOACTIVE INTESTINAL CONTTRACTOR IN RAT, PIG AND HUMAN TISSUES

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Endothelins (ET) are potent vasoactive peptides. Three isoforms of ET have been described, ET-1, ET-2 and ET-3 (Yanagisawa & Masaki 1989) which have a high degree of sequence similarity with the snake venom sarafotoxin S6B. Recently, a fourth endothelin has been described, mouse vasoactive intestinal contractor (VIC) (Ishida et al 1989).

Our aim was to use quantitative autoradiography to identify and compare specific binding sites for these five peptides in consecutive sections from rat, pig and human tissues. In vitro receptor autoradiography was carried out as previously described (Davenport et al 1989 a,b,c). Consecutive sections were incubated with the following iodinated peptides ET-1, ET-2, ET-3, human big ET[1-38], sarafotoxin S6B or mouse VIC (Amersham International plc). Non-specific binding was assessed by coincubating adjacent sections with the corresponding unlabelled peptide.

Binding sites for ET-1, ET-2 and ET-3 were present in the heart of all three species (nerves < atria < ventricle < coronary artery) and kidney (vasa recta < medulla < cortex). A difference between the species was observed within the glomeruli where high levels of binding were detected in rat but no detectable binding was observed in pig and human. In the adrenal, the highest levels of ET-1, ET-2 and ET-3 binding were present in the zona glomerulosa of all species. In pig and human, higher levels of binding were present in the two inner zones of the cortex compared to the medulla. This pattern was reversed in the rat. In the rat brain highest densities of ET-1, ET-2 and ET-3 sites were present in the cerebellum and binding was detected in the spinal cord. Binding sites were also detected for sarafotoxin S6B and mouse VIC in all species but binding of big ET was low or below the level for detection.

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Supported by British Heart Foundation, Wellcome Trust (APD) and Squibb Cardiovascular Fellowship (DJN).

115P EFFECT OF CHRONIC β-ADRENOCEPTOR-ANTAGONIST TREATMENT ON ISOPRENALINE-EVOKED CHANGES IN FUNCTION AND SUBSET COMPOSITION OF CIRCULATING MONONUCLEAR LEUKOCYTES

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We have recently demonstrated rapid increases in β_2-adrenoceptors (AR) and decreases in function of circulating mononuclear leukocytes (MNL) in correlation with changes in MNL subset composition following β-AR agonist administration to healthy human subjects (Van Tits et al., 1990). The increase in β_2-AR is a β_2-AR mediated effect which can be blocked by the β_2-AR antagonist ICI 118,551, but not by the β_1-AR antagonist bisoprolol. However, it is not known what effect β-AR antagonists have on subset composition and function of circulating MNL. Thus, in the present study we investigated the effects of β-AR antagonist treatment in healthy volunteers on the above mentioned phenomena.

Volunteers received either bisoprolol (1x10 mg/day, n=8) or celiprolol (1x200 mg/day, n=7) or propranolol (4x40 mg/day, n=8) for 9 days. Before and after β-AR antagonist treatment, volunteers were infused with isoprenaline (ISO) and changes in MNL subset composition, β_2-AR density and in vitro proliferative responses to mitogens were assessed.

Bisoprolol-treatment did not affect basal MNL β_2-AR density, while propranolol-treatment slightly increased and celiprolol-treatment slightly decreased basal MNL β_2-AR density. The ISO-induced increase in MNL β_2-AR density was not significantly altered by bisoprolol- or celiprolol-treatment, but completely abolished by propranolol-treatment.

β-AR antagonist treatment did not influence basal MNL subset composition. ISO-infusion caused a decrease in T-helper cell number and increases in T-suppressor/cytolytic and NK-cell numbers. These ISO-induced changes in MNL subset composition were not changed by the β-AR antagonist treatment except for the increase in NK-cells, that was blocked by propranolol treatment.

β-AR antagonist treatment did not significantly alter in vitro MNL proliferative responses to mitogens. ISO-infusion caused a depression of the proliferative responses. While bisoprolol- and celiprolol-treatment had no influence, propranolol-treatment blocked the ISO-induced depression of the proliferative responses.

We conclude that the ISO-induced increase in MNL β_2-AR density and depression of in vitro MNL proliferative responses to mitogens can be blocked by chronic propranolol-treatment without affecting a redistribution of T-cell subsets.

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116P β-ADRENOCEPTOR-MEDIATED INOTROPIC EFFECTS OF DOPAMINE AND EPININE IN HUMAN ISOLATED RIGHT ATRIUM

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Dopamine (DA) and its N-methyl derivative epinine (EP, the active metabolite of ibopamine) are sympathomimetic agents used in the treatment of cardiac failure (Goldberg & Rajfer, 1985; Henwood & Todd, 1988). However, little is known concerning the interaction and efficacy of these drugs at β_1- and β_2-adrenoceptors (AR) in the human myocardium. Thus, in right atrial tissue derived from patients undergoing coronary artery bypass grafting we have characterized the β-AR subtype(s) mediating the positive inotropic effects of DA and EP. None of these patients had received catecholamines or β-AR antagonists at least three weeks prior to operation.

Cumulative concentration-effect curves for the positive inotropic effect of DA and EP were assessed on isolated electrically driven (1.0 Hz) right atria (equilibrated at 37°C in Krebs-Henseleit solution containing 5μM phenoxybenzamine) in the presence or absence of the β_1-AR antagonist CGP 20712A (CGP, 300nM) or the β_2-AR antagonist ICI 118,551 (ICI 30nM).

ICI and CGP caused similar rightward shifts of the concentration-effect curves for the positive inotropic effect of EP (pD_2-values: control = 5.49±0.13; after ICI = 4.59±0.11; after CGP = 4.57±0.11; n=7-9), while only CGP significantly shifted the concentration-effect curves of DA to the right (pD_2-values: control = 4.94±0.10; after ICI = 4.90±0.12; after CGP = 3.79±0.14; n=9-11). EP caused nearly the same maximum increase in force of contraction as isoprenaline, while the DA-induced maximum increase in force of contraction was only 70-75 % that of isoprenaline. In receptor binding experiments using (-)-[^{125}I]-iodocyanopindolol EP was found to have similar affinities to β_1-AR (human right atrial membranes in the presence of 50nM ICI) and β_2-AR (human lung membranes in the presence of 300 nM CGP), while DA revealed a three fold higher affinity to β_1-AR.

In conclusion, we have provided evidence that in human right atrium the positive inotropic effect of DA is mediated almost exclusively via β_1-AR, while that of EP is mediated by both β_1- and β_2-AR to about the same degree.

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117P DIFFERENTIAL ALTERATIONS OF ADENYLATE CYCLASE REGULATION BY G_i IN HUMAN DILATED AND ISCHAEMIC CARDIOMYOPATHY

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In the failing human heart, there is a decreased number of cardiac β-adrenoceptors with a concomitant loss of effectiveness of β-adrenoceptor agonists (Bristow et al., 1982). Since not only β-adrenoceptor agonists but also phosphodiesterase inhibitors are less effective in failing myocardium (Böhm et al., 1988) another defect such as an increase of G_i (Feldman et al., 1988) has been suggested. In order to further characterize the alterations in the human failing heart, the positive inotropic effects of isoprenaline (Iso) and milrinone (Mil) were studied in isolated, electrically driven papillary muscle strips from patients with dilated (DCM) and ischemic (ICM) cardiomyopathy as well as in nonfailing (NF) human myocardium. Basal, guanine-nucleotide Gpp(NH)p-, forskolin- and iso-stimulated adenylate cyclase activity (AC) as well as G_s and G_i were studied with cholera toxin- and pertussis toxin-catalyzed ^{32}P ADP-ribosylation, respectively. Cardiac β-adrenoceptors were studied with radioligand binding experiments using [^{3}H]-CGP 12177.

In DCM and ICM the number of β-adrenoceptors was reduced similarly compared to NF by 60 %; concomitantly, iso-stimulated AC was reduced. The amount of G_s was not altered in ICM or DCM compared to NF. However, there was a 36 % increase of $G_{i\alpha}$ in DCM compared to ICM or NF. In the same hearts, basal as well as Gpp(NH)p-stimulated AC was reduced. The positive inotropic effects of Iso and Mil were markedly reduced in ICM and DCM compared to NF. The positive inotropic effect of both compounds, however, was in DCM more reduced than in ICM.

It is concluded that in DCM compared to ICM, G_i is increased by 36%, whereas β-adrenoceptors are similarly down-regulated. Since basal and Gpp(NH)p-stimulated AC and the positive inotropic effect of Iso and Mil are more strongly reduced in DCM, the increase in G_i might be functionally relevant in the reduced sensitivity to cAMP increasing positive inotropic agents in DCM.

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118P DOES ENDOTHELIN 1 OR 3 FACILITATE PRESSOR RESPONSES MEDIATED BY POSTJUNCTIONAL α-ADRENOCEPTORS?

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The endothelin (ET) family of the peptides, ET1, ET2 (Trp6-Leu7) and ET3 are all potent constrictors of vascular smooth muscle . A facilitatory interaction between α-adrenergic receptor activation and ET induced vasoconstriction has been suggested (Tabuchi et al. 1989; Boarder 1989). The aim of the present study was to investigate the effect of ET *per se* as well as its proposed facilitatory properties. Arterial blood pressure (BP) at different doses of ET1 and ET3, respectively, were assessed in the pithed rat model. In addition, the pressor response to preganglionic sympathetic nerve stimulation (PNS) and to phenylephrine at subthreshold doses of ET1 and ET3, respectively, were analysed.
Experiments were performed on 75 pithed (Gillespie & Muir 1967) male Sprague-Dawley rats pretreated with atropine and pancuronium. ET1 (200-1600 pmol/kg) and ET3 (400-3200 pmol/kg) were infused for 10 min and BP and heart rate (HR) were recorded from the left cannulated carotid artery. One group of animals was subjected to six PNS (0.25-0.8 Hz, 1 ms, 65 V for 20 s) and another group of rats was given six bolus injections of phenylephrine (25-50 nmol/kg i.v.).
Both ET1 and ET3 dose-dependently increased systolic and diastolic BP. The increase in the MABP during the highest doses of ET1 and ET3 were 127.3 ± 8.8 mmHg and 111.8 ± 9.6 mmHg, respectively. The subthreshold dose of ET1 (400 pmol/kg) or ET3 (800 pmol/kg) was administered before and during the third PNS and the third injection of phenylephrine, respectively. Compared to the control recording, the absolute BP responses during stimulation were higher during ET1 and ET3 infusion, respectively, but the relative increases were not markedly influenced (109.7 ± 6.1 % and 101 ± 5.3 % of control, respectively.) However, the lower dose of ET1 tended to facilitate the pressor response to PNS at a low frequency, whereas there tended to be an inhibition of the BP response to PNS after the highest dose of ET1 infused. The absolute BP increase to bolus injections (25 and 50 nmol/kg) of phenylephrine was enhanced during ET1 infusion. However, the pressor response to the low dose of phenylephrine was only slightly increased by ET1 (102.3 ± 4.1 % of control).
We conclude that subthreshold doses of ET1 and ET3 do not markedly potentiate either the PNS induced pressor response or the postjunctional BP response to α_1-adrenergic receptor stimulation in the pithed rat model. The obtained discrepancy with what has prevously been suggested might be due other mediators involved in the sympathetic neurotransmission.
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119P 'ATYPICAL' β-ADRENOCEPTORS IN THE RAT GASTRIC FUNDUS

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There is evidence that 'atypical' β-adrenoceptors mediate relaxation in gastrointestinal smooth muscle preparations including guinea pig ileum (Bond *et al*, 1986) and rat distal colon (McLaughlin & MacDonald, 1989). These 'atypical' β-adrenoceptors are characterised by relative resistance to β-adrenoceptor antagonists and by the high potency of a novel class of β-adrenoceptor agonists (Arch *et al*, 1984). As it has previously been reported that relaxatory responses to noradrenaline in rat gastric fundus (RGF) are resistant to α- and β-adrenoceptor antagonists (Dettmar *et al*, 1986), the present study investigated whether these resistant responses were also mediated by 'atypical' β-adrenoceptors.

Longitudinal smooth muscle strips of RGF were suspended in Krebs' PSS at 37°C containing cocaine (3μM), ascorbic acid (30μM) and EDTA (30μM). Strips of RGF were contracted using a submaximal concentration of methacholine, added directly to the organ baths. Cumulative concentration-response curves (CRC's) to agonists were constructed. Responses to noradrenaline and isoprenaline were measured in the presence of propranolol (1μM) and prazosin (0.1μM), respectively.

Noradrenaline relaxed the RGF in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 6.7 ± 0.15 (n=8). Prazosin (0.01 to 1μM) antagonised the noradrenaline CRC, the slope of the Schild plot being 0.53 ± 0.28, 95% C.L. (n=23). Isoprenaline also relaxed the RGF in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 7.7 ± 0.21 (n=6). The relaxations to isoprenaline were antagonised by propranolol (0.01 to 1μM), the slope of the Schild plot being 0.55 ± 0.24, 95% C.L. (n=18). In the presence of prazosin (0.1μM), the 'atypical' β-adrenoceptor agonist BRL 37344 relaxed the RGF with a pIC_{50} (mean ± se) of 5.22 ± 0.09 (n=8). In the presence of prazosin (0.1μM) and propranolol (1μM), BRL 37344 relaxed the RGF with a pIC_{50} (mean ± se) of 5.33 ± 0.15 (n=10), and the rank order of potency was isoprenaline (1.0) > noradrenaline (0.39) > BRL 37344 (0.11). BRL 37344 (1μM) produced a marked desensitisation of responses to isoprenaline, causing a mean rightward 11-fold shift in the CRC to isoprenaline ($p < 0.05$, n=5).

The weak, non-competitive antagonism displayed by prazosin and propranolol and the cross-desensitisation by BRL 37344 would suggest that the catecholamines act at 'atypical' β-adrenoceptors. However, the relatively low potency of BRL 37344 also suggests that the atypical adrenoceptors are not necessarily identical with those present in other tissues.

We thank Dr. M.A. Cawthorne for the supply of BRL 37344.

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120P SALMETEROL AND FORMOTEROL: ARE THEY BOTH LONG-ACTING β_2-ADRENOCEPTOR AGONISTS?

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Salmeterol (SALM.) is a potent, long-acting β_2-adrenoceptor agonist on airways smooth muscle and in human lung tissue (Bradshaw et al., 1987). Formoterol (FORM.) has also been described as a long-lasting β_2-agonist bronchodilator (Löfdahl & Svedmyr, 1988). We have now compared directly, the potencies and durations of action of FORM., SALM. and salbutamol (SALB.) in vitro and in vivo.

Guinea-pig superfused tracheal strips (GPT) were contracted with $PGF_{2\alpha}$ (2.8μM) or by 10s trains of electrical field stimulation (5Hz, 0.1ms, supramaximal voltage; Coleman & Nials, 1989). Conscious guinea-pigs were challenged for 15-30s with aerosolised histamine (9mM) to cause reproducible bronchoconstriction (Ball et al., 1987). Human lung fragments (HLF) were passively sensitised and challenged with specific antigen (Butchers et al., 1979); histamine (HIST) and leukotriene (LT) C_4/D_4 release was measured by bioassay and radioimmunoassay.

FORM. was the most potent agonist in relaxing GPT, with equipotent concentrations (EPC, isoprenaline = 1) of 0.06 [range 0.013-0.28, n=2] and 0.03 (95% CL 0.02-0.06, n=4) on the $PGF_{2\alpha}$ and electrically-stimulated (ES) preparations respectively, whilst equivalent values for SALM. were 0.9 (0.6-1.2, n=12) and 0.4 (0.2-0.7, n=14), and for SALB., 2.0 (1.2-3.3, n=7) and 3.0 (1.5-6.5, n=8). After termination of infusion, effects of FORM. and SALB. reversed rapidly, with an RT_{50} (time to 50% recovery from an EC_{50} concentration) of <20 min, whereas there was no decline in responses to SALM. within 45 min ($F_{2\alpha}$-GPT) and 770 min (ES-GPT). In the conscious guinea-pig, FORM. had a threshold effective concentration of 0.01mM, compared with 0.12mM (SALM.) and 0.2mM (SALB.). However, whilst the effects of SALM. lasted for at least 6h, FORM. and SALB. were effective for <3h and <1.5h respectively. In HLF, the EC_{50} for FORM. in inhibiting HIST. and LT release was 0.15nM and 0.26nM. Corresponding values for SALM. were 3.0nM and 0.94nM and for SALB., 35.8nM and 11.5nM. The effects of FORM. and SALB. were readily reversed on washing (Rt_{50} < 6h and <2h). In contrast, responses to SALM. persisted for at least 20h.

We conclude that formoterol is a relatively short-acting β_2-adrenoceptor agonist, being intermediate between salbutamol and salmeterol.

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121P SALMETEROL, BUT NOT FORMOTEROL, PERSISTS AT β_2-ADRENOCEPTORS *IN VITRO*

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Salmeterol (SALM.) and formoterol (FORM.) have both been reported to be longer acting than salbutamol (SALB.) after aerosol administration to man (Ullman & Svedmyr, 1988; Löfdahl & Svedmyr, 1988). However, experimental studies (Nials et al, 1990), including those in human lung tissue, have shown that whilst submaximal responses to SALM. persist for many hours, those to FORM. are relatively short-lived.

We have now determined the duration of action of supramaximal concentrations (30 and 300x EC_{50}) of FORM. (0.01 and 0.1μM) and SALB. (1.0 and 10μM) on the electrically-stimulated guinea-pig trachea preparation (Coleman & Nials, 1989). Under these conditions, although inhibitory responses were of extended duration, there was still clear recovery with FORM., [Rt_{50} (time to 50% recovery) values being 82 and 370 min (n=4)] and with SALB. [57 and 115 min (n=4)]. Administration of sotalol (10μM) caused substantial reversal of the residual FORM. and SALB. effects. Washout of sotalol resulted in transient reassertion of FORM. (0.01 and 0.1μM) and of SALB. (10μM), agonist activity. In contrast, responses persisting after an EC_{50} concentration of SALM. (10nM) were also reversed by sotalol (10μM), but showed full and maintained reassertion after sotalol withdrawal.

We have also compared the effects of SALM., SALB. and FORM. on the binding of [^{125}I] iodopindolol (^{125}IPIN) to β_2-adrenoceptors in rat lung membranes (Barovsky & Brooker, 1980). Affinity constants (K_i; mean ± SEM, n>3) were calculated from plots of β-agonist concentration against inhibition of ^{125}IPIN binding. SALM. (Ki 53±8nM) had an apparent affinity similar to that for FORM. (76±20nM), both being greater than SALB. (Ki 2.5±0.2μM). Agonist dissociation from β_2-adrenoceptors was assessed on membrane preparations pre-incubated for 30 min with a concentration calculated to occupy ~90% of receptors. The membranes were then diluted (1:250) with vehicle containing ^{125}IPIN (100pM) and the binding of the radioligand was measured at intervals. FORM. and SALB. dissociated rapidly (<5 min), the ^{125}IPIN binding rate being similar to that in control preparations. However, ^{125}IPIN binding was greatly inhibited in SALM.-treated tissues, there being no evidence of dissociation from β-adrenoceptors over a 60 min. period.

These results demonstrate further differences between the long-acting β_2-adrenoceptor agonist, SALM. and shorter-acting compounds such as FORM. and SALB. and suggest that the mechanism by which SALM. achieves its long duration of action is not shared by FORM.

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122P VASCULAR CONTRACTIONS TO ISOPRENALINE ARE MEDIATED BY AN α_2-ADRENOCEPTOR

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Peripheral vascular α_1- and mixed α- responses from dogs with pacing-induced heart failure (CHF) are enhanced (Forster et al., 1989a). In addition, isoprenaline (IP) produced concentration-dependent contractions of the dorsal pedal artery and the saphenous vein which were attenuated by phentolamine and CHF (Forster et al., 1989b). Accordingly, we examined the mechanism of the vascular IP response.

Vessels from 5 dogs were removed before (C) and at CHF and cut into rings; alternate rings were denuded (D). In the presence of propranolol ($3x10^{-6}$M), concentration-effect curves were constructed to IP. One D ring and one intact (I) ring remained as controls and no α antagonist was added. To an additional pair of rings (D and I), concentration-effect curves to IP were generated in the presence of increasing concentrations of yohimbine (10^{-8} - 10^{-6}M). The remaining pair was treated with prazosin (10^{-8} - 10^{-6}M). Results showing tension generated to IP and antagonism (antag) by yohimbine and prazosin are shown below. For tension, the results are mean±SEM and for antag, -ve log IC_{50} (geometric mean and 95% cl: that concentration which caused a reduction in max tension by 50%) for 4-11 results.

Table 1 — Antagonism of IP-induced vascular contractions

	Dorsal pedal artery			Saphenous Vein		
	Max tension (grams)	Antag by Yohimbine	Antag by Prazosin	Max tension (grams)	Antag by Yohimbine	Antag by Prazosin
CI	3±0.45	7.1(6.8-7.5)	5.9(5.2-6.5)	4.05±0.70	8.7(8.5-9.0)	5.6(5.4-5.8)
CD	1.48±0.41+	7.0(6.9-7.3)	no antag	2.51±0.42*	8.8(8.5-9.0)*	5.5(5.0-5.6)
CHFI	2.95±0.60	no antag	undetectable	1.87±0.46*	7.0(6.9-7.2)*	no antag
CHFD	2.08±0.40	7.3(7.1-7.4)	6.5	1.25±0.19	7.8(7.6-8.0)	no antag

+P,0.05, D versus I; *P<0.05, CHF versus C. Student's t-test

The results show that 1) IP always caused a dose-dependent contraction. 2) In C, D caused a decrease in responsiveness to IP. 3) IP responses were significantly attenuated in the vein at CHF. 4) Neither yohimbine nor prazosin were competitive antagonists and IC_{50} values for yohimbine were significantly reduced in the vein at CHF. These results suggest that IP selectively interacts with an endothelium-dependent α_2-adrenoceptor in the sv. In addition, at CHF, the α_2-adrenoceptor function in veins is decreased.

Supported by the Heart and Stroke Foundation of Ontario
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123P RS-15385-197, A POTENT AND SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST

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RS-15385-197, ((+)-(8a ,12a ,13a)-5,8, 8a, 9, 10, 11, 12, 12a, 13,13a-decahydro-3-methoxy-12-methylsulfonyl-6H-isoquino[2,1-g][1,6] naphthyridine hydrochloride) is the most potent and selective α_2-adrenoceptor antagonist thus far reported (Clark et al, 1989). We now report the selectivity profile of the compound which is a tetracyclic which has been resolved as an active enantiomer; other enantiomers (eg RS-15385-198) are >1000 fold less potent at α_2-adrenoceptors. RS-15385-197 is a potent and selective inhibitor of the binding of [^{3}H]-idazoxan and [^{3}H]-yohimbine (Table 1). RS-15385-197 (10^{-4}M) did not inhibit the uptake of [^{3}H]noradrenaline into isolated synaptosomes isolated from rat cerebral cortex.

Table 1. Binding profile of RS-15385-197

Receptor	*Radioligand*	*Tissue*	*pKi*	*Receptor*	*Radioligand*	*Tissue*	*pKi*
α2	[^{3}H]-Yohimbine	baboon cortex	10.1	ß1	[^{3}H]-Dihydroalprenolol	guinea-pig heart	<5
α2	[^{3}H]-Idazoxan	baboon cortex	10.1	ß2	[^{3}H]-Dihydroalprenolol	guinea-pig lung	<5
α2	[^{3}H]-Yohimbine	human platelet	9.9	D1	[^{3}H]-SCH23390	rat striatum	5.1
α2	[^{3}H]-Idazoxan	rat cortex	9.2	D2	[^{3}H]-Spiperone	rat striatum	6.2
α2	[^{3}H]-Yohimbine	rat cortex	9.5	5-HT1A	[^{3}H]-8-OH-DPAT	rat cortex	6.5
α1	[^{3}H]-Prazosin	rat cortex	5.7	5-HT2	[^{3}H]-Ketanserin	rat cortex	5.1

In functional tests RS-15385-197 was a potent antagonist of the effects of the α_2-adrenoceptor agonist, UK 14304, in field stimulated guinea-pig ileum preparations. The antagonism was reversible and apparently competitive with a pA2 value of 9.7 ± 0.1 (n=5) and a slope of 1.00. In contrast RS-15385-198 had a pA2 of 6.5 ± 0.1 (n=5). In rat vas deferens preparations RS-15385-197 was a competitive antagonist of UK 14304 (pA2 9.3 ± 0.1) and antagonized the contractile effects of phenylephrine (pA2 6.1) indicating >1000 fold selectivity for α_2-adrenoceptors. RS-15385-197 did not directly affect the contractile responses to field stimulation in guinea-pig ileum, or prostatic or epididymal portions of rat vas deferens and was not a partial agonist. RS-15385-197 would appear to be the most selective agent reported to date to explore the functional role of α_2-adrenoceptors.

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124P PERIPHERAL ACTIONS OF PERTUSSIS TOXIN PRETREATMENT IN THE RAT

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Pertussis toxin (PT) ADP-ribosylates the guanine-nucleotide binding proteins G_i and G_o (Dolphin, 1987) and is reported to inhibit endothelium-dependent relaxations in guinea-pig pulmonary artery (Weinheimer & Osswald, 1989). In this study, the effects of PT were examined on endothelium-dependent relaxations of rat aorta and on pre-junctional inhibition by alpha$_2$-adrenoceptor agonists in rat atrium and vas deferens.

Two different methods of treatment with PT were employed: exposure of tissues in vitro to PT (1µg/ml) for 2 hours, or pretreatment with PT (6-40µg/kg, i.v.) 3 days prior to experimentation.

The effectiveness of PT treatments was assessed as the ability to prevent acetylcholine (ACh)-induced negative inotropic responses in 1Hz-paced left atria. Incubation in vitro with PT (1µg/ml) failed to alter the negative inotropic actions of ACh, but pretreatment with PT (6µg/kg, i.v.) abolished the actions of ACh. In subsequent experiments, animals were pretreated with PT i.v.

In aortic rings, KCl 40mM produced contractions of 0.82±0.07g (n=6), 0.69±0.16g (n=4) and 0.63±0.08g (n=4) in tissues from vehicle animals and animals pretreated with PT 6µg/kg and 40µg/kg respectively (no significant differences). PT (6µg/kg) did not significantly alter the relaxations to histamine or ACh in KCl contracted tissues, and PT (40µg/kg) significantly increased the maximum relaxation to histamine.

In prostatic portions of rat vas deferens, PT (40µg/kg) pretreatment failed to alter the inhibition by the alpha$_2$-adrenoceptor agonist xylazine of the isometric contraction to a single stimulus. In right atria incubated with ^{3}H-noradrenaline, PT (40µg/kg) pretreatment failed to alter the inhibition by xylazine of 2Hz stimulation-evoked release of tritium.

Since PT even at doses 6 times higher than necessary to abolish the negative inotropic response of ACh in rat atria, did not affect endothelium-dependent relaxations of rat aorta, nor the peripheral prejunctional inhibitory actions of xylazine, it seems unlikely that G_i or G_o are involved in these responses.

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125P INOSITOL PHOSPHATE FORMATION IN DEVELOPING AND ESTABLISHED PERINEPHRITIS HYPERTENSION IN THE RABBIT

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Alterations in phosphatidylinositol metabolism with increased inositol phosphate (IP) formation have been reported in animal models of hypertension (Heagerty et al.1986; Eid & de Champlain,1988). However, the role of IP in the generation and maintenance of hypertension is uncertain. In this study IP levels were examined during the development of hypertension in the perinephritis hypertensive rabbit. Hypertensive rabbits (HT) and uninephrectomised controls (C) were prepared as described previously (Hamilton & Reid, 1983) and studied either 1,2, or 6 weeks after surgery. Mean arterial pressure (MAP) was measured and the aorta removed. The aorta was cut into 1 mm rings and incubated for 3.5 hours with 0.5 uCi of [^{3}H]-myoinositol and 10^{-2}M LiCl . Noradrenaline (NA) (10^{-6}M-10^{-4}M)was added and samples incubated for a further 30 minutes. Samples were assayed for IP according to Heagerty et al. (1986). NA-stimulated IP formation was expressed as mean ± SEM (n=5-9). Comparisons between HT and C were made using repeated measures ANOVA or unpaired t-test.

MAP was significantly higher in HT than C at all times examined being 87±4,96±3 and 123±7 mmHg in HT at 1,2 and 6 weeks and 74±3,71±3 and 85±2 mmHg in C. Basal values of IP were: 1 week HT 347±42,C 212±37 cpm mm^{-1}, 2 week HT 298±27, C 304±47 cpm mm^{-1}, 6 week HT 222±30, C 248±31 cpm mm^{-1}. NA-stimulated IP formation increased in a dose-dependent manner in all animals. In the 2 week group there was a significantly greater increase in HT animals compared to C at all concentrations of NA ($p<0.05$). However, at 1 and 6 weeks there were no significant differences between HT and C. NA-stimulated but not basal IP levels were also increased in femoral artery at 2 weeks.

NA-stimulated IP production in aorta as a percentage of basal levels

Group (HT)	NA (M)10^{-6}	$3x10^{-6}$	10^{-5}	$3x10^{-5}$	10^{-4}	(C) NA (M)10^{-6}	$3x10^{-6}$	10^{-5}	$3x10^{-5}$	10^{-4}
1 week	123± 8	128± 4	141± 6	164±10	179± 6	100±11	114± 5	158± 9	182±19	224±22
2 week	164±20	182±22	229±40	266±24	313±35	105±14	125±12	141±16	169±16	230±24
6 week	143± 9	175±21	192±31	220±22	246±32	110± 5	134± 7	159±12	180±11	212±12

NA-stimulated IP production was increased at 2 weeks during the development of hypertension but did not appear to be the primary mechanism initiating the rise in blood pressure and was not significantly elevated in established hypertension in this model.

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126P TYPE-2 GLUCOCORTICOID RECEPTORS DIFFERENTIALLY REGULATE THE RELEASE OF ADRENOCORTICOTROPHIN VARIANTS FROM ANTERIOR PITUITARY CELLS

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Glucocorticoids regulate secretion from anterior pituitary corticotrophs. Type-2 glucocorticoid receptors are present in the pituitary and have been implicated in this inhibitory role. We have studied the corticotrophin releasing factor (CRF)-stimulated release of phosphorylated and non-phosphorylated adrenocorticotrophic hormone (ACTH) from 7 day cultures of rat anterior pituitary cells and its regulation by the glucocorticoid dexamethasone (DEX). After preliminary extraction with C-18 SEPAK, immunoreactive ACTH species were separated using reversed-phase, high-performance liquid chromatography (C-18 stationary phase modified by heptafluorobutyric acid in the mobile phase) as described previously (Bennett et al, 1981). This revealed two main forms; a non-phosphorylated form of ACTH (ACTH-n) coeluted with synthetic $ACTH_{1-39}$ and the other eluted 4 min earlier. The latter was likely to be a phosphorylated form of ACTH (ACTH-p) because prior treatment with alkaline phosphatase increased its elution time to that of ACTH-n and $ACTH_{1-39}$.

Stimulation with CRF for 15 min, increased the ACTH secreted by $6X10^6$ cells from 0.61±0.07ng to 1.18±0.10 ng (mean±s.e.m., n=5), and this was unaffected by DEX (100 nM) added 5 min prior to the CRF. However, DEX reduced the ratio of ACTH-n/ACTH-p secreted in the presence of CRF from 0.74±0.15 to 0.33±0.11. These values were not significantly different, because in the absence of CRF, the ACTH-n/ACTH-p ratio varied greatly among experiments. Expressing the ACTH-n/ACTH-p ratio measured in the presence of CRF, as a fraction of that in its absence, corrects for this and shows that DEX significantly ($P<0.01$) reduced the ratio from 2.17±0.27 to 0.84±0.16. Thus DEX favoured the release of ACTH-p. In 2 separate experiments, DEX reduced the ratio ACTH-n/ACTH-p, measured in the presence of CRF, from 0.73±0.06 to 0.45±0.06 (mean±S.D.). Addition of the type-2 glucocorticoid antagonist, RU38486 (1μM), 5 min prior to DEX, returned this to 0.69 ±0.07, which is close to that found in the absence of DEX.

These findings suggest that the release of phosphorylated and non-phosphorylated variants of peptide hormones can be differentially regulated by brief exposure to glucocorticoids, and that this is mediated via a receptor with type-2 characteristics.

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127P ENDOTHELIN-LIKE IMMUNOREACTIVITY IN HUMAN PLASMA: DEVELOPMENT OF A RADIOIMMUNOASSAY AND COMPARISON OF LEVELS IN HYPERTENSION

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Endothelin-1 (ET-1) is a potent vasoconstrictor of human blood vessels in vitro (Brain et al 1989, Davenport et al 1989a), and in vivo (Hughes et al 1989). We have found an extensive distribution of binding sites for [^{125}I] ET-1 which parallels the density of endothelial cells - the putative source of the peptide (Davenport et al 1989 a,b,c), suggesting the majority of these binding sites could be exposed to locally synthesised ET-1. The aim was to determine to what extent the peptide is released into the circulation and whether levels are changed in hypertension.

Forearm venous blood was collected from adult, non-smoking males with normal ECGs and renal function (mean age 42, range 30-54) prior to starting medication for the treatment of hypertension and from age-matched male volunteers. Peptide was extracted from acidified plasma prior to radioimmunoassay (Amersham International plc).

The mean concentration of ET-like immunoreactivity (ET-like IR) measured in plasma from normotensive volunteers was 5.1 ± 0.5 pmol/l (n=25) which falls within the range of human plasma levels previously reported. No significant change was detected in plasma levels from hypertensive patients (5.7 ± 0.5 pmol/l). There was no significant correlation between ET-like IR and age, cholesterol levels or diastolic blood pressure in either group. We were able to detect elevated levels of ET-like IR in plasma from a small number of patients before (12.4 ± 2.7 pmol/l, n=4) and after renal dialysis (19.9 ± 5.6 pmol/l). Human coronary arteries have a threshold for vasoconstriction of about 600 pmol/l with an EC_{50} of 17 nmol/l (Davenport et al 1989a). If these values are representative of most vascular beds it would suggest ET-1 is more likely to function as a locally released rather than as a circulating hormone.

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Supported by British Heart Foundation, Wellcome Trust (APD) and Squibb Cardiovascular Fellowship (DJN).

128P PREDICTION OF *IN VIVO* DISPOSITION FROM HEPATIC MICROSOMAL AND HEPATOCYTE DATA

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Despite the widespread use of *in vitro* systems to study hepatic drug metabolism there has been little effort expended upon the potential of hepatic microsomes and/or isolated hepatocytes to predict *in vitro* disposition. We have investigated the relationships between *in vitro* and *in vivo* metabolism of ethoxycoumarin (EC), a commonly used enzyme marker of the hepatic microsomal monooxygenase system (Ullrich & Weber, 1972).

Hepatic microsomes and isolated hepatocytes were prepared from untreated male Sprague-Dawley rats using standard methodology. EC O-deethylation activity was determined over a range of 1-1000μM substrate concentrations measuring hydroxycoumarin formation by hplc with fluorimetric detection (Waghela & Houston, 1985). The kinetics were consistent with two classes of binding sites as reported by other workers. In our animals the difference between the Km for the high and low affinity sites was two orders of magnitude in microsomes (5.4 and 1408 μM, respectively) and one order of magnitude in cells (17 and 251μM, respectively). The capacity of the low affinity site was approximately one order of magnitude greater than the capacity of the high affinity site in microsomes (22 and 1.4 n moles/min/mg protein, respectively) and 4-fold in cells (123 and 33 p moles/min 10^6 cells, respectively).

Five separate groups of rats, unanaesthetised, with previously implanted cannulae in the carotid artery and hepatic portal vein received both an intravenous bolus and an intravenous infusion of EC over a three hour time period. Carotid blood samples were assayed for EC by hplc with UV detection (Waghela and Houston, 1985) and demonstrated that by varying the bolus dose and infusion rate (2-20 μmoles/h) steady-state concentrations of 1.2-31μM were achieved. The increase in steady-state concentration with increase in infusion rate was disproportionate and could be analysed in terms of a Michaelis-Menten relationship with a Vmax of approximately 0.5 μmoles/min/250g body weight. In terms of unbound concentration, the Km and intrinsic clearance of EC was calculated to be 3.6μM and 137 ml/min/250g body weight, respectively.

In vitro data was scaled up to provide prediction of *in vivo* pharmacokinetic parameters by use of microsomal protein yield, hepatocyte yield, liver weight and degree of nonspecific binding within the microsomes and hepatocytes. Good agreement could be obtained between the *in vitro* estimates of Km obtained from the high affinity site for EC metabolism and the *in vivo* value when unbound concentration was used (5.4 and 3.2μm for microsomes and cells respectively). Scaling of other *in vitro* values was less successful and highlighted the need for precise scale up parameters. The role of the low affinity, high capacity site *in vivo* would appear to be minimal.

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129P THE EFFECT OF THE ISOMERS OF 202-791 AND THE $GABA_B$ AGONIST BACLOFEN ON GLUTAMATE RELEASE FROM DEPOLARISED CULTURED RAT CEREBELLAR NEURONES

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It has been suggested that high voltage activated neuronal Ca^{2+} channels can be divided into two distinct types, the dihydropyridine sensitive L-type channel and the dihydropyridine insensitive N-type channel (Tsien et al, 1988). Because of the generally observed lack of sensitivity of transmitter release to dihydropyridine antagonists it has been said that N and not L-type channels are responsible for the supply of Ca^{2+} for release at presynaptic terminals.

We have examined the effect of the dihydropyridine agonist and antagonist enantiomers of 202-791, (Hof et al, 1985) and the $GABA_B$ agonist baclofen on glutamate release from cultured rat cerebellar granule neurones. ^{3}H-glutamine was used to label a releasable pool of glutamate and released ^{3}H-glutamate was separated as described in Dolphin and Prestwich (1985). As dihydropyridines bind more effectively to depolarised membranes, their effect on glutamate release stimulated by 2 min pulses of 50mM K^+ from neurones maintained in normal medium (2.5mMK^+, 1.8mM Ca^{2+}) was compared to Ca^{2+}-stimulated glutamate release from neurones maintained in a depolarising (50mMK^+, OmM Ca^{2+}) medium. The dihydropyridine antagonist (-)-202-791 (1μM) (10mM stock dissolved in 70% ethanol) inhibited K^+-stimulated glutamate release by 22% ± 5% (mean ± SEM, n=9). This inhibitory response was greatly enhanced by maintaining the neurones in depolarising medium and stimulating release with 5mM Ca^{2+}; under these conditions, glutamate release was almost completely (99%) blocked by 0.5μM (-)-202-791. The IC_{50} for (-)-202-791 in depolarising medium was between 0.1 and 1nM. This is comparable to the effect of (-)-202-791 seen on L-type channels in cardiac muscle. This enhancement of the effect of dihydropyridines is again observed using the agonist enantiomer. (+)-202-791 (5μM) produced an increase in K^+-stimulated glutamate release of 63 ± 12% (10) whereas in depolarised neurones, the stimulatory effect of 1μM (+)-202-791 was 172 ± 38% (3). Inhibition of K^+-stimulated glutamate release and Ca^{2+}-stimulated release by 100μM(-)-baclofen was very similar, being 26 ± 2% (9) and 29 ± 2% (4) respectively.

These results provide evidence that L-channels as well as N-channels are present near release sites and are able to support transmitter release. Glutamate release from depolarised neurones remains sensitive to baclofen, suggesting that both types of high voltage activated channels are capable of inhibition by $GABA_B$ receptor activation, and that both may contribute to $GABA_B$ mediated inhibition of transmitter release under suitable conditions.

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130P DOES PHARMACOLOGICAL STIMULATION OF CENTRAL $GABA_A$ RECEPTORS STIMULATE FEEDING IN SATIATED PIGS?

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Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter in mammalian CNS acting at two distinct receptor subtypes, namely, GABA-A and GABA-B (Bowery, 1988). Recent experiments carried out in rats and sheep suggest that a central GABA-A receptor mediated mechanism may be involved in the control of food intake (see Morley, 1987). The present study was undertaken to investigate whether a similar mechanism may also regulate food intake in pigs.

Prepubertal large white pigs of either sex (n=21, b.w. 30-55kg) were implanted, under halothane anaesthesia, with cannulae aimed at the lateral cerebral ventricle for chronic icv injections. The pigs lived in metal cages and performed operant responses (pressing switch panels with their snouts) to obtain food and water. The occurrence of feeding and drinking was continuously monitored on a data logger. Food and water were available ad libitum. ICV injections of normal saline (control) or drug was carried out each weekday morning, and the criterion for drug administration was that the pig had not eaten in the preceding 30 min.

The GABA-A receptor agonist muscimol (25-200 nmol) icv produced a dose-related increase in food intake. The 25nmol dose had no effect on feeding. However, muscimol (50nmol) caused a significant increase in feeding ($P<0.01$) during the first 30 min after injection, while the 100 and 200nmol doses increased food intake ($P<0.01$) during the first 60 min. Thus, e.g. the pigs ate 778.8 ± 64.9g of food in the first 30 min after muscimol (50nmol) compared with 23.7 ± 18.7g after saline. The effect of muscimol (100nmol) was completely abolished by the simultaneous administration of the GABA-A receptor antagonist bicuculline (100nmol). GABA (20-1600nmol) icv also produced a dose-related increase in food intake. However, only doses of GABA of 800nmol and above caused significant increases in food consumption ($P<0.01$) in the first 15 min after injection. The effects of GABA (1600 nmol) was abolished by the simultaneous administration of bicuculline (50nmol). Analysis of the daily (24h) food intake in pigs treated with muscimol and GABA showed no significant differences from control data. To eliminate the possibility that icv administration of these drugs could influence food intake by a peripheral mode of action, muscimol (100nmol) and GABA (1600nmol) were given iv to 4 pigs. IV administration of these drugs produced no effects on food intake, thus indicating a central mode of action. Both GABA and muscimol had no effects on water intake when administered icv or iv.

The present results indicate that pharmacological stimulation of central GABA-A receptors induces feeding in the satiated pig and extend previous observations in sheep and rats. Further studies are required to determine whether GABA plays a physiological role in the regulation of food intake.

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131P ANTICONVULSANT TOLERANCE AND RECOVERY DURING REPEATED ADMINISTRATION OF THE GABA UPTAKE INHIBITOR NO-328

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The aim of the study was to examine the tolerance-inducing potential of a new GABA uptake blocker, NO-328. This drug was compared with two benzodiazepines (BZs), midazolam and diazepam. The study was carried out using adult, male Tuck No 1 mice (25-40 g). Pentylenetetrazole (PTZ) was used as the convulsive challenge and infused, i.v.,until a predetermined end point was observed. This model has been used extensively in the study of anticonvulsant tolerance with BZs (Haigh & Feely, 1988). From the volume and concentration of PTZ infused and the weights of the mice a minimum convulsant dose (MCD), and thus a convulsive threshold, could be determined (see Gent et al, 1985). The changes in this 'threshold' produced by single and repeated, twice daily, doses of all three drugs were determined; groups of 5 mice were used throughout. Doses and times for testing (and the BZs used) were chosen as a result of preliminary experiments which determined single dose time profiles and log-dose response curves.

Results (MCD of PTZ, mg/kg): CONTROLS (vehicle only, day 1), 41.0 (0.8)

	1st dose	12h	24h	48h	72h
DIAZEPAM (0.6 mg/kg), mean (s.e.m.)	98.3 (1.6)	61.3 (1.6)	54.1 (2.0)	54.9 (2.7)	51.4 (3.3)
MIDAZOLAM (0.75 mg/kg), mean (s.e.m.)	109.4 (3.1)	79.7 (2.5)	71.6 (8.6)	79.7 (4.0)	58.7 (0.8)
NO-328 (2.0 mg/kg), mean (s.e.m.)	95.5 (4.4)	94.2 (3.1)	63.2 (4.1)	80.8 (3.2)	89.0 (3.6)

Tolerance was assessed by measuring changes in MCD over time; significance of results (changes in MCD) was assessed using single classification analysis of variance. Both BZs showed significant tolerance ($P < 0.05$) after one dose and this was maintained for the duration of the study. NO-328 showed a significant reduction ($P < 0.05$) in anticonvulsant effect after 2 doses. However, during continued dosing recovery occurred and was almost complete by 3 days. In supplementary studies, recovery was maintained during 8 days dosing and a similar pattern of tolerance and recovery was seen with 1.0 mg/kg NO-328. On the basis of the single dose time profile, it is unlikely that this recovery results from accumulation of the drug. Although it was less potent than both BZs, the recovery from tolerance suggests that NO-328 may merit further investigation as an anticonvulsant.

We wish to thank Novo (Denmark) for NO-328 and financial assistance.

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132P THE PERIPHERAL-TYPE SITE FOR BENZODIAZEPINES MAY BE ASSOCIATED WITH THE UPTAKE CARRIER FOR ADENOSINE

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Diazepam has been shown to potentiate adenosine by binding to the uptake carrier for adenosine and blocking it (Wu et al., 1980; Phillis & O'Regan, 1988). The present study attempts to characterize the structure/activity profile for this effect of the benzodiazepines (BDZ).

Rat vas deferens was suspended in Krebs bicarbonate medium gassed with 95% O_2 ± 5% CO_2 at 37°C. Field stimulation at 5 or 10 Hz with pulse width 0.1 - 0.5 ms and supramaximal voltage was applied for 2 s every 5 min. Adenosine (1 - 100 µM) added 1 min before stimulation caused a dose-related depression of the isometrically recorded twitch response. Subsequent incubation in 10 µM diazepam for 30 min resulted in a shift to the left of the adenosine log. dose - response curve by 0.721 ± 0.072 log. unit (s.e.mean, n=8). At 1 µM, diazepam caused a smaller potentiation of adenosine of 0.288 ± 0.064 log. unit (n=4). These results are similar to those reported by Clanachan & Marshall (1980). Diazepam has high affinity for both the centraltype and peripheral-type BDZ sites. However, clonazepam binds selectively to the central-type site and Ro5-4864 binds selectively to the peripheral-type site (Braestrup & Squires, 1978; Schoemaker et al., 1981). In the present experiments, 10 µM clonazepam failed to potentiate adenosine whereas 1 and 10 µM Ro5-4864 caused shifts to the left of the adenosine log. dose - response curve by 0.317 ± 0.040 (n=4) and 0.964 ± 0.046 (n=5) log. unit, respectively. The antagonist at the central-type site, flumazenil 10 µM, did not reduce the potentiation of adenosine by 10 µM diazepam but the selective ligand for the peripheral-type site, PK11195 (LeFur et al., 1983), at 3 µM did significantly reduce the potentiation due to both 10 µM diazepam and 10 µM Ro5-4864 to 0.424 ± 0.060 (n=6) and 0.582 ± 0.029 (n=5) log. unit, respectively.

Thus, the structure/activity profile for BDZ potentiation of adenosine in the rat vas deferens strongly suggests that the peripheral-type site for BDZ is associated with the uptake carrier for adenosine. If a similar situation obtains in the c.n.s. where peripheral-type sites are also present, the overall effect of a BDZ might be comprised of potentiation of adenosine as well as potentiation of GABA at the central-type site. These two effects would occur in different proportions according to the BDZ concerned and could, therefore, account for differences in clinical properties between BDZs.

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133P RP 59037, A NOVEL PARTIAL AGONIST AT THE GABA-BENZODIAZEPINE RECEPTOR: BINDING AND BEHAVIOURAL STUDIES IN THE RODENT

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The widespread use of benzodiazepines as anxiolytic drugs has been criticised due to their sedative side-effects and their propensity to produce dependence (Lader, 1987). It has been suggested that partial agonists at the GABA-benzodiazepine receptor may not show these drawbacks and thus be more suitable therapeutic agents (Haigh & Feely, 1988). We now report the partial agonist properties of a cyclopyrrolone derivative, RP 59037 (2-(7-Chloro-2- naphtyridin-1,8-yl)3-(5-methyl-2-oxohexyl)isoindolin-1-one).

In radioligand binding studies, RP 59037 displaces the binding of [^{3}H]-flunitrazepam to rat cortical membranes with an IC_{50} of 1.6 ± 0.2 x 10^{-9} M. This compound had no effect on the binding of [^{3}H]-muscimol to the GABA recognition site on the GABA-benzodiazepine receptor, whereas the binding of [^{35}S]TBPS to the picrotoxin site was enhanced by 37%. This latter test, widely used to predict efficacy, would suggest that RP 59037 may be a partial agonist. In vivo, RP 59037 displaces the binding of [^{3}H]-flumazenil in the rat cerebral cortex with an ID_{50} of 3.51 mg/kg po. In behavioural tests, RP 59037 had marked activity (Table 1) in two different models of anxiety, a classical conflict test (Geller & Seifter, 1960) and the elevated plus-maze (Pellow et al., 1985). This compound also had potent anticonvulsant activity against pentylenetetrazole-induced seizures in rats. In tests of myorelaxant and sedative effects, RP 59037 was much less active than diazepam at impairing performance in the rotarod test in rats and in mice treated with a subnarcotic dose of ethanol. The behavioural profile of RP 59037 is qualitatively comparable to that of CGS 9896, a partial agonist of reference (Brown et al., 1984), although RP 59037 seems to be more potent than this latter compound.

In conclusion, RP 59037 is a potent and specific ligand at benzodiazepine binding sites, active in animal models predictive of anxiolytic and anticonvulsant properties, and devoid of sedative effects. It is thus a promising candidate for the treatment of anxiety in man.

Table 1

			RP 59037	diazepam	CGS 9896
conflict (rat)	MED	(mg/kg po)	1	8	10
plus-maze (rat)	MED	(mg/kg po)	0.63	5	-
pentetrazole (rat)	ED_{50}	(mg/kg po)	0.66 (0.37 - 1.19)	5.90 (3.70 - 9.51)	29.5 (18.3 - 47.4)
rotarod (mouse)	ED_{50}	(mg/kg po)	93.0 (35.0 - 243)	1.50 (0.50 - 2.90)	>400
ethanol (mouse)	ED_{50}	(mg/kg po)	>800	1.27 (0.69 - 2.34)	133 (46.0 - 386)

Data are presented as the Minimal Effective Dose (MED) or the ED_{50} with 5 - 95% confidence limits.

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134P LOCAL CEREBRAL GLUCOSE UTILISATION DURING SPONTANEOUS AND "FLUMAZENIL-PRECIPITATED" WITHDRAWAL FROM DIAZEPAM

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There is controversy over whether the benzodiazepine antagonist, flumazenil (FZL), precipitates a withdrawal syndrome (Cumin et al. 1982), prevents withdrawal anxiety (Baldwin & File 1988) or develops its own inverse-agonistic activity (Little et al. 1987) after chronic benzodiazepine treatment. This study was designed to distinguish between these possibilities using quantitative ^{14}C-2-deoxyglucose autoradiography in rat brain. Male hooded rats were assigned to 6 groups (n=6-8 per group), and each given daily injections of vehicle (Groups 1 & 2) or diazepam (DZP; 5 mg/kg)(Groups 3 to 6) for 28 days. On the test day the conscious rats received 2 i.v. injections, (a) & (b), 10 min and 1 min respectively before measurements were initiated. The injection combinations ((a),(b)) were: Group 1 (vehicle, vehicle); Group 2 (vehicle, FZL 5 mg/kg); Group 3 (DZP 0.3 mg/kg, vehicle); Group 4 (DZP, FZL); Group 5 (vehicle, vehicle); Group 6 (vehicle, FZL). The rate of glucose use was determined in 68 brain regions.

Table 1 Effect of Flumazenil and/or Chronic Diazepam on Glucose Use in Limbic Structures

Group:	1	2	3	4	5	6
Structure	Control	Acute FZL	Chronic DZP	Precipitated Withdrawal	Spontaneous Withdrawal	Spontaneous Withdrawal + FZL
Mamillary Body	95±4	97±3	84±3	117±6*,+	95±4	114±4*,x
Anterior Thalamus	103±4	106±3	88±4	122±5*,+	102±4	122±2*,x
Subiculum	84±4	83±4	78±4	88±4	78±3	86±4
Hippocampus	79±3	71±4	71±2	76±2	71±3	74±4
Cingulate Cortex IV	97±4	98±2	89±3	112±4*,+	94±3	110±2*,x

Data are presented as mean glucose use (μmol/100g/min)±SEM. Data were analysed by 2-way ANOVA followed by the Bonferroni method. * $p<0.05$ vs Control; + $p<0.05$ vs Chronic DZP; x $p<0.05$ vs Spontaneous Withdrawal.

Neither FZL administration to drug-naive rats, nor spontaneous withdrawal from DZP affected glucose use in any brain region examined. However, FZL administered to rats chronically treated with DZP or to rats already in spontaneous withdrawal raised glucose use in many limbic structures (Table 1). The patterns of raised glucose use in limbic structures were similar to those induced by FG 7142 (Pratt et al. 1988). This may suggest that, after chronic DZP treatment, FZL changes from being a BZ antagonist to having partial inverse-agonistic properties.

We thank the Wellcome Trust for financial support.

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135P INFLUENCE OF DIAZEPAM ON THE ANALGESIC EFFECTS OF ELEVATED PLUS-MAZE EXPOSURE IN MICE

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The suggested link between some forms of non-opioid analgesia and anxiety has been supported by our observation of this type of pain inhibition in mice exposed to the elevated plus-maze (EPM) test of anxiety (Lee & Rodgers, 1989). To further examine this relationship, the present studies assessed the influence of the benzodiazepine anxiolytic, diazepam (DZP), on EPM analgesia. 13-20 week old male DBA/2 mice (Bantin & Kingman, Hull) were housed in a temperature controlled room (24±1°C) under a reversed LD cycle. Testing was conducted under dim red light during the dark phase of the cycle. The EPM was based on that validated for mice by Lister (1987). Tail flick latencies (TFL) were assessed by traditional radiant heat assay both before (pre) and after (0, 5 & 10 min) EPM exposure. DZP (Roche Products Ltd) was ultrasonically dispersed in distilled water to which Tween 80 had been added; water/Tween alone served as vehicle control. Injections were performed i.p. in a volume of 10ml/kg. For both studies, a 5 min EPM exposure period was used, with mice initially placed on the central square of the EPM. In Expt. 1, mice were treated with DZP (0, 0.5 or 1mg/kg) 30 min prior to testing; higher acute doses produced significant movement suppression and were not used. In Expt. 2, untreated mice were exposed to the EPM on Day 1, following which they received 7 daily injections of DZP (0, 2 or 4 mg/kg). On Day 9, mice again received DZP and were exposed to the EPM 30 min later. Data were analyzed by ANOVA and appropriate follow-up tests. Results are summarized in Table 1.

Table 1 Effects of acute and chronic diazepam treatment on EPM analgesia. Data are given as mean TFL (s.e.mean). * p<0.05, ** p<0.01 vs pre-EPM ; + p<0.05, ++ p<0.01 vs vehicle control.

	Acute Diazepam			Chronic Diazepam		
	Vehicle	0.5mg/kg	1.0mg/kg	Vehicle	2.0mg/kg	4.0mg/kg
Pre-EPM	2.2(0.1)	2.1(0.1)	2.2(0.1)	1.9(0.1)	1.9(0.1)	1.9(0.1)
0 post	2.9(0.3)**	2.4(0.1)*++	2.5(0.1)+	2.4(0.1)**	2.0(0.1)++	2.0(0.0)++
5 post	3.0(0.2)**	2.3(0.1)++	2.5(0.1)*++	2.1(0.1)**	2.0(0.1)	1.9(0.1)
10 post	2.8(0.2)**	2.2(0.1)++	2.6(0.2)**	2.2(0.2)**	2.2(0.1)**	2.1(0.1)*

In Expt. 1, EPM exposure resulted in analgesia which lasted for at least 10 min, the period of TFL testing ($F(3,87) = 8.8$, $p<0.01$). This effect was attenuated, but not totally blocked, by DZP ($F(2,29) = 4.62$, $p<0.05$). In Expt. 2, analysis confirmed Day 1 analgesia in all groups ($F(3,87) = 9.47$, $p<0.01$). Chronic DZP treatment produced complete inhibition of analgesia immediately after EPM exposure, partial inhibition at 5 min and no effect at 10 min ($F(6,87) = 2.2$, $p<0.05$). The latter finding may suggest involvement of more than one intrinsic analgesia system. Present data confirm that anxiety is an important factor in non-opioid EPM analgesia. Other classes of anxiolytics are currently being tested in this model.

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136P CHARACTERISATION OF CHOLECYSTOKININ OCTAPEPTIDE STIMULATED ENDOGENOUS DOPAMINE RELEASE FROM RAT NUCLEUS ACCUMBENS *IN VITRO*

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Cholecystokinin has been found to coexist with dopamine in some mesolimbic neurones (Hokfelt et al, 1980). Electrophysiological evidence shows that sulphated cholecystokinin (CCK8S) excites neurones in the nucleus accumbens (White and Wang, 1984). Tritiated dopamine release is also stimulated by CCK8S (Vickroy and Bianchi, 1989). The use of selective agonists and antagonists suggest that both these effects are mediated via a CCK_A type of receptor. In the present study, the effects of CCK8S on endogenous release of dopamine from slices of rat nucleus accumbens and striatum were examined using HPLC with electrochemical detection. CCK8S (10^{-9} - 10^{-5}M) had no effect on basal or potassium stimulated dopamine release from slices of rat striatum. In the nucleus accumbens CCK8S (10^{-8} to 10^{-5}M) caused a dose dependent increase in the potassium stimulated release of dopamine. Pentagastrin (10^{-8} to 10^{-5}M) was without effect on the release of dopamine. In the presence of low concentrations of L364718 (10^{-9} to 10^{-8}M) which would specifically antagonise the CCK_A receptor (Chang and Lotti, 1986), the effects of CCK8S were completely abolished. In contrast to this blocking action, in the presence of either 10^{-8} or 10^{-7}M L365260, a specific CCK_B receptor antagonist (Kemp and Woodruff, 1989), the CCK response remained intact. In addition, when 10^{-8}M L364718 was applied to the preparation in the presence of CCK, it caused a decrease in evoked dopamine release. These data suggest that the cholecystokinin receptor mediating the release of dopamine in the nucleus accumbens resembles the CCK_A receptor in peripheral gastrointestinal systems rather than the central CCK_B receptor.

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137P THE CCK ANTAGONISTS DEVAZEPIDE AND L-365,260 PROMOTE WEIGHT GAIN IN WEANLING RATS

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Cholecystokinin octapeptide (CCK) reduces food intake in a variety of animal species and in humans. Recent studies have suggested that endogenous CCK may induce satiety by stimulating CCK receptors in the brain. Thus the selective brain type CCK receptor (CCK-B) antagonist L-365,260 (Bock *et al.* 1989; Lotti *et al* 1989; Kemp *et al.* 1989) was 100 times more potent than the selective peripheral CCK receptor (CCK-A) antagonist devazepide (formerly MK-329, L-364,718) in increasing food intake and preventing satiety in rats (Dourish *et al.* 1989). In the present study we examined whether chronic treatment with CCK receptor antagonists may increase body weight gain in weanling rats. 21 day old male Sprague Dawley rats were housed in groups of 4 and allowed ad libitum access to standard food pellets and water. They were randomly divided into groups of eight and injected between 4.00 and 5.00 p.m. each day for 40 days with either devazepide (0.001, 0.01, 0.1mg/kg s.c.), L-365,260 [3R(+)-N(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N1(3-methyl-phenyl)urea (0.0001, 0.001, 0.01mg/kg s.c.) or 0.5% methylcellulose vehicle. The percentage increase in body weight was calculated for each animal and data were analysed by a two-way ANOVA. The table shows the percentage increase in body weight.

Effect of devazepide and L-365,260 on weight gain in weanling rats following 40 days of daily dosing.

		Devazepide (mg/kg)			L-365,260 (mg/kg)		
	Vehicle	0.001	0.01	0.1	0.0001	0.001	0.01
% increase	295.9	361.8*	378.2*	340.0	369.9*	393.0**	382.6**
SEM	14.3	14.5	10.4	23.3	22.1	16.5	18.1

Significant differences determined by Tukey test following significant ANOVA. * $p < 0.05$, ** $p < 0.01$.

Both CCK antagonists caused a significantly greater increase in body weight gain in weanling rats than that observed in controls [devazepide $F(3,29) = 4.43$, $p < 0.01$, L-365,260 $F(3,29) = 6.88$, $p < 0.001$]. The doses of the selective CCK-B antagonist L-365,260 which increased growth are low suggesting that the increase in body weight gain may be mediated by CCK-B receptors. This finding is in agreement with results from satiety studies in adult rats in which L-365,260 was more potent than devazepide (Dourish *et al.* 1989). Therefore CCK antagonists may have a role as growth promoters in weanling animals.

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138P ANXIOLYTIC PROFILE OF THE CHOLECYSTOKININ ANTAGONIST DEVAZEPIDE IN MICE

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Acute injection of benzodiazepines antagonizes the effect of cholecystokinin (CCK) in the periphery and the CNS (Bradwejn and De Montigny, 1984). Thus, it was predicted that CCK receptor activation might induce anxiety. The results of recent clinical studies support this hypothesis. Thus, i.v. injection of CCK-4 provoked panic attacks in normal volunteers (De Montigny, 1989) and reproduced the symptoms of spontaneous panic in anxious patients (Bradwejn et al, 1988). These findings suggested that CCK antagonists might have anxiolytic properties. Therefore, we examined the effects of the CCK antagonist devazepide (formerly MK-329) in a simple animal model of anxiety in the mouse, the black/white exploration box. Male DBA/2 mice (20-25g) were injected i.p. with vehicle (0.5% methylcellulose) or 0.00005-0.5mg/kg devazepide and 30 minutes later placed in the black/white box where their behaviour was assessed as previously described (Costall et al, 1986). Data were analysed by ANOVA and Dunnett's test. Drug effects on line crossing (LX) were also representative of effects on rears and time spent in the white side and are shown in the table.

Effect of the CCK antagonist Devazepide on LX in the black/white box

	Devazepide (mg/kg)					
	0	0.00005	0.0005	0.005	0.05	0.5
LX in						
White	58.2(15.2)	35.1(4.7)	53.4(12.1)	55.4(3.7)	33.5(4.8)	36.8(7.4)
Black	107.1(9.1)*	87.6(8.7)*	71.5(13.2)	61.3(19.0)	97.7(15.2)*	60.0(21.3)

Data are mean(SEM); *=$p<0.05$ from white

These data indicate that (1) vehicle controls displayed an increase in line crossings, rearing and time spent in the black section; (2) Devazepide (0.0005,0.005 and 0.5mg/kg) reduced this preference for the black side to non-significance, indicating anxiolysis at these doses; (3) CCK receptors appear to be involved in the expression of anxiety and therefore CCK antagonists may be useful anxiolytic drugs.

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139P EVIDENCE FOR THE INVOLVEMENT OF CCK-A RECEPTORS IN THE MEDIATION OF OPIOID SOCIAL CONFLICT ANALGESIA IN MICE

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Recent studies have shown that the selective CCK-A antagonist devazepide (formerly MK-329) and the selective CCK-B antagonist L-365,260 enhance the analgesic efficacy of morphine in rats (Dourish et al, 1988,1989). L-365,260 was 5-20 times more potent (depending on the analgesia model) than devazepide in enhancing morphine analgesia suggesting mediation of the response by CCK-B receptors (Dourish et al,1989). By contrast, devazepide completely blocks the opioid form of social conflict analgesia in mice (Hendrie et al,1989). However, the doses of devazepide required to block social conflict analgesia were 500 times lower than those required to enhance morphine analgesia. Therefore, it appears that the morphine-enhancing properties of devazepide are due to blockade of CCK-B receptors whereas the prevention of social conflict analgesia is mediated by CCK-A receptors. In order to test this hypothesis we examined the effects of the selective CCK-B antagonist L-365,260 on opioid social conflict analgesia in mice. Male DBA/2 mice (20-25g) were assayed for baseline tail flick latency (TFL) and injected intraperitoneally with L-365,260 (0.0005-5.0mg/kg) or vehicle(0.5% methylcellulose) 30 minutes prior to being placed into the home cage of an aggressive individually housed BKW male mouse and exposed to 35 bite attacks. This procedure has been found to induce profound analgesia in the attacked animal which is unequivocally mediated by opioidergic mechanisms (Rodgers and Randall, 1985). Subsequently, TFL's were assessed immediatly after the termination of, and at 20,40 and 60 minutes post-encounter. The data obtained mean(SEM) in vehicle controls are shown in the table.

	Minutes post-encounter			
Baseline	0	20	40	60
2.7(0.09)	4.9(0.5)*	3.9(0.4)*	3.2(0.3)	2.9(0.2)

* = $p<0.05$ from baseline control

Analysis of variance and Dunnett's test revealed there to be significant analgesia at 0 and 20 minutes post-encounter in controls, a profile which was unaffected by 0.0005,0.005,0.05,0.5 or 5.0mg/kg L-365,260.
These data demonstrate that blockade of CCK-B receptors by the selective CCK-B antagonist L-365,260 fails to alter the expression of the opioid form of social conflict analgesia in DBA/2 mice. Therefore, the results support the hypothesis that blockade of opioid social conflict analgesia in mice by devazepide is mediated by CCK-A receptors.

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140P MUSCARINIC RECEPTOR MEDIATED PHOSPHOINOSITIDE METABOLISM IN CEREBELLAR GRANULE CELLS

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Primary cultures of cerebellar granule cells have proven to be valuable preparations to investigate the mechanism of glutamate release (Gallo et al., 1982). They also possess a variety of receptors linked to phosphoinositide metabolism (Xu & Chuang, 1987). We have examined the properties of muscarinic receptor stimulation in these cells with a view to understanding the relationship between the signalling system and calcium homeostasis in neurones.
Granule cells were prepared from neonatal rats 6-8 days old and cultured in serum containing medium with elevated potassium to provide the necessary depolarising conditions required for cell survival.
Cells were labelled with [^{3}H]inositol for 48 hours and [^{3}H]inositol phosphates ([^{3}H]-IP) separated using anion exchange columns. In other experiments, the mass of inositol(1,4,5)trisphosphate (Ins(1,4,5)P_3) was analysed by a radioreceptor method (Challiss et al., 1988). Muscarinic receptors were also identified in intact cells using the radioligand [^{3}H]-N-methylscopolamine [^{3}H-NMS].
Cells examined at 7 days in vitro possess a high density (283±48 fmol/mg protein) of muscarinic receptor sites identified with ^{3}H-NMS (K_D 0.18±0.01 nM). The sites are not M_1 receptors as they possess low affinity for pirenzepine, but their definitive classification remains to be established.
Muscarinic stimulation with carbachol leads to a large accumulation of [^{3}H]IP (15-20 fold in the presence of 5mM Li^+) and this response was seen as early as 4 days and maintained until 12 days derived in culture. The onset of this response coincides with attainment of an equilibrium in the incorporation of [^{3}H]inositol into the phospholipid. Other agonists, noradrenaline, 5-hydroxytryptamine and histamine also stimulated phosphoinositide metabolism, but to a lesser degree. Mass assays of Ins(1,4,5)P_3 revealed basal levels of 6.5±1.7 pmol/mg protein that were elevated by 1mM carbachol to 19.4±0.75 pmol/mg protein at 5 s that decayed to a plateau (9.7±1.4 pmol/mg protein) at 1 min and was maintained for at least 5 min.
These data provide evidence for a muscarinic receptor mediated production of the second messenger Ins(1,4,5)P_3 in a relatively homogeneous primary culture of neurones. They may represent a particularly useful cellular system to investigate receptor mediated polyphosphoinositide metabolism, intracellular Ca^{2+} homeostasis and the possible modulation of transmitter release from neurones.

E.M. Whitham is a Wellcome Trust student and this work is supported by The Wellcome Trust.

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141P XANTHINE AFFINITIES FOR THE ADENOSINE RECEPTOR MEDIATING INHIBITION OF TRANSMISSION AT THE RAT HIPPOCAMPUS

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The xanthine amine congener, 1,3-dipropyl-8-(4-(2-aminoethyl)amino)carbonylmethyloxyphenyl)xanthine (XAC), has a similar potency as antagonist of the adenosine receptors mediating inhibition of transmission at the frog neuromuscular junction, rat neuromuscular junction and rat hippocampus (Sebastião et al., 1989). However, the potencies of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and 1,3-dipropyl-8-(4-carboxymethyloxyphenyl)xanthine (XCC) as antagonists of the inhibitory adenosine receptor at the frog (Sebastião & Ribeiro, 1989) and rat (Ribeiro & Sebastião, 1990) neuromuscular junctions are different. In the present work the affinities of DPCPX and XCC for the adenosine receptor mediating inhibition of transmission at the rat hippocampus were investigated.

The experiments were carried out at 30°C on hippocampal slices (400 µm thick) of the rat. Orthodromic population spikes in the pyramidal layers of the CA_1 region were recorded through 3 M KCl-filled microelectrodes of 5-10 MΩ resistance. The Schaffer collateral and commissural afferents were stimulated through a bipolar electrode placed in the stratum radiatum. The bathing solution (pH 7.4) contained (mM): NaCl 115, KCl 2.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 2.2, $NaHCO_3$ 25, glucose 10. The solutions were continuously gassed with 95% O_2 and 5% CO_2

In Table 1 are shown the pA_2 and Ki values for DPCPX and XCC, obtained from the antagonist-induced shifts to the right of the concentration-response curves for the inhibitory effect of 2-chloroadenosine on the amplitude of the orthodromically evoked population spikes in CA_1 pyramids of the hippocampus.

Table 1 Potency of DPCPX and XCC at the rat hippocampus

	pA_2 (±s.e.mean)	slope*	Ki
DPCPX (n=6)	9.38 ± 0.02	0.99	0.42 nM
XCC (n=11	8.27 ± 0.04	1.03	5.4 nM

*Slope of the regression line of the Schild plot

The potencies of DPCPX and XCC as antagonists of the adenosine receptor at the rat hippocampus were similar to those obtained at the rat neuromuscular junction (DPCPX: Ki=0.54nM, XCC: Ki=10nM - Ribeiro & Sebastião, 1990). This suggests that the adenosine receptors mediating inhibition of transmission at the peripheral and central nervous systems in the same animal species are similar.

We thank Drs J.W. Daly and K.A. Jacobson for gifts of XCC

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142P CHARACTERISATION OF [^{3}H]-SQ29,852 RECOGNITION SITES IN RODENT BRAIN

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The presence of the components of the renin-angiotensin system in brain tissue has been previously demonstrated. Here we report the characterisation of the recognition sites labelled by the tritiated derivative of the potent angiotensin converting enzyme (ACE) inhibitor SQ29,852 (Karanewsky *et al.*, 1988).

Female Hooded Lister rats (200-250g) were killed by cervical dislocation and the brains removed. The amygdala, cerebral cortex, hippocampus and striatum were dissected and pooled separately. Pooled tissues were homogenised (Polytron, setting 7, 10 sec) in 20 vol Hepes (50mM)/Krebs buffer (pH 7.4) and centrifuged for 10 min (48000 x g, 4°C). The pellet was resuspended in the buffer and centrifuged as above. The membrane homogenate was formed by resuspending the pellet in buffer at approximately 50mg original wet weight/ml. In the binding experiments each assay tube contained 650µl of competing drug/buffer and 100µl [^{3}H]SQ29,852 (63Ci/mmol): 250µl of tissue homogenate was added to initiate binding, which was allowed to proceed for 30 min at 37°C before termination by rapid filtration through Whatman GF/B filters followed immediately by washing with 12ml ice-cold buffer (wash time 8 sec). Bound radioactivity was quantified by liquid scintillation spectroscopy at an efficiency of approximately 47%. All results are means ± S.E.M., n = 3, except where stated.

Scatchard analysis of [^{3}H]SQ29,852 saturation experiments (non-specific binding defined by 10µM SQ29,852) indicated the existence of an homogenous population of [^{3}H]SQ29,852 recognition sites in the cortex (B_{max} = 147±18fmol/mg protein, pKd = 8.61±0.14), amygdala (B_{max} = 242±22fmol/mg protein, pKd = 8.69±0.02), hippocampus (B_{max} = 875fmol/mg protein, pKd = 8.59, n = 2) and striatum (B_{max} = 3317±192fmol/mg protein, pKd = 8.42±0.04). In both the hippocampus and striatum, the ACE inhibitors captopril and unlabelled SQ29,852 competed potently for [^{3}H]SQ29,852 binding sites, captopril being the most potent. The mean IC_{50} values for captopril and SQ29,852 were 1.6±0.1nM and 4.3±0.3nM in the hippocampus and 4.1±0.2nM and 6.3±0.3nM in the striatum respectively.

It is concluded that [^{3}H]SQ29,852 labels a recognition site that is pharmacologically similar to ACE.

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143P EFFECT OF PERTUSSIS TOXIN ON CARBACHOL-STIMULATED RISES IN $[Ca^{2+}]_i$ AND INOSITOL PHOSPHATE ACCUMULATION IN SH-SY5Y NEUROBLASTOMA CELLS

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Activation of muscarinic M3 receptors expressed on human SH-SY5Y neuroblastoma cells leads to stimulated phosphoinositide metabolism, Ca^{2+} mobilization from intracellular stores and Ca^{2+} entry through a Ni^{2+} sensitive channel (Lambert and Nahorski 1989a, 1989b). Here we examine the possibility that a pertussis toxin (PTX)-sensitive G-protein may regulate components of this response.
Cells were pretreated with 100ng/ml PTX for 22-26 hours. Experimental incubations were carried out at 37°C in Krebs-Henseleit buffer pH7.4. $[Ca^{2+}]_i$ measurements were made in fura2 loaded cells and calculated according to Grynkiewicz et al (1985). Total [^{3}H]-Inositol phosphate (IP) accumulation (over 30min in the presence of 5mM Li^+) was measured in 1hr [^{3}H]-Inositol (4μCi/ml) prelabelled cells. [^{3}H]-rauwolscine binding to whole cells was performed over 30min and non-specific binding was defined in the presence of 10μM phentolamine. The effect of adrenaline (1 and 10μM) on forskolin (10μM) stimulated cAMP accumulation was measured over 10mins in the presence of 1mM IBMX. [cAMP] was determined by protein binding assay (Brown et al 1971). All data shown are Mean±SEM (n=3-4).
Carbachol caused a biphasic increase in $[Ca^{2+}]_i$ (Basal 54±8nM rising to 505±20nM then declining to a steady state of 145±7nM with 1mM carbachol). PTX (100ng/ml) pretreatment neither influenced the peak (internal stored Ca^{2+}) or plateau (influx) phase $[Ca^{2+}]_i$ nor carbachol stimulated [^{3}H]-IP accumulation (EC_{50}31±6μM in control and 43±16μM in PTX treated cells). In parallel experiments the effectiveness of PTX to inactivate G_i-protein was confirmed by the total reversal of alpha$_2$ adrenoceptor (shown to be expressed by these cells by the presence of specific [^{3}H]-rauwolscine binding) mediated inhibition of forskolin stimulated cAMP production in whole cells.
This study demonstrates in SH-SY5Y cells, that whilst a PTX sensitive G-protein is involved in the alpha$_2$ adrenoceptor mediated inhibition of cAMP accumulation such a G-protein is not involved in muscarinic receptor linked rises in $[Ca^{2+}]_i$ or phosphoinositide metabolism.

This work was supported by The Wellcome Trust.

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144P INTRACELLULAR STORES OF CA^{2+} IN ELECTRICALLY PERMEABILISED SH-SY5Y CELLS ARE DISCHARGED FOLLOWING MUSCARINIC RECEPTOR AND G-PROTEIN ACTIVATION

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D-Inositol 1,4,5-trisphosphate (IP_3) is a Ca^{2+}-mobilizing second messenger derived from the action of phospholipase C on phosphatidylinositol 4,5-bisphosphate (PIP_2) (Berridge & Irvine, 1989). In general, studies dealing with the Ca^{2+}-mobilizing properties of inositol phosphates have utilized disrupted cells in order to overcome the inability of these compounds to traverse the plasma membrane. Recently we have been examining factors affecting Ca^{2+}-release from electrically permeabilized SH-SY5Y human neuroblastoma cells. In these cells phospholipase C is activated by muscarinic agonists, such as carbachol and by GTP analogues, such as GTP[S] (Wojcikiewicz & Nahorski, 1989). Ca^{2+}-release from cell suspensions (4-10mg protein/ml) was monitored either with a Ca^{2+}-selective electrode or by redistribution of $^{45}Ca^{2+}$ sequestered, in an ATP-dependent manner, into intracellular stores.
Using the Ca^{2+}-selective electrode, IP_3,in a dose-dependent manner, rapidly increased pCa^{2+} from 6.5 (basal) to 5.9 (maximal effect). In addition, carbachol (1mM) and GTP[S] (0.1mM) also stimulated Ca^{2+}-release by a similar extent. However, the effects of these agents were slower in onset and were more prolonged than those of IP_3. Mastoparan (20μM), which inhibits PIP_2 hydrolysis (Wojcikiewicz & Nahorski, 1989), blocked the effects of carbachol and GTP[S], indicating that Ca^{2+}-release resulted from activation of phospholipase C. This was confirmed by measurement of IP_3 concentration in the cell suspensions using a radioreceptor assay (Challiss et al., 1988); the kinetics of Ca^{2+}-release followed closely changes in IP_3 concentration, and in the presence of carbachol and GTP[S], IP_3 concentration reached approx. 0.5μM.
GTP[S] dramatically enhanced the ability of carbachol to release Ca^{2+}. This was seen most clearly in experiments with $^{45}Ca^{2+}$, in which carbachol alone maximally released 32% of sequestered $^{45}Ca^{2+}$ (EC_{50}=61±12μM,n=19) and carbachol in the presence of GTP[S] released maximally 42% of $^{45}Ca^{2+}$ (EC_{50}=0.34±0.09μM,n=17).
These data show that stimulation of muscarinic receptors and/or G-proteins in permeabilized SH-SY5Y cells leads to an IP_3-dependent release of intracellular Ca^{2+}. This system represents a valuable model with which to investigate the mechanisms and control of this form of signal transduction.

This work was supported by The Wellcome Trust.

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145P GUANINE NUCLEOTIDE SENSITIVITY OF [^{3}H]-ILOPROST BINDING SITES

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[^{3}H]-Iloprost, a stable analogue of prostacyclin (PGI_2), is widely used as a radioligand for PGI_2 receptors (Edwards et al, 1987; Hall & Strange, 1984). PGI_2 receptors are G-protein coupled receptors, and thus the binding of the agonist ligand [^{3}H]-iloprost to these receptors might be expected to exhibit guanine nucleotide sensitivity. In this study we demonstrate for the first time that [^{3}H]-iloprost binding is indeed sensitive to guanine nucleotides.

Human platelets, NG108-15 cells (passage 16-25) or NCB-20 cells (passage 12-20) where homogenized in 20mM HEPES, 10mM EDTA, pH 7.5 using a glass Dounce. The homogenates were stirred on ice for 10 min and then washed three times in 20mM HEPES, pH 7.5. [^{3}H]-iloprost binding was assayed by a modification of the method of Edwards et al. (1987), using 20mM HEPES, 1mM $MgCl_2$ pH 7.5 as the assay buffer.

A proportion of the specific binding of low concentrations of [^{3}H]-iloprost (0.5 - 5nM) was suppressed by 10^{-4}M GppNHp. This proportion was 20 - 30% in platelet membranes, 50 - 60% in NG108-15 cell membranes and 60-70% in NCB-20 cell membranes. The rank order of potency for a range of nucleotides suppressing ^{3}H-iloprost binding to NG108-15 cell membranes was GTP γ S $\geq$ GppNHp > GTP > GDP = GMP. ATP, UTP, and CTP were without effect at concentrations up to 1mM. The apparent EC_{50} for GppNHp was 10μM. It is likely that the apparent lack of potency of GTP is due to its hydrolysis to GDP during the incubation. In platelet, NG108-15 and NCB-20 cell membranes direct binding curves for [^{3}H]-iloprost and curves for the displacement of low concentrations of [^{3}H]-iloprost (2 - 3nM) by unlabelled iloprost were shifted to the right when performed in the presence of 10^{-4}M GppNHp. These shifts were consistent with the conversion of a minor population of high affinity ($K_H \sim$ 1nM) guanine nucleotide-sensitive sites to lower affinity ($K_L \sim$ 10nM) guanine nucleotide-insensitive sites.

Thus [^{3}H]-iloprost binding to membranes from platelets, NG108-15 and NCB-20 cells displays guanine nucleotide sensitivity, consistent with the binding being to a G-protein coupled receptor. This supports the use of [^{3}H]-iloprost as a ligand to identify PGI_2 receptors.

This work was supported by a Programme Grant from the Wellcome Trust.

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146P POLYCATIONS INDUCE RAT HIND-PAW OEDEMA

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Inflammatory compounds of special interest are those endogenous mediators which may be released or activated during injury. Naturally occurring polycations are released from activated leucocytes and platelets (Peterson et al., 1985). We report here the inflammatory response induced by poly-l-arginine (Polarg, 43KD) or poly-l-lysisne (85 KD) in the rat hind-paw oedema and its modulation by various inhibitors.

Oedema was induced in male Wistar rats (160-180g) by a single subplantar injection of 0.1ml of Polarg (0.01-1 mg) or poly-l-lysine (1 mg) in sterile saline in the left hind-paw under light ether anaesthesia. The paw volume was measured 0.5, 1, 2, 4, 6 and 18 hours after the subplantar injection using a hydroplethysmometer (Model 7150, Ugo Basile, Italy). The results were expressed by comparison of the area under the time-course curve (up to 6h) and expressed as % of inhibition. Each value represents the mean $\pm$ s.e.m. of 5-7 rats/group.

Polarg induced a dose-dependent oedema which had a rapid onset (at 30min, 0.91$\pm$0.09 ml, n>25) and long duration (at 12h, 0.53$\pm$0.1 ml, n>25). Methysergide (2mg/kg, i.p., 30min before) caused 24.8$\pm$4.1% inhibition (n=5, p<0.05). LNMMA (1mg/paw and 2mg/paw, zero time) caused 27.6$\pm$5.2% and 49.4$\pm$5.3% inhibition respectively (n=5, p<0.05). Heparin (50 U/paw, zero time) caused 63$\pm$11% inhibition (n=10, p<0.001). Indomethacin (10mg/kg i.p., 60 min before), dexamethasone (1mg/kg, s.c., 60min before), BW755c (50mg/kg, p.o., 60min before), mepyramine (2mg/kg, i.p., 30min before) or WEB 2086 (20mg/kg p.o., 30min before) caused no significant inhibition of the Polarg-induced oedema. At the doses used above, dexamethasone, indomethacin and BW755c inhibited carrageenan induced paw oedema (1mg/paw, n=5). Poly-l-lysine (1mg/paw) was less potent than Polarg in inducing oedema (at 30min, 0.61$\pm$0.02 ml, n>25; at 12h, 0.27$\pm$0.08 ml, n>25).

The heparin-induced inhibition of Polarg oedema is probably due to neutralisation of the Polarg positive groups as previously reported for the rabbit skin (Needham et al., 1988). Whether the LNMMA-induced inhibition is due to a decrease in blood flow or a decrease in vascular permeability has yet to be established.

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147P INTERLEUKIN-1 ACTIVATES A G-PROTEIN IN EL4.NOB1 MURINE THYOMA CELLS: A POSSIBLE SIGNAL TRANSDUCTION MECHANISM

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Interleukin-1 (IL-1) is a macrophage-derived cytokine with a range of activities which suggest a key role in inflammation. The molecular mechanism by which IL-1 activates cells is obscure. Here we describe a series of experiments which show that IL-1 activates a G protein in membranes from an IL-1 receptor (IL-1R)-rich cell line commonly used to bioassay IL-1: EL4.NOB1 murine thymoma cells.

Incubating EL4.NOB1 membranes with human recombinant(hr)IL-1α or IL-1β caused a time- and concentration-dependent increase in binding of the non-hydrolysable GTP analogue GTPγ[^{35}S]. The effect was detectable from 1 min where IL-1α (10ng/ml) increased nucleotide binding from 7120±621cpm in control membranes to 12012±320 in IL-1α-stimulated membranes. IL-1β proved 10 times less potent, which agrees with the receptor affinity for hrIL-1α and hrIL-1β in EL4.NOB1 cells (Bird et al, 1987). Specific neutralising antisera to IL-1α and IL-1β abolished the response. Experiments in which the concentration of nucleotide was varied showed that the effect of IL-1 was due to an increase in the affinity of the binding sites and not their number. IL-1α was also found to increase the hydrolysis of [γ-^{32}P]GTP in the membranes with a time and dose dependency similar to increased nucleotide binding. At 10ng/ml IL-1α for example the rate of GTP hydrolysis was increased from 62.2±4 pmol/mg/min in control membranes to 83.2±3 pmol/mg /min in IL-1-stimulated membranes. Parallel experiments in which [^{125}I]IL-1α binding was measured revealed that 6% fractional occupancy of IL-1R caused maximal nucleotide binding, indicating a considerable degree of signal amplification.

Pre-treating membranes for 30mins with activated pertussis toxin (10ug/ml) partially blocked the IL-1α-induced increase in both nucleotide binding and hydrolysis, implying a pertussis toxin sensitive component in the response. Cholera toxin (10ug/ml) was without effect. Using [^{32}P]NAD labelling of membranes both toxins at these concentrations were shown to ADP-ribosylate proteins in EL4.NOB1 membranes.

These findings suggest that IL-1 in EL4.NOB1 murine thymoma cells activates a G protein. The significance of this finding for IL-1 receptor signalling will be discussed.

Acknowledgements: hrIL-1α and hrIL-1β were the kind gifts of Dainippon Pharmaceutical Co.,Osaka, Japan and Dr C. Dinarello, Tufts' University, Boston, U.S.A. respectively. The authors would like to thank the Medical Research Council and the Arthritis and Rheumatism Council for financial support.

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148P INTERACTION BETWEEN INTERLEUKIN 1 AND THE FIBRINOLYTIC SYSTEM IN THE DEGRADATION OF COLLAGEN BY RABBIT CHONDROCYTES

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Cartilage cells or articular chondrocytes are now recognised as playing an active role in the degradation of their own extracellular matrix in chronic arthritis. The degradation is believed to be due to the action of metalloproteinases which are elaborated from chondrocytes in response to cytokines, such as interleukin 1 (IL-1). To investigate the mechanisms involved in collagen degradation, we have utilised a model where rabbit articular chondrocytes are grown on a ^{14}C-labelled collagen matrix and consequently, the degradation can be quantified by measuring the release of radiolabelled collagen fragments into the surrounding medium, as described by Gavrilovic et al (1985).

Type 1 collagen, isolated from rat skin, was acetylated with ^{14}C-acetic anhydride and 200µg added to each well of 24-well plates in a volume of 0.1ml. The collagen matrix was then allowed to polymerise and then dried. Chondrocytes were isolated by enzymic digestion of articular cartilage from the shoulders and femurs of New Zealand White rabbits. They were plated at high density (10^7 cells/75 cm^2 flask) and grown to confluence. Experiments were then performed in medium containing 1% acid-treated foetal calf serum. The digestion was quantitated by measuring the liberated radioactive fragments in the medium by liquid scintillation counting. This was expressed as the % degradation (mean ± s.e. mean), determined by measuring the counts in the residual matrix after digestion with bacterial collagenase (0.5mg/ml).

Over a 48 hour period there was minimal basal degradation (4.5 ± 0.3%, n=6) and the presence of human recombinant IL-1β alone, at concentrations up to 10ng/ml (570 pM), did not increase the degradation. When IL-1β (570 pM) was added in combination with plasminogen (100µg/ml) or plasmin (100µg/ml) there was 93.0 ± 1.6% (n=6) and 91.3 ± 0.7% (n=6) degradation of the collagen matrix respectively. Plasminogen or plasmin alone had a small but significant ($P<0.01$) effect on the degradation of the collagen by the chondrocytes (18.1 ± 1.1% for plasminogen and 8.3 ± 0.4% for plasmin). In the presence of plasminogen, IL-1β caused a dose-dependent degradation of the collagen matrix which was maximal at 20pM. The lowest concentration of IL-1β used (4pM) in combination with plasminogen, caused 31.4 ± 2.8% degradation of the collagen. Furthermore, when fibrin (1µg/ml) was added to this concentration of IL-1β and plasminogen, there was 96.1 ± 0.1% degradation of the matrix. Fibrin in combination with plasminogen alone did not enhance the degradation of the collagen matrix by these chondrocytes.

These results indicate that there is a co-operation between IL-1 and the fibrinolytic system in the degradation of collagen by rabbit articular chondrocytes.

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149P EFFECTS OF DOSING REGIMEN OF TISSUE PLASMINOGEN ACTIVATOR ON THE EXTENT AND DYNAMICS OF FIBRINOLYSIS

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The importance of coronary thrombolysis is becoming widely accepted as necessary to salvage ischaemic myocardium and reduce mortality, (ISIS-2, 1988). At present, for clinical efficacy to be achieved with recombinant tissue plasminogen activator (r-tPA), dosing regimens involving prolonged intravenous (i.v.) infusions have been used.

The aim of the present study was to evaluate the thrombolytic efficacy of equivalent doses of r-tPA given as an i.v. bolus compared to an i.v. infusion in the anaesthetised rabbit model of jugular vein thrombosis (Collen et al, 1983). Using ^{125}I-Fibrinogen to label a 30 min aged thrombus, rate (counts s^{-2}), onset (min), duration of lysis (min) and extent of fibrinolysis(%) were determined directly using an external gamma probe placed over the jugular vein. Double-chain rt-PA (Wellcome Biotech Ltd., Duteplase) was administered either as a 30ml i.v. infusion over 1h (0.06 - 1.2 M.I.U/Kg), or as a 10ml i.v. bolus over 10 min (0.12 - 0.6 M.I.U/Kg), (n=48). Results are shown in the table below. All data are mean ± s.e.mean. *P<0.05 unpaired t-test, bolus vs infusion.

DOSE (M.I.U./Kg)	% LYSIS	RATE (CS^{-2})	ONSET (min)	DURATION (min)
0.06 (INFUSION)	17.3 ± 9.2	0.04 ± 0.03	-	-
0.12 "	32.0 ± 7.2	0.10 ± 0.03	10.7 ± 3.7	70.7 ± 2.4
0.30 "	38.0 ± 6.0	0.10 ± 0.02	6.3 ± 1.8	87.7 ± 2.9
0.60 "	47.7 ± 7.2	0.16 ± 0.02	8.7 ± 1.0	84.0 ± 3.7
1.20 "	77.8 ± 6.1	0.36 ± 0.06	4.0 ± 1.0	101.7 ± 2.3
0.12 (BOLUS)	16.7 ± 4.0	0.12 ± 0.03	5.3 ± 1.3	60.0 ± 8.1
0.30 "	33.6 ± 4.6	0.21 ± 0.04*	7.0 ± 1.7	62.7 ± 6.6*
0.60 "	46.8 ± 7.6	0.31 ± 0.04*	5.0 ± 1.2	64.7 ± 6.2*

No difference in the final % lysis was observed between infusion and bolus at equivalent doses. Time to onset of lysis was unchanged between sub-groups. Bolus administration significantly increased the maximal rate of lysis with two of the doses studied. The duration of lysis was shorter compared to infusion which accounts for the unchanged final % lysis. However, the duration of lysis following a bolus of Duteplase far exceeds that predicted from the short plasma $t_{1/2}$ (5min>). This data indicates a possible therapeutic potential for bolus administration of Duteplase in thrombotic disorders.

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150P 5-HT AND BRADYKININ PLEURISY IN THE RAT IS INSENSITIVE TO DEXAMETHASONE PRETREATMENT

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Following intrapleural injection of PAF in the rat, the number of leukocytes present in pleural lavage fluid after 30 min is reduced, and a small exudate develops (Peers, 1988). These responses, unlike the oedema which follows PAF, 5-HT or bradykinin (BK) injection into the paw, are insensitive to pretreatment with dexamethasone (Cirino et al, 1989). I now report similar findings with intrapleural 5-HT and BK.

Male Wistar rats (200-250 g) were ether anaesthetised, and pleural injections (0.1 ml) made as previously described (Peers, 1988). 30 min later the rats were killed, the pleural cavity washed and the lavage collected. The volume of exudate was estimated gravimetrically and the number of leukocytes present counted microscopically.

30 min after 5-HT (10 ug) or BK (50 ug) injection, the number of leukocytes present in the recovered exudate was reduced (P>0.001, Table 1). Coinjection of methysergide inhibited reponses to 5-HT but not BK. Indomethacin (p.o. - 1hr) and dexamethasone (s.c. - 3h) had no effect.

Table 1. Effects of drugs on 5-HT and BK pleurisy

	saline		5-HT 10 ug		BK 50 ug	
Pretreatment	exudate (g)	cells ($x10^6$)	exudate (g)	cells ($x10^6$)	exudate (g)	cells ($x10^6$)
saline	0.05±0.02	4.0±0.5	0.45±0.04	1.5±0.1	0.33±0.03	1.2±0.2
methysergide 10ug	0.03±0.01	4.4±0.4	0.13±0.05*	6.0±0.1*	0.31±0.02	1.0±0.4
indomethacin 5mg/kg	0.02±0.01	4.6±0.7	0.30± 0.04	1.8±0.2	0.35±0.08	1.8±0.3
dexamethasone 1mg/kg	0.04±0.02	4.8±0.7	0.43±0.07	2.2±0.4	0.33±0.03	1.3±0.3

*p<0.05, Student's t test. n=5-20 rats per group.

These observations extend the finding that 'cell disappearance' is an acute response to intrapleural injection of PAF (Peers, 1988; Tarayre et al, 1985). The mechanism of 'cell disappearance and the reason why responses in the pleural cavity are unaffected by a steroid teatment which significantly reduces paw oedema are as yet unknown.

SHP is supported by the Wellcome Trust.
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151P THE EFFECTS OF ENDOTHELIN-1 ON GUINEA-PIG ISOLATED WORKING HEARTS

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Endothelin-1 (ET-1) is a potent and long lasting vasoconstrictor in coronary arteries and has a direct effect on vascular smooth muscle (Yanagisawa *et al.* 1988). Receptors for ET-1 have been found in the media of porcine and human coronary arteries and high affinity binding sites reported on cardiac membranes (Power *et al.* 1989; Gu *et al.* 1989). In addition ET-1 also has a direct effect on cardiac myocytes causing positive inotropic responses (Ru Hu *et al.* 1988). The effects of ET-1 and another coronary vasoconstrictor LTC_4 were compared.

Hearts from male Dunkin-Hartley guinea-pigs (350-450g) were perfused using the modified Langendorff apparatus with Krebs buffer gassed with 95% O_2, 5%CO_2. One of two methods of perfusion were used: i) a constant flow of 8ml min^{-1} was maintained and changes in perfusion pressure (PP) measured ii) a pressure of 40cmH_2O was maintained and the variable coronary flow (CF) was measured by means of a drop counter. The coronary vascular resistance (CVR) was determined by: peak PP/peak CF - basal PP/basal CF. In both preparations cardiac developed tension (CDT) was measured by a force displacement transducer attached by a thread to the apex of the left ventricle.

In both the variable and constant flow systems ET-1 and LTC_4 (3-100pmol) induced similar dose-dependent increases in PP and CVR respectively. The ET-1-induced reductions in coronary flow were longer lasting than that of LTC_4. The effects of 30pmol ET-1 recovered in 10 mins, while that of 100pmol lasted longer than 20 mins. A decrease in CDT was observed for both ET-1 and LTC_4; this appeared to be associated with a decrease in resting tone.

ET-1 (30pmol) induced the release of both TxA_2 (measured as TxB_2) and PGI_2 (measured as 6-oxo $PGF_{1\alpha}$). Raised levels of TxA_2 (1.0 ± 0.06ng ml^{-1}) occurred within 1-3 mins after the bolus injection of ET-1. However, larger quantities (6.0 ± 1.4 ng ml^{-1}) of PGI_2 were released within 2 mins and remained raised up to 3 mins later. In the presence of indomethacin (2.8μM) the increase in PP induced by ET-1 (30pmol) was significantly increased at 2 and 5 mins (27.0 ± 4.0 and 23.0 ± 4.5 mmHg respectively) above control values (15.0 ± 2.6 and 11.4 ± 2.6 mmHg respectively). The duration of response was also extended.

ET-1 was equiactive with LTC_4 but caused a longer-lasting response. The coronary constriction to ET-1 is accompanied by a reduction in both CDT and resting tension, which may be partly due to a direct effect of ET-1. The above results suggest that a cyclo-oxygenase product, probably prostacyclin, modulates ET-1-induced coronary constriction.

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152P HYPOTENSION PRODUCED BY ENDOTHELINS UNDERGOES CROSSED TACHYPHYLAXIS IN BOTH ANAESTHETISED AND CONSCIOUS RATS

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Endothelins are a family of isopeptides (ET-1, ET-2, ET-3, ET-β, sarafotoxin 6b) of 21 amino acids with two disulfide bridges. In rats, these compounds produce short lasting hypotensive responses which for ET-1 and ET-2 are followed by pressor effects. The aim of this investigation was to assess whether successive administrations of the same or different ETs evoked reproducible decreases in blood pressure in rats.

Male normotensive Sprague Dawley rats (250 to 270 g) were anaesthetised with ether, placed under artificial ventilation, pithed and prepared for carotid artery blood pressure (MAP) measurements. A continuous infusion of vasopressin (7.5 mIU/kg/min) was started after a 30 min equilibration period. When a steady-state pressor response was attained, four successive i.v. bolus injections (at 10 min intervals) of ET peptides were given to the same animal, each isoform being studied in separate groups. The doses studied were 0.25 nmol/kg for ET-1 and 0.5 nmol/kg for ET-2 and ET-3. Acetylcholine (ACh) (0.5 μg/kg) was given before each ET administration. In groups of pentobarbitone anaesthetised rats a 10 min i.v. infusion of either saline, ET-1, ET-2 or ET-3 (0.1 nmol/kg/min) was initiated, then, ET-1 (0.25 nmol/kg, i.v. bolus) was injected 8, 30 or 120 min later. The same protocol was applied to study ET-1 (0.5 nmol/kg/min i.v. over 10 min; bolus: 0.5 nmol/kg) in conscious spontaneously hypertensive rat (SHR).

In vasopressin-supported pithed rats, the first injection of the chosen dose of ET-1, ET-2 and ET-3 produced transient hypotensive effects of similar magnitude (-44 ± 2, -44 ± 1, -45 ± 3 mmHg, respectively ; initial MAP 119 ± 3, n = 15). The responses to the fourth injection of these peptides were +18 ± 5, -10 ± 4, 12 ± 1 mmHg (initial MAP 156 ± 3), respectively. In contrast, the hypotensive effects of ACh were not changed after the fourth injection of ETs. Eight min after starting a 10 min i.v. infusion of ET-1, ET-2 or ET-3, the control hypotensive response (-54 ± 2 mmHg) to ET-1 (0.25 nmol/kg i.v. bolus) was reduced by 100, 52 and 72%, respectively. In saline or ET-1 pretreated conscious SHRs, the responses to an i.v. bolus of ET-1, were -51 ± 5 and -10 ± 6 mmHg (n=5), respectively. In these experiments, 1.5 h later the hypotension to ET-1 returned to control values.

These results indicate that in conscious or anaesthetised rats, tachyphylaxis develops to repeated injections of the same or different endothelins. The existence of a crossed tachyphylaxis between different ET isoforms may imply that ETs hypotension is mediated by a single receptor becoming refractory to stimulation upon repeated exposure to ETs. Alternatively or additionally, stimulation of ET receptors might liberate an easily depletable endogenous hypotensive substance.

153P INHIBITION OF ENDOTHELIN-1-INDUCED RAT GASTRIC DAMAGE BY CALCITONIN GENE-RELATED PEPTIDE

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Sensory neuropeptides, including calcitonin gene-related peptide (CGRP), have been located within capsaicin-sensitive primary afferent neurones in the gastric mucosa (Green & Dockray, 1988). A protective role for such neuropeptides has been suggested since capsaicin-pretreatment augments mucosal damage induced by a number of ulcerogens (Holzer 1988). We have now investigated the effects of endogenous neuropeptides and locally administered CGRP on gastric damage induced by the vascular peptide endothelin-1 (ET-1; Yanagisawa et al, 1988) following local infusion (Whittle & Esplugues, 1988).

Male rats (250g body weight) were treated over 3 days with capsaicin under halothane anaesthesia following aminophylline and terbutaline administration, 2 weeks before study (Esplugues et al, 1989). Rats were anaesthetised with pentobarbitone, the stomach exposed and the left gastric artery cannulated with a 23g teflon cannula, and acid-saline (2ml, 100mM HCl) was instilled into the gastric lumen. Systemic arterial blood pressure (BP) was measured from a carotid artery. Close-arterial infusion of ET-1 (4-20 pmol kg^{-1} min^{-1}) for 10 min, with the stomach being removed 20 min later, induced dose-dependent mucosal injury characterised macroscopically as vasocongestion and haemorrhage. The ET-1-induced mucosal injury was substantially enhanced in capsaicin-pretreated rats; thus damage following ET-1 (20 pmol kg^{-1} min^{-1} i.a.) was increased from 32 ± 9 % of total mucosal area (n=4) to 79 ± 5 % (mean ± s.e. mean, n=4; $P<0.01$) as determined by computerized planimetry. Concurrent close-arterial infusion of rat α- CGRP (10-50 pmol kg^{-1} min^{-1}) induced a dose-dependent reduction of the mucosal injury induced by ET-1. Thus, CGRP (50 pmol kg^{-1} min^{-1} i.a.) induced a 64 ± 8 % inhibition (n = 4; $P<0.05$) of the damage induced by ET-1 (20 pmol kg^{-1} min^{-1} i.a.). Whereas this local intra-arterial dose of CGRP did not alter BP (-4 ± 8 mmHg n=3), i.v. infusion of this dose lowered BP by 37 ± 2 mmHg (n=3, $P<0.05$), thus suggesting extensive metabolism of this neuropeptide within the gastric circulation or liver.

These findings indicate that depletion of sensory neuropeptides by capsaicin augments damage induced by the ET-1, while local administration of CGRP inhibits mucosal injury. The local release of endogenous CGRP may therefore be involved in the protective response of the gastric mucosa to challenge. Thus, an imbalance between these opposing vasoactive peptides in the gastric microcirculation could play a role in the pathogenesis of mucosal injury.

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154P EFFECTS OF INDOMETHACIN ON REGIONAL HAEMODYNAMIC RESPONSES TO ENDOTHELINS AND SARAFOTOXIN-S6B IN CONSCIOUS RATS

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The *in vitro* vascular effects of endothelin-1 (Et-1) are modified by indomethacin (De Nucci *et al.*, 1988). We have investigated the effects of indomethacin on the responses to Et-1, Et-2 and Et-3, and the structural homologue, sarafotoxin-S6b (S6b) *in vivo* in conscious rats. Male, Long Evans rats were anaesthetised (sodium methohexitone, 60 mg kg^{-1} i.p., supplemented as required) and had pulsed Doppler probes (Haywood *et al.*, 1981) implanted around left renal and superior mesenteric arteries, and the distal abdominal aorta (to measure hindquarters flow). At least 7 days later animals were briefly re-anaesthetised (sodium methohexitone 40 mg kg^{-1} i.p.) and had intravenous and intraarterial catheters implanted. Experiments began the following day. Animals received 2 doses (4 and 40 pmols $i.v.^{-1}$) of Et-1, -2, and -3 and S6b. On the second day indomethacin administration (5 mg kg^{-1}; 5 mg kg^{-1} h^{-1}) was begun 30 min before peptides were given. The table summarizes the initial changes following the high dose of the peptides. The later pressor and regional vasoconstrictor effects of the peptides were unaffected by indomethacin.

Table 1 Cardiovascular changes 20-30 s after peptide administration in the absence (-I) or presence (+I) of indomethacin in conscious, Long Evans rats (n=8); values are mean (s.e.m.)

			Et-1	Et-2	Et-3	S6b
Mean blood pressure (mmHg):		-I	-22(2)*	-9(4)	-13(3)*	-28(2)*
		+I	-18(3)*	-7(3)	-15(4)*	-13(3)*+
Conductance (%):	Renal	-I	4(4)	3(8)	24(7)*	20(7)*
		+I	15(4)*+	3(4)	22(8)*	16(4)*
	Mesenteric	-I	3(6)	-10(7)	4(9)	27(12)
		+I	-19(4)*+	-24(4)*+	-19(6)*+	-15(7)+
	Hindquarters	-I	98(11)*	64(17)*	69(6)*	120(11)*
		+I	96(14)*	48(9)*	61(10)*	64(13)*+

* $P<0.05$ versus baseline (Friedman's test); + $P<0.05$ -I versus +I (Wilcoxon's test).

The early renal effects of Et-1, the hindquarters vasodilator effects of S6b, and the initial mesenteric actions of all four peptides were changed by indomethacin.

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155P EFFECTS OF MUSCARINIC ANTAGONISTS ON ARECOLINE-INDUCED HIPPOCAMPAL THETA RHYTHM AND HYPERTENSION IN THE ANAESTHETISED RAT

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In the anaesthetised rat, muscarinic agonists produce an increase in the frequency of hippocampal theta rhythm (Barnes et al, 1989) and, in the presence of peripheral muscarinic blockade, an increase in blood pressure (Pazos et al, 1986). The muscarinic receptor subtypes within the brain mediating these effects are not known and therefore we have compared the potencies of the M_1 selective antagonist, pirenzepine (PZ) and the non-selective antagonist, scopolamine (SCOP) to antagonise these responses.
The studies used male Lister-Hooded rats (300-350g), anaesthetised with isoflurane. In the EEG experiments, a bipolar electrode was positioned in the CA1 region of the hippocampus and connected to a Grass 7P511 amplifier. The EEG was sampled and digitised by an ADC, for periods of 60s (at a rate of 128 samples/s, epochs of 2s and an inter-epoch gap of 3s), and stored on computer for subsequent analysis by fast Fourier transform. Experiments were performed after the administration of N-methylscopolamine (NMS, 0.5mg/kg iv) and chlorisondamine (0.1mg/kg iv). In the cardiovascular experiments, blood pressure was measured via the carotid artery and experiments were carried out following the administration of NMS (0.5mg/kg iv). For all experiments, arecoline (0.3mg/kg) was administered iv at 25mins intervals, prior to and 10min following the intraventricular administration of 2 cumulative doses of antagonist. Endogenous theta wave frequency ranged from 2.8±0.6 to 4.3±0.2Hz, which was increased by arecoline to 5.8±0.3Hz (before PZ) and 6.5±0.3Hz (before SCOP) ($p<0.001$ vs no-drug control, ANOVA).

Table 1: Effect of PZ and SCOP (nmol) on arecoline-induced theta rhythm (Hz) and hypertension (BP)

PZ dose	Hz	PZ dose	BP (mmHg)	SCOP dose	Hz	SCOP dose	BP (mmHg)
0	5.8±0.3	0	39.6±3.4	0	6.5±0.3	0	50.2±5.4
400	5.3±0.1	104	31.9±1.0	132	5.6±0.7	11	44.0±13.5
600	2.3±0.6*	206	11.0±6.7*	264	2.2±0.3*	33	12.7±4.3*

Both PZ and SCOP dose-dependently reduced the increase in theta wave frequency and the hypertension induced by arecoline (Table 1; mean values±SEM, *$p<0.01$ vs arecoline control). SCOP was less than 10 times more potent than PZ to antagonise both the responses, a difference which is similar to their relative affinities for the M1 receptor subtype (Watson et al, 1986). Slightly higher doses of PZ were required to antagonise the theta wave response than the hypertension, but this difference was also seen with SCOP and therefore cannot be attributed to receptor selectivity. The conclusion is that the enhanced theta wave frequency and hypertensive responses induced by arecoline are likely to be mediated by the M1 muscarinic receptor subtype.

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156P CORTICAL AND HIPPOCAMPAL MUSCARINIC RECEPTORS ARE DIFFERENTIALLY AFFECTED IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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Subcortico-cortical cholinergic alterations are common features of Alzheimer's disease (AD) and Parkinson's disease (PD). Neuronal loss in the substantia innominata is observed, and reductions in cortical cholinacetyltransferase activity correlate with the degree of dementia in both diseases (Perry *et al.*, 1985). We have examined whether altered cholinergic function is associated with similar changes in muscarinic receptor binding in the temporal cortex and hippocampus of patients with AD or PD.

Brains were obtained from 9 patients with AD and from 9 matched controls without neurological or psychiatric diseases as well as from 9 patients with PD, 4 of whom had suffered from dementia, and 9 controls. The Parkinsonian patients had all received L-dopa therapy up to the time of death, two patients had also received anticholinergic drugs. Using washed membranes saturation analysis was performed for [^{3}H]-quinuclidinylbenzilate binding (QNB; total number of muscarinic receptors; concentrations 9.4 pM - 0.3 nM) and [^{3}H]-pirenzepine binding (PZ; M-1 receptors; concentrations 0.5 - 64 nM). Non-specific binding was defined by 1µM atropine.

Table 1. B_{max} values for muscarinic receptor binding (fmol/mg protein; means ± s.e.mean)

	TEMPORAL CORTEX		HIPPOCAMPUS			TEMPORAL CORTEX		HIPPOCAMPUS	
	QNB	PZ	QNB	PZ		QNB	PZ	QNB	PZ
CONTROLS	814 ± 30	580 ± 23	361 ± 15	241 ± 17	CONTROLS	844 ± 24	659 ± 19	336 ± 18	215 ± 17
ALZHEIMER	802 ± 33	590 ± 16	298 ± 14*	224 ± 16	PARKINSON	952 ± 13*	755 ± 10*	358 ± 23	220 ± 13

*) Significantly different from controls ($P < 0.05$, Mann-Whitney U test)

In AD no changes in densities of muscarinic receptors measured by specific binding of QNB and PZ were observed in the temporal cortex, QNB binding was reduced and PZ binding was unchanged in the hippocampus. By contrast, both demented and non-demented Parkinsonian patients had increased concentrations of the total number of muscarinic receptors and M-1 receptors in the cortex and no receptor alterations in the hippocampus. Alterations of K_D values were not seen.The results suggest that muscarinic receptors are differently affected in AD and PD. This may reflect fundamental differences in the pathophysiological process underlying dementia in these diseases.

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157P COPING WITH RAPID CHANGES IN THE RATE OF SUBMISSIONS TO THE BRITISH JOURNAL OF PHARMACOLOGY

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In January, 1987, after many years of debate, the ***British Journal of Pharmacology*** abandoned its rule requiring Authors' surnames to be published in alphabetical order. It was anticipated that this would result in about 50-60 more manuscripts being submitted annually, an increase of approximately 10%. Between then and June, 1988, however, there was a 50% increase in submissions with a similar rise in the numbers of acceptances and publications (Figure 1). The volume of associated correspondence rose by over 70%. This marked increase, which has been sustained, was linked to a major promotion of the Journal by the publisher and necessitated changes in every facet of Journal activity. It proved possible, however, to deal effectively with the large and unexpected demand by the implementation of only a few, relatively minor modifications to existing arrangements for dealing with papers at all stages, from submission to final publication, and only a modest expansion of existing facilities and staffing. The steps taken to cope with this substantial growth in submissions and publications will be illustrated.

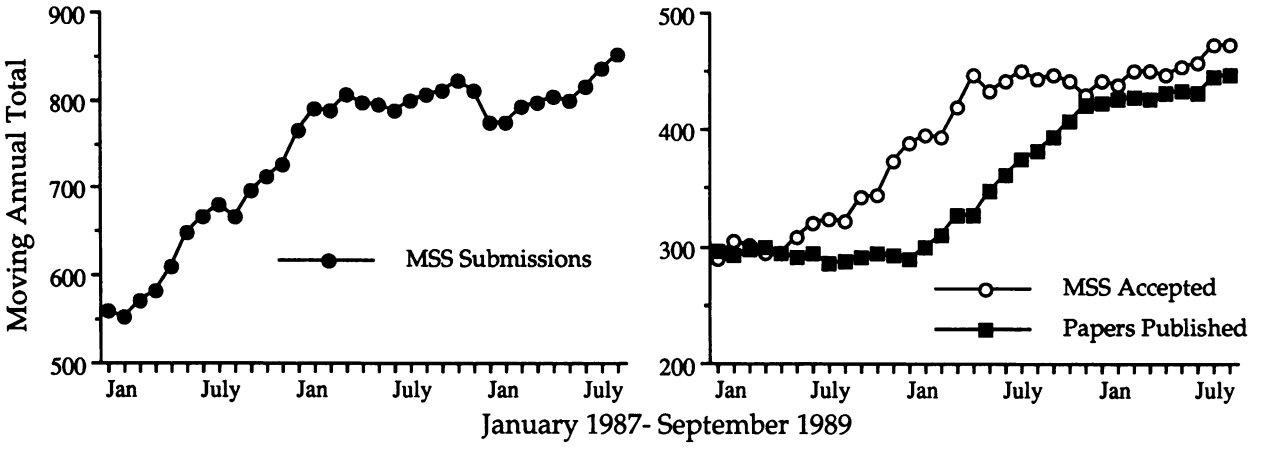

Figure 1. Numbers of submissions, acceptances and publications in consecutive twelve-month periods.

158P A TEST TO DETECT POSSIBLE ERRORS IN AGONIST AFFINITY ESTIMATION

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Recent theoretical studies have questioned the pharmacological estimation of agonist affinity. They show that the receptor inactivation method (Furchgott, 1966) can overestimate affinity when receptor isomerisation (Colquhoun, 1987) or ternary complex (Mackay, 1988; Leff & Harper, 1989) mechanisms operate, while the comparative method (Barlow *et al.*, 1967), for partial agonist analysis is more reliable. This led us (Leff & Harper, 1989) to propose a test to detect conditions unfavourable to accurate estimation of agonist affinity. The test involves analysing a partial agonist by both the receptor inactivation and comparative method. Under unfavourable conditions, the affinities estimated by the two methods should be different. This communication describes the practical application of this test.

Firstly, the action of a partial agonist operating by each of the isomerisation and ternary complex mechanisms was computer-simulated. The theoretical data so generated were then analysed, using operational model-fitting (Black *et al.*, 1985), by the inactivation and comparative methods to quantify the magnitude of error in affinity estimation that could occur. This analysis showed that for a partial agonist with 85% of the activity of a full agonist, the inactivation method could produce an affinity (pK_A) estimate 0.7 $\log_{10}$ units higher than that produced by the comparative method. It also showed that the former method could overestimate E_m, the maximal effect parameter, by some 3- to 5-fold under those conditions. Secondly, experiments were conducted using the guinea-pig isolated left atrial preparation to exemplify the test in a muscarinic receptor system. The test agonist was pilocarpine, which produced on average 83% of the activity of the full agonist, carbachol. In a single preparation, cumulative concentration-effect curves were constructed first to carbachol, then to pilocarpine, then again to pilocarpine after phenoxybenzamine treatment ($3x10^{-7}$-$1x10^{-6}$M, 30 min). Thus pilocarpine was analysed in comparison with carbachol and by receptor inactivation in the same tissue. The resulting parameter estimates, obtained by operational model-fitting, and statistics are tabulated below, showing no statistical differences in the estimates provided by the two methods.

Table 1	Comparative Method		Inactivation Method		Mean Difference (s.e.; paired t-test)	
	E_m	pK_A	E_m	pK_A	E_m	pK_A
Mean (n=8)	96.73	5.03	97.32	4.95	0.59 (3.3; P>0.5)	0.08 (0.1; P>0.5)

There was no evidence that conditions unfavourable to muscarinic agonist quantification apply in the guinea-pig atria. These results cannot be regarded as general, but they question the theoretical objections raised about pharmacological methods for agonist quantification.

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159P INFLUENCE OF PHORBOL ESTER AND OTHER PROTEIN KINASE C-RELATED DRUGS ON AGONIST-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN CULTURES OF ADRENAL CHROMAFFIN CELLS

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We have previously shown that bradykinin and histamine stimulate both catecholamine release and inositol phosphate production in bovine adrenal chromaffin cells (Plevin and Boarder, 1988., Owen et al, 1989) and are interested in in the role of protein kinase C in this system. In a number of reports it has been suggested that agonist enhancement of phospholipase C activity, producing inositol phosphates and diacylglycerol, may activate a short inhibitory feedback loop by diacylglycerol activation of protein kinase C. In some cases it has been shown that protein kinase C activation by phorbol esters can inhibit agonist stimulation of phospholipase C. Here we report some experiments intended to evaluate the existence of this loop in chromaffin cells.

Cells were purified by centrifugation and differential plating following collagenase digestion of bovine adrenal medulla (Owen et al, 1989). After 3-6 days culture on 'Primaria' plastic cells were loaded with [^{3}H]inositol at 1μCi/ml for about 48h. Stimulation of cells was in the presence of lithium (10mM), and total inositol phosphates (IP) were separated batchwise on Dowex-1. Incubation with agonists was for 30 minutes, preceeded where appropriate by a 10 minute preincubation. Phorbol ester and related drugs were present both during the preincubation and incubation periods. The presence of 1μM tetradecanoylphorbol acetate (TPA) during the 10 min preincubation and 30 min incubation had no consistent effect on basal IP but substantially reduced bradykinin and histamine stimulated IP: control, 4625 319; bradykinin 100nM, 9854±664; bradykinin 100nM plus TPA 1μM, 4934±401; histamine 10μM, 26546±734; histamine 10μM plus TPA 1μM, 5494±637; (figures are d.p.m. of [^{3}H]-IP, n=4±S.E.M.). This TPA effect could be seen at 10nM and was mimicked by mezerein (1μM) but not by 4-methoxy TPA. The results suggested that protein kinase C mediated feedback may be effective in limiting agonist stimulation of phospholipase C in these cells. If this is so then downregulation of protein kinase C by 24h TPA pretreatment might enhance the agonist stimulation of IP accumulation. However 24h pretreatment with TPA (1μM) also reduced both bradykinin and histamine stimulated IP. If agonist induced diacylglycerol accumulation has the potential to attenuate agonist stimulated IP response, then inhibitors of diacylglycerol breakdown might be expected to enhance this attenuation, using diacylglycerol kinase and lipase inhibitors (50μM R 59022 from Janssen and 10μM RG 80267 from Revlon respectively) either separately or together had no effect on bradykinin stimulated IP accumulation; with histamine the kinase inhibitor attenuated the IP response by virtue of its H_1 antagonist activity.

These results show that the mechanisms exist in this cell preparation for an inhibitory feedback loop, but they fail to provide any evidence that such a feedback loop is activated by agonist stimulated inositol phospholipid breakdown.

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160P MEDIATORS OF PRIMARY IRRITANT DERMATITIS IN MINIATURE SWINE

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Topical application of irritant substances to human skin leads to an increase in cutaneous blood flow (CBF) (Dowd et al., 1987). Interleukin (IL)-1, which is present in normal human skin, can release vasoactive mediators and cause erythema on intradermal injection (Dowd et al., 1988). The possible involvement of IL-1 in irritant dermatitis has now been investigated using miniature swine, since the structure and functional responsiveness of porcine skin is reported to be similar to that of man (Hensby et al., 1984).

The presence of biologically active amounts of IL-1 in porcine skin was first investigated by use of an EL-4-NOB-1 assay as previously described (Fincham et al., 1988). IL-1-like activity was found to be present in homogenates of both surface stratum corneum and epidermal slices (0.1 mm depth), the amounts detected being 459 ± 144 and 372 ± 86 pg IL-1α equivalents/mg wet weight of tissue respectively (n = 6).

Changes in CBF following topical application of 50 μl of 10% hexyl nicotinate in propylene glycol/isopropanol or 5 mg of benzalkonium chloride in dimethylsulphoxide to the flanks of 4 miniature swine were then measured with a laser Doppler flowmeter (Pf2, Perimed Sweden). Responses to vehicle alone were also recorded. Although both irritants provoked a visible erythematous response and an increase in CBF, the time courses differed (Table). Benzalkonium chloride also caused oedema which was apparent by 2 h.

Irritant	N	Time of Maximal response	Mean maximal responses ± s.e.mean (calculated as: CBF + irritant (mV) / CBF + vehicle (mV))
Hexyl nicotinate	4	45 - 60 min	5.4 ± 0.5
Benzalkonium chloride	4	4 - 5 h	4.7 ± 0.6

Thus IL-1 present in the epidermis of miniature swine may, if released into the dermis, contribute to the erythematous response induced by irritants in this species. Changes in IL-1 levels before and after application of benzalkonium chloride and hexyl nicotinate are now being measured.

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161P SODIUM AUROTHIOMALATE INHIBITS RECOMBINANT HUMAN INTERLEUKIN-1β-INDUCED DEGRADATION OF RAT FEMORAL HEAD ARTICULAR CARTILAGE *IN VITRO*

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Interleukin-1 (IL-1) may maintain the immune response and induce cartilage erosion in the rheumatoid joint. Sodium aurothiomalate (ATM) reduces mouse thymocyte proliferation in response to monocyte-derived IL-1 (Drakes et al., 1987) and also inhibits recombinant human IL-1ß (rhIL-1ß) induced collagenase release by rabbit chondrocytes in vitro (Hunneyball et al., 1988). Other gold compounds but not ATM inhibit IL-1α induced glycosaminoglycan (GAG) loss from bovine nasal septum (BNS) in vitro (Rainsford, 1989). We report the action of ATM on rhIL-1ß induced GAG loss and synthesis inhibition in rat femoral articular cartilage (RFC) in vitro and compared this to thiomalate (TM) and d-penicillamine (d-PEN).

RFC were cultured as previously described (Clay et al., 1989) in antibiotic supplemented DMEM with and without rhIL-1ß (3-1000ng/ml), or rhIL-1ß with drug for 5 days. Cartilages were pulsed with 0.25 uCi [35-S]04 16h before washing and papain digestion. Media and cartilages were assayed for GAG content (Farndale et al., 1986) and counted by liquid scintillation. rhIL1-ß stimulated GAG loss into the medium (EC50 13.1+/-2.2ng/ml) and inhibited [35S]04 incorporation (EC50 12.0+/-1.6ng/ml). ATM reversed these effects with dose related shifts in rhIL-1ß concentration-response curves at 100uM, 30uM and 10uM. An example of ATM action with or without rhIL-1ß at 100ng/ml is given (table 1). TM and d-PEN had no effect at the same concentrations.

Table 1. Influence of ATM with or without rhIL-1ß (100ng/ml) on GAG loss (ug released/mg wet wt.) and [35S]04 incorporation (cpm per cartilage) by RFC (n=6-12). Means of drug were compared to rhIL-1ß, (*p<0.05, ***p<0.001) and rhIL-1ß to basal (+p<0.05, +++p<0.001 ANOVA RS-1, BBN Software).

	without rhIL-1ß		with rhIL1-ß	
	GAG(ug/mg)	[35S]04(cpm)	GAG(ug/mg)	[35S]04(cpm)
Basal	3.47 +/- 0.15	3826 +/- 117	3.61 +/- 0.12	4091 +/- 155
rhIL-1ß	-	-	7.90 +/- 0.31+++	1822 +/- 175+++
ATM 3 uM	ND	ND	7.11 +/- 0.84	1994 +/- 290
10 uM	3.42 +/- 0.09	3700 +/- 162	6.99 +/- 0.32	2398 +/- 126*
30 uM	3.33 +/- 0.25	4071 +/- 220	5.46 +/- 0.33***	3168 +/- 126***
100 uM	3.73 +/- 0.40	4266 +/- 304	5.27 +/- 0.31***	3367 +/- 350***

Thus, ATM inhibited rhIL-1ß stimulated GAG loss and reversed rhIL-1ß inhibition of GAG synthesis in RFC in vitro. This is gold dependent as both thiomalate and d-penicillamine were inactive and ATM had no effect on RFC alone. The difference between the effects on RFC and BNS may reflect the IL-1 form, the species or cartilage source.

The rhIL-1ß was kindly supplied by Roussel UCLAF, Romainville, France.

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162P COMPARATIVE STUDY OF THE PYROGENICITIES OF INTERLEUKIN 1β AND INTERLEUKIN 6 IN CONSCIOUS RATS

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Interleukin 1β (IL-1β) causes fever in rats following both peripheral and central administration (Dascombe et al, 1989). IL-1β stimulates the release of another cytokine interleukin 6 (IL-6) from many cell types including astrocytes and microglial cells (Frei et al, 1989). In this study, the effects of peripheral and central injections of IL-1β and IL-6 on body temperature in rats were compared to determine whether IL-6 could mediate the febrile response to IL-1β.

Male Sprague Dawley rats (150-300g) were injected intravenously (tail vein) or intracerebroventricularly using cannulae chronically implanted into the third ventricle under sodium pentobarbitone anaesthesia (60mg/kg i.p.) at least 7 days earlier. Colonic temperature was measured in conscious, hand held rats before i.v. or i.c.v. administration of cytokines, and intermittently for the following 3h at an ambient temperature of 25 ± 1°C. Cytokines were dissolved in sterile saline for peripheral studies, and in water for injection for central studies.

I.v. human IL-1β (DuPont, 5μg/kg, n=6) caused an increase in colonic temperature from 90 min after injection until 3h (thermal response index for 3h (TRI_3) saline -1.06 ± s.e. mean 0.29 °Ch, IL-1β 0.49 ± 0.42 °Ch, P<0.05). Human IL-6 (ICI Pharmaceuticals, 5 and 25μg/kg, n=6) had no effect on colonic temperature following i.v. administration. I.c.v. IL-1β (10ng in 2μl, n=6) caused a rise in body temperature 45 min after injection which lasted the study period (TRI_3 water 2.44 ± 0.22 °Ch, IL-1β 4.57 ± 0.50 °Ch, P<0.05). IL-6 (100ng in 2μl, n=4) also caused an increase in colonic temperature (TRI_3 water 1.36 ± 1.2°Ch, IL-6 4.22 ± 1.39°Ch, P<0.05); pyrexia was not observed with 1 and 10ng IL-6 (n=5). Flurbiprofen (100μg in 4μl i.c.v. 15 min before cytokine) attenuated the response to 100ng IL-6 (P<0.05, n=6).

These results confirm that IL-1β acts as a pyrogen following i.v. and i.c.v. administration. IL-6 is a less potent pyrogen following i.v. injection in rats than IL-1β. This observation is consistent with the hypothesis that peripheral IL-6 is not a major endogenous pyrogen (Gehua et al, 1989). The febrile response to IL-6, being blocked by flurbiprofen, appears to be mediated by cyclo-oxygenase products.

EJR is an ONO Pharmaceuticals Research Student.

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163P INHIBITION OF EOSINOPHIL OXYGEN RADICAL PRODUCTION BY TYPE IV- BUT NOT TYPE III-SELECTIVE cAMP PHOSPHODIESTERASE INHIBITORS

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3′,5′-cyclic adenosine monophosphate (cAMP)-specific phosphodiesterase (PDE) exists as two distinct isozymes, designated PDE III and PDE IV (Reeves et al., 1987), which can be differentiated by selective inhibitors. cAMP-elevating agents - including -adrenoceptor agonists and E-series prostaglandins - have been shown to inhibit leukocyte oxygen radical production and granule enzyme secretion induced by a variety of stimuli, and certain of these effects were mimicked or enhanced by non-selective PDE inhibitors such as theophylline and 3-isobutyl-1-methyl xanthine (Fantozzi et al., 1984; Yukawa et al., 1989). We have investigated the effects of a $_2$-agonist and selective inhibitors of PDE isozymes upon stimulated active oxygen metabolite production by eosinophils in order to determine whether cAMP levels may regulate eosinophil activity and to identify the PDE isozyme responsible for the catabolism of cAMP in these cells.

Guinea pig eosinophils were obtained by peritoneal lavage of human serum-treated guinea pigs (1 ml/animal/week i.p.) and purified by centrifugation on 5-step discontinuous Percoll density gradients. Eosinophils were recovered from the 1.080/1.085 g/ml and 1.085/1.090 g/ml interfaces, washed and suspended in Ca^{2+}/Mg^{2+}-free HEPES-buffered Krebs-Ringer bicarbonate buffer, pH 7.4 (KRB). Cells (10^6/assay) were added to a final volume of 1 ml KRB, containing 1 mM $MgSO_4$, 1 mM $CaCl_2$ and 30 U superoxide dismutase, in the presence or absence of drugs, and incubated at 37°C for 2 min prior to addition of 500 ug opsonised zymosan (OZ). Oxidant production was measured as hydrogen peroxide (H_2O_2) by horseradish peroxidase-catalysed oxidation of scopoletin, as described (Root et al., 1975).

Pre-treatment of eosinophils with the selective PDE IV inhibitors, rolipram and denbufylline, for 2 min caused concentration-dependent inhibitions of OZ-stimulated H_2O_2 generation with maximal inhibitions of 71 ± 5.5% (mean ± sem, n=3) and 67 ± 3.7% (n=4), and EC_{50} values of 16 ± 9.4 nM and 38 ± 22 nM, respectively. In contrast, the selective PDE III inhibitor, SK&F 94120, caused no significant inhibition (n=3). The $_2$-agonist, salbutamol, also caused a concentration-dependent inhibition with maximal inhibition of 38 ± 1.5% and EC_{50} of 53 ± 17 nM (n=3). A 2 min preincubation with 10 nM denbufylline potentiated the effect of salbutamol, the maximal inhibition being increased to 64 ± 7.9% and the EC_{50} decreased to 2.6 ± 1.3 nM (n=3). We conclude that cAMP may be involved in the modulation of eosinophil activity and that the PDE responsible for regulation of cAMP in these cells is predominantly type IV.

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164P GLUCOCORTICOID INDUCTION OF THE ANTI-PHOSPHOLIPASE PROTEIN, LIPOCORTIN I, IN THE RAT

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The lipocortins are a family of calcium and phospholipid binding proteins, originally described as glucocorticoid-inducible and with phospholipase A_2 inhibitory properties (Flower, 1988). Anti-peptide antibodies have been raised to epitopes which are reported to be specific for lipocortin 1 (Carey et al, 1989). Using these antibodies we have investigated the effect of dexamethasone on the expression of lipocortin 1 in various tissues of the rat.

Alderley Park male rats (n=6) were dosed s.c. daily with dexamethasone (3.0 and 0.1mgkg^{-1} body weight) or saline. After 7 days body weights were recorded, the rats sacrificed and body organs removed and weighed. Expression of lipocortin 1 in the detergent soluble fraction of the organs was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and Western blotting using antibodies described previously. (Forder et al).

Dexamethasone treatment caused a significant reduction in body weight (25.5±1.4% with 3.0mgkg^{-1}, 16.4±1.8% with 0.1mgkg^{-1}; mean±s.e.m., n=6). Similar observations were seen in the total wet weight of the pituitary (21.4±3.1% and 14.6±3.5%), adrenal (50.0±3.1% and 33.9±4.5%), lung (49.2±2.3% and 33.4±4.4%) and spleen (64.5±1.5% and 46.7±2.4%) respectively. Dexamethasone induction of lipocortin 1 expression was observed in lung (327±9% with 3.0mgkg^{-1}, 88±25% with 0.1mgkg^{-1}; n=5) and adrenal tissue 83±46% and 45±20% respectively). Conversely, suppression was observed in spleen tissue (87±1% with 3.0mgkg^{-1}, 40±7% with 0.1mgkg^{-1}; n=5). Lipocortin 1 was not detected in hypothalamic tissue in these experiments. In the pituitary, although immunoreactive lipocortin was detected no effect was observed with dexamethasone treatment.

These data support the hypothesis that glucocorticoids induce expression of lipocortin 1 in lung, whilst expression in the spleen is diminished. The treatment regime used caused suppression of ACTH secretion from the pituitary (Carey et al, unpublished observations) but this was not accompanied by an elevation of lipocortin 1 expression. These results suggest that expression of lipocortin 1 is not associated with suppression of the hypothalamic/pituitary adrenal axis by glucocorticoids.

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165P INHIBITION OF LYMPHOCYTE PROLIFERATION AND ACTIVATION BY KETOTIFEN AND CYCLOSPORIN A

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We have studied the effects of ketotifen and cyclosporin A on mitogen-stimulated proliferation of human peripheral blood lymphocytes. Additionally we have investigated their effects on intracellular Ca^{++} increases in lymphocytes and Jurkat and U937 cells.

Lymphocytes were prepared from the peripheral blood of young healthy volunteers and stimulated for 4 days with various concentrations of the T-cell mitogens concanavalin A and phytohemagglutinin and the B-cell mitogen Pokeweed mitogen; proliferation was assessed by [^{3}H]thymidine incorporation.

All three mitogens stimulated lymphocyte proliferation with bell-shaped concentration-effect curves. Ketotifen inhibited mitogen-stimulated lymphocyte proliferation in concentrations between 1 and 100 µM. The inhibititory effect of all ketotifen concentrations was stronger against submaximal than against optimal or supraoptimal mitogen concentrations. Low ketotifen concentrations only inhibited the proliferation stimulated by the lowest mitogen concentrations. Cyclosporin (0.1-10 µg/ml) also inhibited the concanavalin A-stimulated lymphocyte proliferation. This effect was most pronounced at higher (supraoptimal) mitogen concentrations. The combined treatment with 1 µM ketotifen and 0.1 µg/ml cyclosporin inhibited concanavalin A-stimulated lymphocyte proliferation to a similar extent as would have been predicted for an additive effect.

Intracellular Ca^{++} was assessed by the fluorescent indicator dye Flura-2 as described (Motulsky & Michel, 1988). Ketotifen (100 µM) inhibited Ca^{++} increases in lymphocytes and Jurkat cells (stimulated by concanavalin A and phytohemagglutinin) and in U937 cells (stimulated by ATP) by approximately 40%. Whereas the mitogen-stimulated Ca^{++} increase in lymphocytes and Jurkat cells is mostly influx of extracellular Ca^{++}, the Ca^{++} increase in U937 cells is mostly mobilization of intracellular Ca^{++}. Ketotifen inhibited the ATP-stimulated Ca^{++} increase in U937 cells whether extracellular Ca^{++} was present or not. Cyclosporin did not inhibit the mitogen-stimulate Ca^{++} increase in lymphocytes but rather enhanced it.

We conclude that ketotifen and cyclosporin A inhibit proliferation and Ca^{++} increases in human lymphocytes via different mechanisms.

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166P PHARMACOLOGICAL STUDIES OF PRE- AND POST-SYNAPTIC NEURONAL NICOTINIC RECEPTORS

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Molecular biological studies indicate multiple genetic subtypes of neuronal nicotinic receptors (e.g., Boulter *et al.*, 1986). We wished to find out if pharmacologically-different pre- and post-synaptic subtypes can be distinguished in the rat superior cervical ganglion.

Ganglia isolated from male SD rats (190-230g) were maintained at 30°C perfused with Krebs' solution pre-bubbled with 95% O_2/5% O_2, containing 1µM scopolamine. The grease-gap method of Brown & Marsh (1978) was used to record agonist-induced potential changes of the cell body, or presynaptic terminal region. The results obtained with various agonists and antagonists are shown in Table 1.

Table 1 Agonist ED_{50} and antagonist IC_{50} values (µM) ± s.e. mean (n)

*Antagonist	pre-synaptic	post-synaptic
Pempidine	0.08 ± 0.035 (4)	0.45 ± 0.25(4)
Mecamylamine	0.32 (2)	0.36 (2)
Pentolinium	0.63 (2)	0.90 (2)
$Ph(CH_2)_4NEt_2$	11.3 ± 0.4 (3)	7.4 ± 1.4 (3)
$Ph(CH_2)_4N^+Et_3$	1.5 (2)	8.2 (2)

*agonist = 3µM m-OH $Ph(CH_2)_4N^+Me_3$

Agonist	pre-synaptic	post-synaptic
m-OH $Ph(CH_2)_3N^+Me_3$	1.8 ± 0.5 (3)	5.6 ± 3.2 (3)
coryneine	10.7 (2)	9.9 ± 3.5 (3)
leptodactyline	9.5 (2)	12 ± 3.6 (3)
nicotine mono me-I	27 (2)	26.3 (2)
DMPP	29.9 ± 12.1(3)	23.4 ± 4.3 (3)
β-pyridyl $CH_2N^+Me_3$	31 (2)	24.9 ± 8.6 (3)
hordenine	50 (2)	45.5 ± 12.9(3)
β-pyridyl$(CH_2)_3N^+Me_3$	56.5 (2)	25 (2)

We conclude that these compounds provide no evidence for a distinction between the pre- and post-synaptic receptors in the rat superior cervical ganglion.

Supported by the Medical Research Council. We are grateful to BAT Industries for gift of equipment.

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167P SITE-DEPENDENT DIFFERENCES IN INFLAMMATORY OEDEMA FORMATION IN THE RAT

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Inflammation has been widely studied in the dermis and hind paws of laboratory animals on a largely empirical basis (Morley et al. 1985). In the present study dermal and hind paw oedema formation in response to identical inflammatory stimuli were compared in the rat.
Male CFHB rats (Interfauna, 160-180g) were injected either i.d. in the shaved dorsal skin or into the hind paws. Non-immunological inflammation was studied by injection of 1mg carrageenin (Viscarin 402, Marine Colloids), or 1mg zymosan (Sigma). For the investigation of immunological inflammation rats were injected i.d. in the tail base with 0.2ml saline/Freund's complete adjuvant (FCA, Difco) emulsion ± 1mg bovine serum albumin (BSA, Sigma) followed 14 days later by i.d. or hind paw challenge with 0.1mg BSA. Oedema formation was measured after 1,3,6,24 and 48h as the increase in double skin fold thickness or paw diameter (mm), relative to control saline (0.1ml) responses at contralateral sites.
The dermal response to carrageenin increased throughout up to 48h, whereas the paw responses peaked at 6h and decreased thereafter. The responses to zymosan peaked at 3-6h at both sites. Profound differences were observed between the immunological responses to BSA. In the dermis the response increased gradually and peaked at 24-48h, and in the hind paw an intense early (1-6h) response was seen which decreased by 24-48h. In addition, granuloma formation was observed in all 24-48h dermal responses but was absent in the hind paws.
These studies clearly indicate site and stimulus-dependent differences in inflammatory oedema formation. The soluble stimulus carrageenin gave a prolonged dermal response and a transient hind paw response in agreement with a previous report (Rao et al. 1988), whereas the insoluble stimulus zymosan gave responses of similar time course. Particularly marked differences were observed between the immunological responses to BSA where the dermal response had a time course typical of delayed-type hypersensitivity and the hind paw response resembled a mixed reaction with major anaphylactic/Arthus-type components. These latter findings suggest that the traditional practice of characterisation of immunoinflammatory responses in terms of time course data may be open to question. For all stimuli it is likely that these differences reflect local differences in cell populations, blood flow, mediator formation and clearance properties. Furthermore, these differences may extend to differential sensitivity with respect to the detection of anti-inflammatory agents.

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168P MYOFIBROBLAST-LIKE CONTRACTURE OF FIBROTIC RAT LUNG-STRIP PREPARATIONS PROVOKED BY OXIDISING AGENTS

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Myofibroblast cells in fibrotic tissues may account for contractility, which is evident functionally, e.g. in Dupuytren's contracture, or experimentally, e.g. in isolated connective tissue strip preparations (Majno *et al.*, 1971). Characteristic myofibroblast contracture is provoked by some distinctive agonists, e.g. mepyramine, which may be utilised to indicate the presence of such cells. Using rat granuloma-strip preparations it was shown previously that some soluble inorganic components from industrial metal-fume particles, e.g. chromates, provoked such contracture (Caldas *et al.*, 1986). Hydrogen peroxide had such an effect also, suggesting an association with oxidising activity. As myofibroblasts may develop in fibrotic lung lesions (Fukuda *et al.*, 1987) and could be influenced by inhaled materials, further work to define the effects of a wider variety of oxidising agents and fume components has been performed, using silica-treated lung tissues.

Fibrosis was induced by intratracheal injection of sterile suspension of crystalline silica (DQ12) particles (doses 100 or 250mg in 0.5ml) into lungs of female rats (CSE, 150-200g), under alphaxalone/alphadolone anaesthesia. After 14 days, animals were sacrificed, lungs removed and longitudinal strips (2.5 x 1 x 15mm) were cut from left lobes on a wax block. Strips were suspended in O_2/CO_2 gassed Krebs solution at 35°C and equilibrated for 45 minutes to a tension of 500mg. Contractions were measured by transducer, when inorganic agonists were added. Control preparations were obtained similarly from normal, untreated rat lungs. Fibrosis was verified histologically.

Preparations from fibrotic, silica-treated lungs responded with dose-related, reversible contractions of up to 500mg tension, to mepyramine (1×10^{-6} to 2×10^{-4}M) and to hydrogen peroxide (1×10^{-4} to 1×10^{-3}M) but not to histamine, 5-hydroxytryptamine or acetylcholine, in concentrations up to 1×10^{-3}M). Control strips did not respond to any agonist. Fibrotic lung preparations also responded to sodium chromate and sodium tungstate (both 1×10^{-5} to 1×10^{-3}M). Less consistent contractures occurred to sodium chlorate, sodium hypochlorite and potassium permanganate, only the more fibrotic (250mg silica dose) lung preparations responded. Preparations did not respond to sodium dichromate or nickel salts. Thus, silicotic fibrous tissue contracted in a manner characteristic of myofibroblasts. The responses to inorganic oxy-acid salts were consistent with oxidising activities. Oxidising activity of dichromate has different pH dependence, possibly explaining lack of stimulant activity.

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169P PHOSPHOLIPID FATTY ACID PROFILES IN HUMAN COLON MUCOSA

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Phospholipids (PL) are one of the sites of arachidonate esterification in cell membranes and are a source of eicosanoid production. In human large bowel mucosa we have investigated the size of the lipid pools of phosphatidylcholine (PC), phosphatidylserine/inositol (PS/PI), phosphatidylethanolamine (PE) and sphingomyelin (SM). We have determined individual fatty acids as a percentage of the total lipid fatty acid in PL and have quantitated the arachidonate content. Macroscopically normal large bowel was obtained at operation for carcinoma. PL were extracted from 20mg samples according to the Folch method. Aliquots of the extract were separated on silica-gel plates using chloroform:methanol:acetic acid:water (50:25:7:3). Zones corresponding to standards were extracted using 2x2ml chloroform:methanol:0.2NHCl (1:2:0:8). An aliquot was transmethylated after neutralising and fatty acids measured by capillary column gas-liquid chromatography. Lipid phosphorus was estimated in an aliquot of each extract and protein was measured in each total lipid extract. The arachidonic acid peak was quantitated by comparison with known amounts of methyl ester standard.
The results are means ± SD on 13 mucosal specimens. PC was the predominant PL present (19.3 ± 12.2nmoles phosphorus/mg protein) while PS/PI was (12.4 ± 7.6); PE (5.7 ± 3.4) and SM (5.4 ± 3.6).

Table 1 Selected fatty acids as a % of total major fatty acids in mucosa PL

	Saturated	C18:1	C18:2	C20:3	C20:4
SM	60.4 ± 17.1[a]	23.7 ± 10.5	12.0 ± 5.5	0.17 ± 1.0	1.1 ± 1.0
PC	37.8 ± 6.5	29.5 ± 3.8	15.1 ± 4.8	1.6 ± 0.7[c]	10.7 ± 5.4[d]
PS/PI	35.9 ± 7.3	30.0 ± 3.7	15.6 ± 3.7	1.6 ± 0.7[c]	14.2 ± 4.8
PE	36.1 ± 9.6	36.9 ± 6.5[b]	17.1 ± 5.8	0.6 ± 0.3	2.3 ± 1.5

a P<.002 cf with other FA; b P<.01 cf with PC and SM; c P<.01 cf with SM and PE; d P<.02 cf with SM and PE

The table shows that arachidonate is greater in PC than PE or SM (P<0.02). Comparison with separate PS and PI was not possible. PE also has a higher percentage of C18:1 than other PL and PC a higher percentage of C20:3 than SM or PE. The arachidonic acid content of each PL expressed as nmoles/nmole phosphorus was SM 0.28 ± 0.48; PC 0.5 ± 0.5; PS/PI 0.5 ± 0.5 and PE 0.4 ± 0.3. PC and PS/PI were significantly greater than SM (Wilcoxon's test P<0.005).
We conclude that PL pools differ in their size in human colonic mucosa and the fatty acid distribution varies within each pool. This information may be of more relevance than total PL estimates when measuring the dynamics of lipid turnover and arachidonate cleavage by phospholipases.
We acknowledge funding from the National Association for Colitis and Crohn's Diseases and Leverhulme Trust.

170P CHARACTERISATION OF CYSTEINYL LEUKOTRIENE RELEASE IN HUMAN LUNG MAST CELLS

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Transcellular interactions may be important in regulating the synthesis and release of certain inflammatory mediators and cause difficulties in establishing the cellular origins of mediators such as leukotrienes (LTs) (Lewis et al., 1981; MacGlashan et al., 1982). We have therefore performed experiments which attempted to characterize LT release in human lung cells after IgE-dependent activation.

Human lung cells were obtained by enzymatic digestion (Holgate et al., 1984) and after passive sensitization enriched or depleted in mast cell content using countercurrent centrifugal elutriation and discontinuous density gradient centrifugation (MacGlashan et al., 1982). Cells were challenged for 20 min with 50 $\mu g\ ml^{-1}$ goat anti-human IgE. Histamine release was measured using a radioenzymatic assay, prostaglandin D_2 by RIA and cysteinyl LTs by RIA after separation by HPLC. Mast cells at a final purity of 63 ± 5.5% (n=3) released a net 36.6 ± 2.6% histamine together with 33.9 ± 3.9, 5.6 ± 0.5 and 6.5 ± 0.7 ng per 10^6 mast cells of PGD_2, LTC_4 and LTD_4/LTE_4 respectively, these being similar to values obtained in challenged unpurified cells containing 7 ± 1% mast cells.

In order to investigate the possible interaction between mast cells and other cell types, 250µl aliquots of fractions containing the highest purities of mast cells were added to 250µl aliquots of mast cell depleted fractions. Twenty-nine recombined fractions containing mast cells from 1.9-73% purity were obtained and challenged as above. Histamine, which is exclusively derived from mast cells in these preparations, and cysteinyl LTs were released from all fractions. When analysed by unweighted least-squares linear regression there were significant correlations between the total net release of the cysteinyl LTs (LTC_4, LTD_4 and LTE_4) and the purity of mast cells (r=0.72, P<0.001, n=29) and also the net release of histamine (r=0.85, P<0.01, n=29). The release of LTC_4 was also significantly correlated with the purity of the mast cell preparations and with the net release of histamine (r=0.86 and r=0.83 respectively, P<0.001 in both cases). There was no significant correlation between LT release and any cell type other than mast cells.

These experiments suggest that following IgE-dependent challenge of human lung cells, the release of cysteinyl LTs is dependent upon mast cell activation. However, in view of the difficulties in obtaining homogeneous populations of cells from human lung we cannot exclude the possibility that contaminating cells regulate mast cell LT production, although this would seem unlikely.

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171P CHARACTERISATION OF A PUTATIVE PHOTOAFFINITY LABEL OF THE PROSTACYCLIN RECEPTOR

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ZK119817 ((11,15-$^3H_2$)-(5Z)-(9R)-(16RS)-9-(5-Azido-1-pentinyl)-16-methyl-18,18,19,19-tetradehydro-6a-carba-prostaglandin I_2), is a stable prostacyclin analogue, which possesses a photoactivatable azido group at the end of a 5-carbon side chain. We have investigated whether [^{3}H]-ZK119817 might be a useful photoaffinity ligand for prostacyclin receptor purification.

Adenylate cyclase activity and [^{3}H]-iloprost binding were assayed as described by Edwards et al. (1987). Platelet membranes were incubated with ZK119817 or [^{3}H]-ZK119817 for 20 min at room temperature in the dark, then put on ice and irradiated for 1h, using a 30 watt lamp, wavelength 360 nm at a distance of 20mm. Iloprost (10uM) was used to define non-specific binding. Membranes were washed by two centrifugation steps and the amount of [^{3}H]-ZK119817 bound determined by TCA precipitation.

ZK119817 stimulates adenylate cyclase activity in platelet membranes with an EC_{50} of 2 nM, and produces the same maximal response as iloprost. ZK119817 displaces [^{3}H]-iloprost binding to platelet membranes with an IC_{50} of 11.8nM and a Hill slope of 1. Pretreatment of platelet membranes with 100 nM unlabelled ZK119817 resulted in a decrease in the subsequent specific binding or [^{3}H]-iloprost; this appeared to be due to a decrease in the [^{3}H]-iloprost Bmax (80%) with no apparent change in its affinity (10nM). It was not possible to investigate the binding properties of [^{3}H]-ZK119817 directly, as it exhibits very high filter binding. Nevertheless, treatment of platelet membranes with [^{3}H]-ZK119817 (1-80nM) results in concentration dependent binding, which is suppressed by 80% when the labelling is carried out in the presence of 10uM iloprost. Binding is dependent on UV irradiation and is stable through TCA precipitation, extensive washing and freeze/thawing. The TCA precipitable [^{3}H]-ZK119817 binding can be solubilized in 8mM CHAPS/0.5M NaCl with a 30-40% yield. On gel filtration through G200 this activity elutes with bulk protein and is separated from free [^{3}H]-ZK119817. We have been consistently unable to obtain any further purification of [^{3}H]-ZK119817 labelled protein. In SDS-PAGE all the radioactivity runs close to the solvent front in apparently the same position as free [^{3}H]-ZK119817 and is not fixed by methanol/acetic acid. In chloroform/methanol extractions of [^{3}H]-ZK119817 labelled membranes all the [^{3}H]-ZK119817 seems to be recovered in the lipid phase.

[^{3}H]-ZK119817 seems to behave like an irreversible ligand for the prostacyclin receptor in binding studies and may be useful in pharmacological assays. However it does not form a sufficiently stable bond with the receptor protein to allow its purification. It is at present unclear whether [^{3}H]-ZK119817 forms a covalent but unstable bond with the receptor protein itself, or whether it forms a covalent bond with anular lipid.

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172P NABUMETONE, AN EFFECTIVE ANTI-INFLAMMATORY AGENT, LACKS THE GASTRIC IRRITANCY POTENTIAL OF PIROXICAM OR IBUPROFEN

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Non-steroidal anti-inflammatory drugs (NSAIDs) can induce gastric bleeding in addition to reducing inflammation, actions which may relate, in part, to inhibition of prostaglandin (PG) synthesis. The relationship between inhibition of PG's in the inflammatory exudate with their inhibition in the gastric mucosa and formation of gastric damage by NSAIDs has been studied in a model of carrageenan-induced paw inflammation.

Drugs were administered orally in 0.7% methyl cellulose to male Wistar rats (150-200g; n=8 fasted 18h) 1 hour prior to sub-plantar injection of 1% carrageenan (0.1ml) using the following doses (5 x ID_{25} values obtained from previous carrageenan studies; mg/kg): nabumetone (nab 79), piroxicam (pirox 7) or ibuprofen (ibup 88). After 3 hours, paw oedema and exudate PGE_2 content, gastric mucosal 6-keto-$PGF_{1\alpha}$ production (Melarange and Rashbrook, 1986) and gastric damage (erosion index, EI) were measured. Results were analysed using Student's 't' test or the Mann-Whitney 'U' test.

Nab significantly reduced oedema formation (45%; $P<0.01$) which was not significantly different ($P>0.05$) compared with pirox (51%; $P<0.001$) or ibup (53%; $P<0.001$). Control exudate PGE_2 concentration (2.4±0.3ng/ml) was inhibited by nab (76%; $P<0.001$), pirox (90%; $P<0.001$) or ibup (88%; $P<0.001$). In contrast, nab or ibup produced only 57% ($p<0.01$) and 60% ($P<0.01$) inhibition respectively of control 6-keto-$PGF_{1\alpha}$ production (4.65±0.8ng/section) whereas pirox produced 84% inhibition ($P<0.001$). Gastric damage, however, was not induced by nab (EI 0.25 $p>0.05$) but was significantly increased by both pirox and ibup (EI both 3.13; $P<0.007$ - $P<0.002$) above the control value (EI 0.25).

This study shows that nab is an effective anti-inflammatory agent comparable with both pirox and ibup. Nab, however, demonstrated a better profile compared with pirox or ibup because it had less propensity to inhibit mucosal protective PG's as previously reported (Melarange and Rashbrook 1987) or to cause gastric mucosal damage.

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173P f-Met-Leu-Phe OR PAF, BUT NOT IgE-ANTIGEN COMPLEXES, RELEASE TXB_2 FROM DIFFERENTIATED U937 MONOCYTE CELLS

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Human lung macrophages release thromboxane B_2 (TXB_2) during phagocytosis and in response to f-MET-LEU-PHE (fMLP) or platelet activating factor (PAF). Furthermore, IgE-antiIgE complexes have been shown to release both TXB_2 and several lysomal hydrolases (Fuller et al, 1986). A low affinity receptor for IgE (CD23/FcE-R11) has been demonstrated on macrophages and on a human monocytic cell line (U937). This cell line may be differentiated to macrophage-like cells (Harris et al, 1985), and we have examined changing responsiveness of these cells to fMLP, PAF or IgE-antigen complexes during differentiation.
A rapid release of TXB_2 in response to 1 μM fMLP, 0.1 μM PAF or 2 μM of the calcium ionophore A23187 was seen in cells that had been differentiated with conditioned medium from HTB5637 cells (containing GM-CSF, G-CSF and Il1a), but not by phorbol myristate acetate (PMA), interleukin 4 (IL4) or interferon (γIF). The release of TXB_2 under these conditions was rapid, transient and complete within 5 min. In these cells PMA also triggered TXB_2 release, but in a sustained manner over a period of at least 1 h, and presumably by a calcium independent mechanism. This response showed synergism with calcium ionophore.
Differentiation of U937 cells with PMA or γIF induces expression of CD23/FcE-R11, and using an anti-CD23 monoclonal antibody we have confirmed similar induction by HTB5637 conditioned medium. However, no TXB_2 was released in response to soluble or particle-bound IgE-antigen complexes or to anti-CD23 antibodies. The IgE used was a chimeric antibody with human Fc domain and mouse anti-NIP Fab regions (Brueggemann et al 1987). We conclude from these observations that occupation of CD23/FcE-R11 on U937 cells by IgE-antigen complexes does not trigger release of thromboxane, despite the fact that these cells are competent to release TXB_2 in response to fMLP or PAF. It seems probable that resident human lung macrophages are further differentiated or primed to respond to IgE through the CD23/FcE-R11 receptor, or may even express other receptors that bind IgE and trigger release of TXB_2. The competance of these cells to respond to PAF or fMLP appears independent from their capacity to express receptors for IgE since I14 and γIF induce expression of CD23/FcE-RII, but inhibited release of TXB_2 whether triggered by fMLP, PAF or PMA.

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174P INCREASED EXPRESSION OF FCεRII/CCD23 ON RAT ALVEOLAR MACROPHAGES BY PAF AND ANTIGEN

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The possible modulation of the expression of the low affinity receptor for IgE, FcεRII/CD23, on alveolar macrophages (AM) from sensitized rats, treated or not with the platelet-activating factor (PAF) antagonist BN 52021 and challenged or not with the antigen was investigated. In addition, the direct effect of PAF *in vivo* and *in vitro* on FcεRII/CD23 expression on rat AM was also evaluated. Brown-Norway (BN) rats (200-250 g) were placed twice, at 48 h interval in a plexiglass chamber and exposed to aerosols of a saline solution (NaCl, 0.9%) containing 10 mg/ml ovalbumin (OA). A booster administration (10 mg/ml OA; aerosol) was performed under the same conditions after 14 days. At day 21, sensitized rats were exposed to four successive aerosol administrations of OA (1, 5, 10 and 50 mg/ml in saline) for 15 min each, or as a control group, to saline alone. The antagonist of PAF BN 52021 (10 mg/ml, aerosol for 30 min) was administered 1 h before the antigen challenge. Male Sprague-Dawley (SD) rats were exposed to an aerosol of PAF (500 μg/ml) or lyso-PAF (500 μg/ml) for 30 min as described above. AM from anaesthetized BN and SD rats (Brietal, Lilly, France, 40 mg/kg, ip) were obtained by 5 successive bronchoalveolar lavages with 5 ml of a warmed (37°C) saline solution. *In vitro*, AM from SD rats were incubated for defined time intervals with different concentrations of PAF or lyso-PAF (10 pM to 10 μM). The expression of FcεRII/CD23 was assessed by flow cytometry after staining with the BB10 monoclonal antibody (Capron et al., 1986).
When administered by aerosol, OA induced a bronchopulmonary response in sensitized BN rats of about 10-15 % without booster, and 20-30 % when booster administration was performed. No expression of FcεRII/CD23 on AM from non-sensitized BN rats, challenged or not with OA, was observed. In contrast, a maximum of 74 % of AM expressed FcεRII/CD23 when collected 24 h after antigen stimulation by aerosol, compared to 12 % of the cells following challenge of the rats with the saline solution. Pretreatement of BN rats with BN 52021 markedly reduced (-82 %) the expression of FcεRII/CD23 on AM induced by OA. Aerosol administration of PAF (500 μg/ml) to SD rats induced after 24 h the expression of FcεRII/CD23 on 79 % of the AM. This is to be compared to the 20 % cells expressing of FcεRII/CD23 after aerosol administration lyso-PAF (500 μg/ml). *In vitro*, PAF induced a concentration-and time- dependent increase of FcεRII/CD23 expression on AM from SD rats, which was maximum at 1 μM and after 24 h. In contrast, lyso-PAF was inactive. These results demonstrate that administration of the antigen in sensitized BN rats induces FcεRII/CD23 expression on AM, in a process inhibited by BN 52021. The fact that PAF induces FcεRII/CD23 expression both *in vivo* and *in vitro* suggests a primary role of this lipid mediator in the late phase of the allergic reaction.

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175P 1,4-DIHYDROPYRIDINES, A NEW CLASS OF PAF RECEPTOR ANTAGONISTS

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New compounds were developed from a series of 1,4-dihydropyridines (1,4-DHP) which are devoid of an effect on voltage-operated calcium channels (Sunkel et al., 1988). They were found to selectively block rabbit and human platelet aggregation and secretion and binding of (3H)PAF to human platelet and polymorphonuclear (PMN) PAF-receptors. One of them, named PCA-4248 (2-[phenyl-thio]ethyl 5-methoxycarbonyl-2,4,6-trimethyl-1,4-dihydropyridine-3-carboxylate) showed an IC50 value of 1.05 μM vs. 1.9 nM PAF in rabbit rich plasma platelet aggregation. (3H)Serotonin secretion studies in rabbit platelets were consistent with PCA-4248 being a selective and competitive antagonist since it displaced rightwards log dose-response curves and lacked any effect on thrombin and ionophore A23187-induced release (Casals-Stanzel et al., 1987). (3H)PAF binding studies showed that 1 μM PCA-4248 inhibited specific binding by 74 ± 5 % (n=5), and it also blocked (3H)PAF binding to PMN with a pA2 of 7.4.
Significant inhibition of PAF-induced systemic hypotension in rats was obtained with i.v. doses of PCA-4248 of 0.3 to 1 mg/Kg, IC50 0.45 mg/Kg with PAF 0.33 μg/Kg. Reversal of the hypotension was observed when PCA-4248 was administered after PAF. The extravasation induced by 1 μg/Kg PAF was also blocked (IC50 0.36 mg/Kg i.v.). Inhibition of systemic extravasation induced by soluble aggregates of immunoglobulin G (A-IgG) and endotoxin was (Sánchez Crespo et al, 1982) provided by PCA-4248 at the dose of 1 mg/Kg and lasted for at least one hour when endotoxin was used. Combination of the cytokine tumor necrosis factor (TNF) failed to enhance PAF-induced systemic extravasation. Intradermal extravasation induced by PAF reached a maximum at 30 minutes after injection and was also inhibited by PCA-4248. The survival rate in mice increased from 16% to 78% when a single oral dose of 30 mg/Kg of PCA-4248 was given 5 minutes before challenge with PAF (LD84 = 80 μg/Kg PAF, i.v.). These data indicate that compounds containing a 1,4-DHP structure can display potent antagonistic activity on the PAF-receptor in vitro and in vivo.

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176P PRELIMINARY PHARMACOLOGICAL CHARACTERISATION OF CULTURED HUMAN PROSTATIC STROMAL CELLS

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Benign prostatic hypertrophy (BPH) arises mostly from the proliferation of stromal smooth muscle cells within the prostate gland. Classical pharmacological and radioligand binding studies reveal significant numbers of α_1-adrenoceptors on prostatic stromal cells (James et al. 1989) and α_1-antagonists may be therapeutically useful in BPH. We have isolated and grown stromal cells from the human prostate in order to examine intracellular calcium, $[Ca^{2+}]_i$, responses, partly to establish whether functional α_1-receptors are present on these cultured cells.

For measurement of $[Ca^{2+}]_i$ these prostatic cells were trypsinised, resuspended in growth medium and centrifuged (200g, 5 min). The pellet was resuspended in a modified HEPES-Tyrode's buffer (pH 7.4) and the cells loaded with 6μM fura 2/AM for 30 min at 37°C. Finally the cells were suspended in the same buffer at a concentration of 5 x 10^5 cells/ml with an external calcium concentration of 1mM. Fluorescence measurements were performed in a PTI Deltascan dual wavelength spectrofluorimeter and $[Ca^{2+}]_i$ calculated according to Grynkiewicz et al.(1985). Elevation of $[Ca^{2+}]_i$ was observed under these conditions in response to a variety of agonists. Large changes in $[Ca^{2+}]_i$ were found with histamine (range 260-5727nM; median 1980, n=9), CGRP (range 426-2704nM; median 1785, n=6), bradykinin (range 354-5979nM; median 1430, n=17), substance P (range 280-4740nM; median 1136, n=12) , endothelin (range 408-2340nM; median 951, n=7), thrombin (range 286-1460nM; median 822, n=6), NPY (range 50-2620nM; median 444, n=14) and carbachol (range 108-2370nM; median 383, n=16). Less consistent responses were obtained with 5-HT (range 84-630nM; median 285, n=6), dopamine (range 31-2390nM; median 146, n=15) and noradrenalin (range 34-2180nM; median 106, n=20) while only small responses were found to pilocarpine (range 26-285nM; median 134, n=9), angiotensin II (range 78-169nM; median 131, n=6) and vasopressin (range 75-218nM; median 119,n=6).

It is particularly interesting that these cultured prostatic cells express such a wide spectrum of receptors, though functional α_1-receptors are present only in small numbers, or are of low affinity. Further experiments are being conducted to characterise the classes of receptor mediating the dopamine, histamine and muscarinic responses and to determine whether any of these agonists may act as growth promoters in these cells.

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177P EFFECTS OF SELECTIVE NEUROKININ AGONISTS ON RAT BLADDER MOTILITY *IN VITRO* AND *IN VIVO*

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The mammalian neurokinins, substance P (SP), neurokinin A and neurokinin B contract the rat bladder in vitro and activate micturition in the anaesthetized rat (Maggi et al., 1987). To characterise the receptors involved, we have used selective, potent and metabolically stable neurokinin agonists at NK-1 (GR73632), and NK-2 receptors (GR64349), (Hagan et al., 1989). These agonists were compared to SP and senktide (selective NK-3 agonist, Wormser et al., 1986) for their ability to contract the rat isolated bladder dome strip and to affect bladder motility in vivo (either tonic bladder contraction (TBC) or rhythmic voiding contractions (RVC) after topical administration (see Maggi et al., 1987).

Table 1 EC_{50} values (nM) ±s.e.mean or 95% confidence limits for neurokinin-induced bladder contraction.

Agonist	In Vitro	In Vivo	
		TBC	RVC
SP	57±18	326(238-448)	1480(493-3118)
GR64349	104±18	184(128-263)	146(66-305)
GR73632	27±3	177(128-245)	64(17-194)
Senktide	>3000	>3000	>3000

Both GR73632 and GR64349 produced potent, dose-related contractions of bladder strips (table 1). Senktide was inactive at concentrations up to 3μM. In vivo, both GR73632 and GR64349 were more potent than SP at evoking TBC. These responses were little affected by pretreatment of the bladder with tetrodotoxin (TTX) (30μM, applied topically). This suggests that the direct action of neurokinins on rat bladder smooth muscle can be mediated by NK-1 and NK-2 but not NK-3 receptors. RVC induced by GR73632 and GR64349 were abolished by TTX treatment. GR73632 induced RVC at concentrations lower than those required to produce TBC. These results are in agreement with the hypothesis that, in addition to their direct effects on the bladder, neurokinins may be involved in the initiation or sensitisation of sensory afferent impulses which result in reflex micturition (Maggi et al., 1987).

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178P INFLUENCE OF COCAINE ON ELECTRICALLY EVOKED [^{3}H]-NA OVERFLOW FROM NORADRENERGIC NERVES: TISSUE DEPENDENT EFFECT

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Neuronal uptake is a major route for removal of NA released from noradrenergic nerves but uptake blockade does not increase electrically evoked [^{3}H]-NA overflow in all tissues (Hagan & Hughes, 1981). This discrepancy was investigated using mouse (Tuck No. 1, 30-35g) & rat (Wistar, 150-200g) vas deferens & rat spleen, atria & brain cortex slices. Tissues were incubated with [^{3}H]-NA & washed as detailed elsewhere (Hagan & Hughes, 1981). Electrical stimulation (vas deferens, parallel electrodes, 2.5Hz, 2ms, 400mA for 90 s every 14 min; spleen, 2.5Hz, 1ms, 200mA for 90 s every 14 min; atria 3 Hz, 1ms, 200mA for 30 s every 14 min; brain slices, 3Hz, 2ms, 20mA for 120 s every 20 min) enhanced tritium overflow from all tissues. Drugs were added to the physiological saline when required after an initial period (I) of stimulation and changes were expressed as the ratio of the fractional evoked tritium overflow in the initial period to that in a subsequent period (S). Results are given as means±s.e. mean.

Cocaine (0.1-10 μM) failed to increase evoked tritium overflow from mouse and rat vas deferens and spleen (P>0.05, Student's t-test) while in atria and brain slices, a concentration dependent increase was obtained. The effect of cocaine (10 μM) in the various tissues is shown below; (*P<0.001).

Ratio (S/I)	mouse vas	rat vas	spleen	atria	brain slices
control	0.94±0.03(n=6)	0.78±0.02(n=4)	1.04±0.03(n=5)	0.96±0.7(n=5)	0.94±0.03(n=6)
cocaine	0.95±0.08(n=6)	0.80±0.04(n=4)	0.97±0.07(n=5)	1.50±0.10*(n=5)	1.81±0.14*(n=6)

The effect of cocaine on tritium overflow evoked by propagated nerve impulses was studied in mouse vas deferens. When only half of the vas was incubated with [^{3}H]-NA stimulation through ring electrodes of the bottom 1/5 of the vas which was not incubated with [^{3}H]-NA evoked a fractional overflow of $0.76\pm0.17\times10^{-3}$ whereas, when the whole vas was incubated with [^{3}H]-NA, ring electrode stimulation evoked a fractional overflow of $1.3\pm0.11\times10^{-3}$(n=4). These figures indicate that a considerable amount of tritium is released by nerve impulses propagated from the ring electrodes. Neuronal uptake blockade by cocaine (10μM) had no effect on tritium overflow evoked by impulses propagated from the ring electrodes (S/I ratio; control, 0.95±0.03: treated, 0.90±0.04; P>0.05; n=4).
These results confirm the tissue dependent effect of neuronal uptake blockade on electrically evoked [^{3}H]-NA overflow. In contrast to the situation in rabbit ear artery (Rand et al., 1988), in mouse vas deferens, overflow evoked by field stimulation or by propagated nerve impulses is similarly unaffected by cocaine.

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179P PHARMACOLOGICAL ACTIONS OF NEUROPEPTIDE Y (NPY) ON HUMAN MYOMETRIUM *IN VITRO*

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Neuropeptide Y (NPY) is known to be present in the autonomic nerve terminals supplying the human myometrium (Fried et al., 1986), but its pharmacological actions in this tissue remain yet to be reported. In the present investigation therefore, we have studied the effects of exogenously added NPY on the spontaneous contractions of in vitro preparations of non-pregnant and pregnant human myometrium, and also have immunohistochemically looked for the presence of NPY-containing nerves in this tissue. Non-pregnant and pregnant myometrial samples from the isthmic region of uterus were collected from women undergoing hysterectomy and caesarian sectioning respectively (age range: 19-41). For immunohistochemistry small specimen, 2 x 2 mm, were immersed in Zamboni's fixative (Stefanini et al., 1967) and processed for indirect immunofluorescence according to standard techniques (Van Noorden, 1986). For recording spontaneous activity, thin strips of non-pregnant (n=3) and pregnant (n=7) myometria (10 mm long and 2 mm in diameter) were cut in the longitudinal axis of muscle and set up in a 1 ml. organ bath in Krebs-Henseleit solution bubbled with 95% O_2, 5% CO_2 mixture at 37°C, at a resting tension of 1.0g for recording tension isometrically. A preliminary equilibration period of 120 minutes was allowed in all experiments. Immunohistochemical examination revealed the presence of a dense network of NPY-immunoreactive nerve fibres in the non-pregnant myometrial sections. By contrast, there was a drastic reduction or even a total absence of NPY-immunoreactive nerve fibres in the pregnant myometrial sections. NPY exerted a profound effect on the spontaneous activity of the myometrial preparations. In the non-pregnant myometria, NPY 50 nM caused an increase in the frequency of spontaneous contractions (mean % increase ± SEM: 58 ± 4, n=3) but reduced the force of individual contractions (mean % decrease ± SEM: 55 ± 7, n=3). The frequency of spontaneous contractions in pregnant myometria was lower compared to non-pregnant myometria (duration of interval between two contractions: mean ± SEM = 9.0 ± 1.5 min, in pregnant mymetria; 3.5 ± 1.0 min in non-pregnant myometria). Addition of NPY, 50 nM invariably enhanced the frequency of spontaneous contractions (mean % increase ± SEM: 244 ± 121). But contrary to its effect in non-pregnant myometrium, NPY potentiated the force of spontaneous contractions in pregnant myometria (mean % increase ± SEM: 56 ± 22). The most dramatic effect of NPY was exerted on oxytocin-induced enhancement of the frequency of spontaneous contractions. NPY, 50-500 nM in all experiments greatly reduced the rise in the frequency of spontaneous contractions induced by oxytocin, 3×10^{-2} I.U./ml. In conclusion, the results are consistent with the view that NPY may have a functional role in the human pregnant and non-pregnant uterus (Heinrich et al., 1986).

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180P INHIBITION OF THE SPONTANEOUS ACTIVITY OF MYOMETRIAL STRIPS *IN VITRO* BY PROSTAGLANDIN E ANALOGUES AND ISOPRENALINE

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We have previously reported that butaprost, a selective EP_2 agonist at the prostaglandin E receptor, produced an inhibitory effect on spontaneously contracting human myometrial samples in vitro (Clayton et al 1989). When tested on uterine artery samples in vitro butaprost was without effect suggesting that it would not affect the uterine blood supply in vivo. We have now evaluated another PGE_1 analogue on human myometrial strips and compared this to the butaprost response (Gardiner & Collier, 1980). The effects of the butaprost and TR4752 have also been compared to the β-adrenoceptor agonist, isoprenaline. The β-adrenoceptor is currently the clinical target for uterine spasmolytics.

Samples of human myometrium were obtained from pre-menopausal patients at hysterectomy or from pregnant patients during Caesarean section (duration of pregnancy 39 ± 0.5 weeks). The myometrial strips were set up as previously described (Massele and Senior 1981) and were superfused with Krebs solution (37°C, 95% O_2/5% CO_2) at 2 ml min^{-1}. Compounds were investigated for their ability to inhibit spontaneous activity. The mean tension (g) generated ± s.e.m. was 3 ± 0.3 (non-pregnant) and 5.3 ± 0.7 (pregnant) and the mean time interval (mins) between spontaneous tension peaks was 2.5 ± 0.5 (non-pregnant) and 11.2 ± 1.0 (pregnant) (n = 10).

The non-selective β-adrenoceptor stimulant, isoprenaline, caused inhibition of spontaneous tension changes in myometrial strips from both non-pregnant and pregnant donors which could be antagonised by propranolol 10^{-6}M. Isoprenaline in doses greater than 5×10^{-7}m caused an initial contraction of the tissue which was not antagonised by the presence of propranolol. A maximum inhibition of spontaneous activity occurred using a bolus dose of isoprenaline 10^{-5}m which inhibited the activity for 23 ± 4 min.

The EP_2 receptor agonists, in contrast, produced no initial contractile activity, the response was characterised by inhibition of spontaneous activity. Butaprost 10^{-7}m produced a maximal inhibition of activity of 46 ± 5 mins on tissue from non-pregnant donors and 30 ± 4 mins on tissue from pregnant donors. The more potent analogue TR4572 at a dose level of 10^{-8}m produced maximal inhibition of spontaneous activity of 79 ± 6 mins on non-pregnant tissue and 90 ± 10 mins on tissue from pregnant donors. The naturally occurring prostaglandin E_2 caused maximal inhibition of activity, after initial contraction, of 15 ± 10 mins duration (2×10^{-8}m non-pregnant tissue; 6×10^{-9}m tissue from pregnant donors).

In summary, the rank order of potency of the uterine spamolytics tested TR4752> butaprost > prostaglandin E_2> isoprenaline. The advantage of butaprost and TR4752 over existing tocolytic therapy would be the lack of adrenoceptor stimulant activity; the specificity for the EP_2 receptor avoids the stimulant effect of PGE_2 on other EP receptors present on myometrium.

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181P PRESENCE OF AN ATROPINE RESISTANT COMPONENT IN THE MOTOR TRANSMISSION OF THE ISOLATED HUMAN DETRUSOR MUSCLE

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It is now widely recognised that the motor transmission in the detrusor of mammalian bladder is comprised of a cholinergic and a non-cholinergic component (Ambache & Zar, 1970; Krell et al., 1981). Human bladder seems to be an exception in that the motor transmission in human detrusor is reportedly fully atropine-sensitive and therefore cholinergic (Sibley, 1984; Kinder & Mundy, 1985). The aim of the present study was to ascertain whether the reported full atropine-sensitivity of human detrusor motor transmission is the result of a genuine absence of a non-cholinergic element or is caused by a failure of non-cholinergic transmission due to prolonged electrical field stimulation.

Macroscopically healthy human bladder samples, obtained from patients (n=13) undergoing surgery, were utilised to prepare thin strips of detrusor in its longitudinal axis (1-2 cm in length and 0.15 cm in diameter) were set up for recording tension isometically in 1 ml organ baths between two platinum electrodes in Krebs-Henseleit solution containing 10 μM indomethacin at 37°C and bubbled with 95% O_2 + 5% CO_2 mixture. Electrical field stimulation (EFS): trains of 10 or 90 pulses, 10 Hz, 0.1 ms pulse-duration supramaximal voltage, at 100 s or 30 s interval. Preparations from each patient were subjected to two different experimental protocols, reserving one preparation for each protocol. Protocol 1: trains of 10 pulses every 100 s were applied. When contractile responses stabilised, the preparations were exposed first to atropine in graded concentrations of 0.5, 1.0 and 3 μM, then to physostigmine 1 μM and finally to tetrodotoxin 0.5 μM. The preparations were left exposed to each concentration of the drug until the contractile responses to EFS in its presence had stabilised. Protocol 2: trains of 90 pulses every 30 s were applied. When contractile responses stabilised, the preparations were exposed to atropine 3 μM. In Protocol 1 experiments: The lowest concentration of atropine that produced a maximal inhibition ranged from 0.5-1 μM. Atropine at no concentration fully blocked EFS-evoked responses (% maximum inhibition by atropine Mean ± SEM = 72 ± 3.5). The atropine-resistant responses were not potentiated by 15 min exposure to physostigmine but were readily abolished by tetrodotoxin, 0.5 μM. In Protocol 2 experiments, treatment with atropine blocked rapidly and fully the EFS-evoked responses. The results clearly demonstrate the presence of a non-cholinergic component in the motor transmission of human detrusor and its extinction by prolonged EFS.

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Sibley,G.N.A. (1984) J.Physiol. 354, 431-443

182P INVESTIGATION OF THE NEURONAL "NON-5-HT_3" RECEPTOR MEDIATING CONTRACTION OF GUINEA-PIG ILEUM

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Studies on the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum have shown that the neuronally mediated component of the contractile response to 5-HT is biphasic (Buchheit et al., 1985). The first phase of the response to low concentrations of 5-HT has been shown to be insensitive to ICS205-930 (up to 1μM), whereas the response to high concentrations of 5-HT involved 5-HT_3 receptors since it was antagonised by ICS205-930. The aim of the present study was to make a preliminary pharmacological characterisation of the first phase of the concentration-response curve to 5-HT.

Strips of the longitudinal muscle-myenteric plexus preparation of guinea-pig ileum were mounted in Krebs-Henseleit solution gassed with 95%O_2/5%CO_2 and containing ondansetron (GR38032F; 10μM), spiperone(1μM) and eserine(0.01μM). Agonists were added to the tissues sequentially at 15 minute intervals with a 2 min contact time. Antagonists were equilibrated with the tissues for 30 minutes.

In the presence of spiperone and ondansetron to antagonise 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors, 5-HT produced monophasic contractile responses with an EC_{50} value of 50(11-210)nM (95% confidence limits,n=4). This response was completely blocked by tetrodotoxin(0.3μM) and atropine(1μM) indicating a neuronal, cholinergically mediated effect. Several indole and benzamide compounds were tested as agonists and their effects compared with that of 5-HT (see table).

Compound	Equipotent molar ratio (5-HT = 1)	% 5-HT maximum	Compound	Equipotent molar ratio (5-HT = 1)	% 5-HT maximum
5-Hydroxytryptamine	1	100	BRL24924	26(10-65)	69±21
N-Methyl-5-HT	7.5 (3-18)	81±8	Zacopride	~1600	54±11
5-Methoxytryptamine	86(12-572)	70±9	Cisapride	-	30±6 at 10μM
α-Methyl-5-HT	95(20-432)	80±16	Metoclopramide	-	25±12 at 30μM

In addition, the following compounds were inactive both as agonists and antagonists up to a concentration of 100μM; 2-methyl-5-HT, 5-carboxamidotryptamine and 5-hydroxyindalpine.

Since metoclopramide and cisapride appeared to be weak partial agonists they were tested as antagonists and produced an unsurmountable inhibition of the 5-HT-induced responses with pD_2' values of 5.0 and 7.2 respectively. The responses to dimethylphenylpiperazine(4μM) were not inhibited by high concentrations of metoclopramide or cisapride (10μM and 1μM respectively). In summary, the present study confirms the presence of a 5-HT receptor in guinea-pig ileum which cannot be designated 5-HT_1-like, 5-HT_2 or 5-HT_3, and which can be stimulated by various benzamides.

Buchheit KH, Engel G, Muschler E, Richardson B, (1985) N-S Arch Pharmacol., 319, 231-238.

183P REGIONAL SENSITIVITY OF HUMAN COLON TO NEUROHUMORAL AGENTS

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In vitro studies using human colonic muscle strips have indicated regional heterogeneity in colonic function namely in the mechanical properties of the smooth muscle (Gill et al, 1986; Snape et al, 1988). The aim of the present investigation was to determine whether this regional heterogeneity extended to smooth muscle responsiveness to neurohumoral agents.

Circular (CM) and longitudinal (LM, i.e. taenia) smooth muscle strips of human ascending (proximal) and sigmoid (distal) colon were obtained from specimens resected for tumours. The strips were suspended under a tension of 1 g in 1.5 ml organ baths and superfused with Kreb's bicarbonate buffer. Cumulative dose response curves were obtained to acetylcholine (ACh), noradrenaline (NA) and vasoactive intestinal peptide (VIP), for NA and VIP these were carried out in the presence of bethanechol (2 μM). Potency comparisons were made by calculating mean ED_{50} values from log dose-response curves to the agonists.

The sensitivity of both muscle layers to ACh and NA was independent of the region of origin. However for VIP sigmoid CM was more sensitive than ascending CM (Table 1), also there was a marked difference in sensitivity to VIP between CM and LM. Thus at the highest concentration tested VIP (3 μM) only caused 5.6 ± 1.4%(n = 9) and 15.4 ± 2.1%(n = 5) of a maximal relaxation to NA on ascending and sigmoid LM compared to values of 89.8 ± 14.9%(n = 9) and 98.9 ± 3.9%(n = 8) for ascending and sigmoid CM.

Table 1: Relative sensitivities (ED_{50}, μM) of ascending and sigmoid colonic muscle layers to ACh, NA & VIP.

	CIRCULAR		LONGITUDINAL	
	Ascending	Sigmoid	Ascending	Sigmoid
ACh	0.20 ± 0.06	0.23 ± 0.05	0.31 ± 0.09	0.42 ± 0.08
NA	0.30 ± 0.05	0.28 ± 0.05	0.23 ± 0.05	0.35 ± 0.07
VIP	1.00 ± 0.16	0.32 ± 0.10*	n/a	n/a

Values are mean ± sem of minimum 10 strips, * = $P < 0.05$.
For VIP on LM, ED_{50} value not applicable (n/a) as this value not achieved in dose range tested.
The feeble slow response of LM to VIP is evidence against an inhibitory neurotransmitter role for VIP at this site.

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Snape, W.J., Mayer, E.A., Keolbel, C., Hyman, P.E. & Williams, R. (1988). Gastroenterology 94, A434.

184P COMPARISON OF ENDOTHELIN-1 RESPONSES IN RAT AND GUINEA-PIG TRACHEA AND THEIR MODULATION BY PEPTIDASE INHIBITORS

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Endothelin-1 (ET-1), has been shown to be a potent vasoconstrictor (Yanagisawa *et al.*, 1988) and there are indications that this 21 amino acid peptide contracts non-vascular smooth muscle including respiratory tissue. We have examined the potency of ET-1 on isolated trachea from rat and guinea pig and studied the effects of peptidase inhibition on the activity of this peptide. Tracheal chains consisting of four adjacent rings of trachea from the upper and lower portions of the tract were prepared from male Wistar albino rats (200-250g) and Dunkin-Hartley guinea-pigs (250-300g). Tissues were mounted in 3ml siliconized organ baths containing Krebs solution at 37°C, gassed with 95% O_2, 5% CO_2 and isometric contractions were recorded via a FT03 transducer linked to a Grass polygraph. Cumulative dose response curves to carbachol (0.1 - 100μM) and ET-1 (0.01 - 10μM) were carried out in both the absence and presence of concentrations of peptidase inhibitors known to modulate tachykinin responses.

In the rat trachea ET-1 was a potent constrictor with equivalent activity in both the upper and lower portions of the tract. In guinea-pig trachea, responses to ET-1 were variable and could be divided into three qualitatively distinct responses: (i) normal dose response curves with EC_{50}s in both upper and lower portions of 1-2μM, (ii) flat dose response curves reaching a plateau at 1μM and generating tensions only 25-30% of that induced by carbachol; in these tissues ET-1 produced transient relaxations followed by contraction, and (iii) atypical dose response curves with initial responses to 0.01μM ET-1 >50% of the carbachol response. Prior incubation of the tissues (30 min) with bestatin, phosphoramidon, captopril or thiorphan (10μM) did not potentiate the response of the rat or guinea-pig tracheal muscle to ET-1.

Comparison of the effects of ET-1 and carbachol in rat and guinea-pig tracheal chains. Values are means ± s.e. mean n = 4-12.

		ENDOTHELIN-1		CARBACHOL	
		EC50 (nM)	Tension (g) induced by 10μM	EC50 (nM)	Tension (g) induced by 100μM
RAT	Upper	315 ± 69	0.20 ± 0.049	728 ± 76	0.23 ± 0.042
	Lower	269 ± 67	0.26 ± 0.064	643 ± 110	0.24 ± 0.058
GUINEA-PIG	Upper	975 ± 286	0.53 ± 0.069	555 ± 67	0.75 ± 0.052
	Lower	1813 ± 713	0.41 ± 0.068	607 ± 85	0.63 ± 0.069

In conclusion, ET-1 exhibits consistent contractile responses in rat trachea. In contrast, responses of guinea-pig trachea to ET-1 are variable and less potent than originally reported for this species (Uchida *et al.*, 1988). The peptide inhibitors bestatin, phosphoramidon, captopril or thiorphan do not potentiate ET-1 responses in tracheal muscle from either rat or guinea-pig.

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Yanagisawa, M.,*et al.* (1988) *Nature*, **332**, 411-415.

185P EXAMINATION OF THE EFFECT OF ADENOSINE ON SUPERFUSED TRACHEA FROM NORMAL AND SENSITISED GUINEA-PIGS

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Bronchoconstrictor responses to adenosine (ADO) have been reported in asthmatic patients (Cushley et al. 1983) and in airway perfused lungs from sensitised guinea-pigs (Thorne & Broadley, 1988). The effects of ADO on the isolated guinea-pig trachea, however, are less conclusive since both contractions (Satchell & Smith, 1984) and relaxations (Jones et al. 1980) occur. The present study investigates ADO on the superfused trachea in an attempt to clarify the results from more classical immersion techniques.

Isolated tracheal spirals (3-4cm) from guinea-pigs (400-550g), under a resting tension of 1g, were either immersed in a 15ml organ bath or lowered into a heated jacket (37.5°C) for superfusion. Warmed (37.5°C) and gassed (5% CO_2 in oxygen) Krebs-bicarbonate solution bathed and superfused (5ml/min) the tissues, respectively. Agonists were added to the organ bath either cumulatively or as single boluses, but only as boluses to the superfused tissue. Tissues were exposed to antagonists for 30 min before and then throughout the addition of agonists. The animals were sensitised by i.p. injections of ovalbumen (OA, 5mg in 0.1ml of water for injections) 14 and 12 days (10mg) before killing.

ADO induced concentration-dependent relaxations in immersed preparations with no significant difference between untreated and sensitized tissues. In the superfused tissue, ADO induced a dose-dependent relaxation in normal tissues (100µg, 100±23.7mg) but a contraction at low doses in sensitised tissues (70.0±25.0mg by 100µg). 7.5µM and 100µg boluses of ADO induced small contractions in immersed (30.5±32.9mg) and superfused (67.2±65.0mg) preparations from sensitised tissues. When repeated, however, ADO caused relaxations (116±43.1mg in immersed and 62.4±37.0mg in superfused). Threshold doses of antigen (70ng/ml and 500ng) contracted in both (90.0±21.1mg and 160.0±58.0mg) preparations. 8-Phenyltheophylline (8PT, 3.9µM) significantly antagonised the responses of immersed trachea to ADO, shifting the concentration-response curve to the right by two fold (EC30 from 177 (97.1-322.8) to 380 (290.7-496.8µM). In superfused tissue there was a trend towards a greater ADO-induced contraction with dose-response curves in the presence of 8PT (at 300µg, 90.9±37.7mg compared with -13.0±50.6mg). The contraction to a single bolus dose of ADO (100g) was not significantly affected by 8PT. Upon repeated exposure, ADO still induced a contraction in the presence of 8PT (80.0±9.2mg) compared with a relaxation in untreated tissues. 8PT had no significant effect on the response to OA.

These results suggest that the superfused trachea preparation may be a suitable model to separate and therefore investigate the two opposing components of the response to ADO in the guinea-pig trachea.

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Satchell, D. & Smith, R. (1984) Eur. J. Pharmac. 101, 243
Thorne, J.R. & Broadley, K.J. (1988) Br. J. Pharmac. 93, 278P

186P RUTHENIUM RED: SELECTIVE, REVERSIBLE INHIBITION OF CAPSAICIN-STIMULATED SUBSTANCE P RELEASE FROM PRIMARY AFFERENT NEURONS IN RAT TRACHEA

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Capsaicin predominantly excites primary afferent C-fibres with concomitant release of neuropeptides, including substance P (SP), from both central and peripheral branches (Holzer, 1988). The inorganic dye, ruthenium red (RR), which inhibits transmembrane calcium fluxes (Tapia et al., 1985), has been demonstrated to inhibit capsaicin (Caps)-stimulated SP-like immunoreactivity (LI) from guinea-pig bladder in vitro (Maggi et al., 1988). We have utilized a multi-superfusion system to investigate the effect of RR on Caps-stimulated release of SP-LI from rat trachea in vitro and have further characterized its actions on the release of SP-LI to several other stimulants.

Ten spirally cut trachea from male rats (Wistar 350-400g) were mounted in parallel oxygenated glass chambers and superfused with Krebs' solution (pH 7.4, 37°C gassed with 95% O_2/5% CO_2) at 5 ml/min. to allow equilibration. After 60 min. phosphoramidon (1 µM), captopril (100 µM), and bacitracin (20 mg/l) were added to the Krebs' soln. and the flow rate adjusted to 1 ml/min. After a further 15 min. four fractions of 5 min. from each trachea were simultaneously collected in vials containing a final concentration of 0.1% trifluoroacetic acid. Fractions were then concentrated on Sep-Pak C_{18} cartridges, lyophilised and reconstituted in barbitone buffer (pH 8.6) for radioimmunoassay (RIA) of SP-LI. Drugs were added to the superfusion fluid reservoir for 4 min. during the third fraction. None of the agents used interfered with the RIA.

We have previously reported that Caps (50 mg/kg s.c.), administered neonatally caused a 93.2 ± 6.4% reduction in tracheal SP-LI content and that isosmolar high potassium (K^+, 37-60 mM), Caps (1-100 µM), veratridine (Ver, 10-50 µM) and bradykinin (BK, 0.01-1 µM) caused a dose-related release of SP-LI (Ray et al., 1989).

Removal of calcium ions (Ca^{2+}) from the superfusion fluid reservoir, with addition of 1 mM EGTA, abolished the release of SP-LI to 60 mM K^+, Caps (1 µM) and Ver (25 µM) but had no apparent effect on spontaneous release. RR (0.1-30 µM), when present throughout the experiment, did not affect spontaneous SP-LI release but caused dose-related, reversible inhibition of Caps (1 µM)-stimulated SP-LI release which was complete at 30 µM (IC_{50} = 0.67 µM). Under identical conditions, RR did not significantly affect SP-LI release to 60mM K^+, 25 µM Ver or 1 µM BK.

We conclude that RR does not inhibit transmembrane Ca^{2+} fluxes to all stimulants but that the antagonism of caps-stimulated SP-LI release from primary afferent neurons may be a universal phenomenon.

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187P LACK OF AN EFFECT OF PEPTIDASES ON VASCULAR RESPONSES TO EPITHELIUM-DERIVED INHIBITORY FACTOR

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We have previously demonstrated the release of an inhibitory factor from rabbit intrapulmonary bronchi that is neither a prostanoid nor endothelium-derived relaxing factor (Spina & Page, 1989). To further investigate the nature of this inhibitory factor we have examined the effects of various peptidases and peptidase inhibitors on the relaxant response of vascular smooth muscle to epithelium-derived inhibitory factor (EpDIF) generated from rabbit intrapulmonary bronchi by methacholine.

Male Wistar rats (200-300g) were stunned by a blow to the head and killed by cervical dislocation. The aorta was removed and denuded of endothelium using a cotton wool swab and cut into zig-zag strips and suspended under 500mg tension in an organ bath containing Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 at 37°C. Methacholine (100μM) failed to relax phenylephrine-precontracted aorta, confirming the absence of an intact endothelium.

Male New Zealand white rabbits (1.8-3.0kg) were anaesthetised with Valium (5mg/kg) and Hypnorm (0.4ml/kg), then exsanguinated. Intrapulmonary bronchial tubes (7mm) were excised from the lung, free of alveolar tissue and visible blood vessels. Endothelium-denuded rat aortic preparations were then resuspended inside the rabbit intrapulmonary bronchial tubes. The aortic preparations within the coaxial bioassay assembly were contracted with phenylephrine (10μM) and relaxed by the cumulative addition of increasing concentrations of methacholine in the absence or presence of α-Chymotrypsin (2 units/ml; 10 min), papain (2 units/ml; 10 min), aprotinin (10μM; 30 min), thiorphan(10μM; 30 min) or captopril (10μM; 30 min).

The relaxant potency ($pD_2 = -\log_{10} EC_{50}$) of methacholine was not altered in the presence of the peptidases α-chymotrypsin (absence : 5.92 ± 0.14 vs presence : 5.92 ± 0.12, n = 6, P>0.05, paired t-test) or papain (5.95 ± 0.15 vs 5.92 ± 0.14, n = 7, P>0.05). Furthermore the peptidase inhibitors aprotinin (5.75 ± 0.09 vs 5.68 ± 0.10, n = 8, P>0.05), thiorphan (5.68 ± 0.12 vs 5.90 ± 0.06, n = 7, P>0.05) and captopril (5.51 ± 0.10 vs 5.45 ± 0.06, n = 7, P>0.05) failed to potentiate the epithelial-dependent relaxation induced by methacholine.

These results indicate that EpDIF is not a substrate for various peptidases and is thus unlikely to be a vasodilator peptide.

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Spina, D. & Page, C.P. (1989) Br. J. Pharmac. 97, 424P.

188P INHIBITION OF GUINEA-PIG ALVEOLAR MACROPHAGE SUPEROXIDE ANION GENERATION BY AZELASTINE

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The involvement of alveolar macrophages (AM) in the pathogenesis of hyperreactive airway disease has recently found much attention (reviewed by Rankin, 1989). There is growing evidence that AM of asthmatic patients exhibit an increased generation of oxygen-derived radicals (Cluzel et al., 1987). We have studied, therefore, the effect of azelastine, an orally effective antiasthmatic/antiallergic drug, on the generation of superoxid anion ($^{\cdot}O_2^-$) by guinea pig AM after stimulation. The AM were stimulated by either the allsurface receptor-independent, soluble phorbolester phorbol myristate acetate (PMA) or by the allsurface receptor-dependent particle zymosan. $^{\cdot}O_2^-$-generation was measured by the sensitive and selective lucigenin chemiluminescence assay (Gyllenhammar, 1987). Two kinds of AM were investigated in this study. They were different with regard to their activation status. The one population were resident AM cultered under serum-free conditions over 20 hours. The other population consisted of primed AM. Priming of AM was achieved in vitro by incubation in medium containing fetal calf serum (Hayakawa et al., 1989). There were different chemiluminescence patterns observed in dependence of the stimulus used and cell priming status. The primed AM exhibited a significantly increased $^{\cdot}O_2^-$-generation after PMA-stimulation but not after zymosan-stimulation. PMA-induced $^{\cdot}O_2^-$-generation was inhibited by azelastine in a dose dependent manner (13.9 %, 21.9 %, and 30.1 % inhibition of the peak chemiluminescence for 1 μM, 5 μM, and 10 μM respectively). The time course of the chemiluminescence response was not affected. An inhibition by azelastine was also observed after zymosan stimulation (12.3 %, 15.7 %, and 21.7 % for 1 μM, 5 μM, and 10 μM respectively). Furthermore we could demonstrate, that after PMA stimulation $^{\cdot}O_2^-$-generation of primed AM were significantly more inhibited than of unprimed AM (16.4 %, 36.7 %, and 49.0 % for 1 μM, 5 μM, and 10 μM respectively). These observations with AM are in agreement with the results recently published by Busse et al. (1989), who found an inhibitory effect of azelastine on neutrophil and eosinophil $^{\cdot}O_2^-$-generation. Furthermore we demonstrated that azelastine reduces the $^{\cdot}O_2^-$-generation in primed AM to a greater extent than in unprimed AM and shifts the metabolism of activated AM (probably activated in vivo by a inflammation process) to more moderate levels in this way. It is likely that this property of azelastine may play an important role in the complex mode of its action. Modulating even the functions of irritated cells might be in part responsible for the beneficial effects in the management of asthmatic patients.

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189P COMPARATIVE REGIONAL DISTRIBUTION OF LIPID MEDIATOR METABOLISNG ENZYMES IN BOVINE LUNG

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In addition to releasing their own lipid mediators, airway epithelial cells are thought to be exposed to local hormones released from other inflammatory cells (Holtzman et al., 1988). It has been suggested that the airways have a low capacity to metabolise lipid mediators such as prostaglandins (Yen et al., 1976; Holtzman et al., 1988), but this has been the subject of only limited systematic study. In particular there is no information regarding the airways epithelium which is considered to be the first barrier exposed to mediators generated and released in the airway lumen. We now describe investigations of the regional activities in the lung of 3 enzymes involved in prostaglandin (PG) metabolism.

Experiments were performed using cytosolic 100 000 g supernatants prepared from bovine tracheal and bronchial mucosa and also lung parenchyma. Radioenzymatic assays were performed as described (Hoult et al., 1988) using $PGF_{2\alpha}$ as substrate for 15-hydroxyprostaglandin dehydrogenase (PGDH; E.C. 1.1.1.141), PGD_2 for 11-ketoreductase (11-KR; E.C. 1.1.1.188) and PGE_2 for 9-ketoreductase (9-KR; E.C. 1.1.1.189). Starting prostanoid substrate concentrations were 2 $\mu g\ ml^{-1}$ and analyses were performed by radio-h.p.l.c. (Hoult et al., 1988). The extent of enzymatic conversion measured after 60 min incubation at 37°C is shown in Table 1. Three separate experiments were performed for each enzyme assay.

Table 1.

	product formation (pmol mg^{-1} protein)		
	parenchyma	bronchial mucosa	tracheal mucosa
PGDH*	231.3 ± 83.3	0 ± 0	0 ± 0
11-KR	826.4 ± 129.9	123.3 ± 20.5	140.8 ± 63.0
9-KR	0 ± 0	-	0 ± 0

*Measurement includes products formed by Δ^{13}-reductase.

These data show that in contrast to lung parenchyma, the airways mucosa does not exhibit PGDH activity, despite being a source of PGE_2 which is a potential substrate for this enzyme (Herbert & Robinson, unpublished). Interestingly, PGD_2 11-KR showed modest activity in the mucosal tissues of the trachea and bronchus. It is possible that the mucosal localization of this enzyme is related to the presence of mast cells (a major source of PGD_2) beneath the basement membrane and in the lumen of the airways.

CAH is a SERC CASE scholar in collaboration with Eli Lilly.

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190P HYPEREOSINOPHILIA AND BRONCHIAL REACTIVITY IN THE RAT

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The role of the eosinophil in allergic reactions is not fully defined. Indeed, allergic subjects may develop peripheral eosinophilia, but the variability of this finding precludes the establishment of its precise contribution in the manifestations of bronchial hyperreactivity. To precise this phenomenon, rats were made hypereosinophilic and their bronchial reactivity was compared to that of control animals.
Male Sprague Dawley rats were injected with 100 mg/kg of cyclophosphamide, intraperitoneally (Thomson et al., 1986). Two weeks later, blood parameters, infiltration of eosinophils into the lung tissue and bronchial reactivity to intravenous serotonin were determined. For the assessement of bronchial reactivity, rats were anaesthetized with ethylcarbamate and ventilated (80 breaths/min, 1 ml air/100 g body weight/breath). Responses to cumulated injections of serotonin (1 to 500 µg/kg, i.v.) was assessed after 30 min. Carotid artery was cannulated for the determination of the differential and absolute blood cell counts with a Technicon H-1 system. Lung fragments were fixed in 10 % formaldehyde prior to processing and inclusion in paraffin. Then, the specimens were cut and stained with Biebrich's scarlet to identify the eosinophils.
Rats treated with cyclophosphamide presented a decrease of the number of erythrocytes (7.5 ± 0.1 x $10^6/\mu l$ vs 8.2 ± 0.2 x $10^6/\mu l$ in control rats, $p< 0.05$), an increase of platelets (1284 ± 81 x $10^3/\mu l$ vs 1081 ± 42 x $10^3/\mu l$, $p< 0.05$) and blood eosinophils (0.69 ± 0.18 x $10^3/\mu l$ vs 0.14 ± 0.03 x $10^3/\mu l$, $p< 0.01$). However, no modification of the number of neutrophils and monocytes was observed. In cyclophosphamide-treated rats, the number of eosinophils infiltrated into the lung tissue was also markedly increased (173.9 ± 59.2 cells/mm^2 vs 28.1 ± 4.3 cells/mm^2 in control rats, $p< 0.05$). Furthermore, there was a significant correlation ($p< 0.001$) between the peripheral eosinophils and the number of these cells into the lung tissue. Intravenous serotonin produced a dose-dependent bronchoconstrictor response in control rats. Despites the marked hypereosinophilia noted in cyclophosphamide-treated rats, no alteration of the bronchial reactivity to serotonin was noted.
These results suggest that the activation of eosinophils, rather than the their mere presence in blood and lung tissue is critical for the development of bronchial hyperreactivity.

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191P OZONE INDUCES BRONCHIAL HYPERREACTIVITY TO INHALED SUBSTANCE P BY FUNCTIONAL INHIBITION OF ENKEPHALINASE

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Exposure of guinea-pigs to ozone (0_3) induces bronchial hyperreactivity (BHR) to a wide range of inhaled spasmogens (Lew, et al., 1985; Yeadon & Payne, 1989a, b), but the mechanisms underlying this effect are unclear. We now report that ozone exposure results in a functional loss of enkephalinase which is responsible for subsequent BHR to inhaled substance P (SP).
Male Dunkin-Hartley guinea-pigs (450-550g) were exposed during quiet tidal breathing to 0_3 (3 ± 0.5ppm) for either 30 min or 2h; control animals breathed laboratory air. Under pentobarbitone (60mg/kg i.p.) anaesthesia, the trachea was cannulated and the animal artifically ventilated. Aerosols were generated by a nebuliser in the afferent arm of the ventilator circuit, and changes in pulmonary inflation pressure (PIP) were measured from a side-arm in the tracheal cannula. Sensitivity to inhaled SP was assessed by nebulising aqueous solutions of SP (0.04 - 3000µg/ml) for 10s and measuring the increase in PIP. The SP concentration provoking a 20cmH_2O rise in PIP (PC_{20}) was determined by interpolation. The SP PC_{20} (µg/ml) in control animals was 1011 ± 260 (n=7) but after 0_3 (30 min or 2h) this fell to 17.2 ± 10.9 (n=4) and 5.6 ± 2.1 (n=6) respectively, the latter representing a 180-fold increase in bronchial reactivity ($P<0.01$). Pretreatment with the antioxidant ascorbic acid (1g/kg i.p., 1h) abolished the fall in SP PC_{20} produced by 30 min 0_3, suggesting that the BHR to SP induced by 0_3 was mediated through oxidant damage.
In a separate set of control animals, threshold airway responses were obtained using a 10s nebulisation of SP (100µg/ml). The enkephalinase inhibitors (EI) phosphoramidon, thiorphan and bestatin (1, 5 and 5mg/kg, respectively) were administered i.v. and 5 min later, the aerosol challenge with SP was repeated. The rise in PIP provoked by SP was significantly enhanced from 2.1 ± 0.3 to 40.3 ± 1.1cmH_2O ($p<0.001$, n=4) by EI. However, following 0_3 (2h) exposure threshold bronchoconstrictor responses to SP after EI were not significantly different from those in corresponding vehicle controls. These data suggest that the BHR to SP aerosol after 0_3 in guinea-pigs is accounted for by an oxidant-mediated functional inhibition of enkephalinase activity in the lung. This mechanism may have wider implications for the general phenomenon of BHR.

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192P ASSESSMENT OF BRONCHODILATOR EFFECT OF ROLIPRAM IN THE ANAESTHETISED CAT

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Rolipram (Schering ZK 62711) is an inhibitor of human cardiac ventricle type IV cyclic nucleotide phosphodiesterase (PDE); (Reeves *et al* 1987). Rolipram has also been reported to inhibit PDE from canine cardiac, vascular and tracheal smooth muscle (Silver *et al*, 1988), being relatively more potent in tracheal smooth muscle.

This study examined the effects of rolipram upon 5-HT-induced bronchospasm in anaesthetised cats. Animals were instrumented for measurement of airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}), using a Buxco pulmonary mechanics analyser. Intravenous infusion of 5-HT produced an increase in R_{aw} of 418 ± 37% (mean ± s.e.mean, n=6), from a resting value of 4.0 ± 0.5 cm H_2O ml^{-1} s^{-1}, and a decrease in C_{dyn} of 28 ± 5% from a resting value of 8.8 ± 0.9 ml cm H_2O^{-1}. When a stable level of bronchoconstriction had been attained, 15 breaths of a 1mg ml^{-1} rolipram solution was administered from a deVilbiss ultrasonic nebuliser.

Rolipram produced a partial reversal of 5-HT bronchospasm , giving a peak 52 ± 3% (n=5) reversal of the 5-HT-induced increase in R_{aw}, and a 24 ± 8% (n=5) reversal of the decrease in C_{dyn}. Onset of bronchodilatation was rapid, within 2-3 breaths from the start of nebulisation (4-6s). In the face of continuing 5-HT infusion, bronchoconstriction returned to pre-rolipram levels within 45s.-5min. Coincident with these bronchodilator effects , rolipram induced a consistent positive chronotropic response, and depressed the incomplete tetanic contractions of the soleus muscle. All these effects of rolipram were quantitatively similar in two animals which had been subjected to bilateral adrenal ligation. In contrast, however, in one of the six animals rolipram was without any bronchodilator effect.

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193P BRONCHODILATOR POTENCY, EFFECTIVENESS AND TIME COURSE OF INHALED NEBULISED AH 21-132 IN NORMAL HUMAN SUBJECTS

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AH 21-132 is a benzo-[c][1,6]naphthyridine derivative possessed of two sets of properties that offer hope of effectiveness as an anti-asthmatic agent. In animal models it is a smooth muscle relaxant showing selectivity for airway smooth muscle (Small et al., 1989). It also interferes in the processes thought to underlie bronchial hyper-reactivity (Kristersson et al., 1988). We have assessed the bronchodilator activity of inhaled nebulised AH 21-132 (in a sucrose excipient) in a saline vehicle in 12 normal volunteers (11 male; aged 21-58, median 23.5; weight 75.6 ± 2.6 kg [mean ± s.e.m.]) against a background of maintained bronchoconstriction, induced by inhaled nebulised methacholine (Foster & Atanga, 1988). Each subject's methacholine dosage regimen was individualised, from the information contained within 3 ln dose effect curves plus offset time effect curves, aiming for a maintained 67-75% reduction in baseline specific airway conductance (sG_{aw}, units $(s.cm\ H_2O)^{-1}$).

Three experiments were performed on each subject 1) time course of effect of AH 21-132 2) cumulative ln dose effect curve to AH 21-132 with time course of offset 3) no drug (vehicle) control. The pooled baseline $\log_{10}\ sG_{aw}$ = -0.717 ± 0.013. The loading dose of methacholine (2.1 [1.7, 2.7 mean - s.e.m., mean + s.e.m.] mg) reduced $\log_{10}\ sG_{aw}$ to -1.136 ± 0.020 (62% reduction in baseline sG_{aw}) and in the no drug control experiment the maintenance dose rate of 0.025 ± 0.002 of the loading dose min^{-1} produced a $\log_{10}\ sG_{aw}$ of -1.179 {-1.158, -1.120 95% C.L.} (66% reduction in baseline sG_{aw}) with a slope of 0.000357 ± 0.000275 min^{-1} (R^2 0.02). Against this background inhalation of AH 21-132 12 mg over 8 min produced bronchodilatation to -0.959 ± 0.032, an effect which was fully developed within 13 min. Inhalation of AH 21-132 2, 8 & 32 mg (cumulative doses) at 30 min separation produced bronchodilatation to -1.111 ± 0.028, -0.983 ± 0.039 & -0.797 ± 0.031 respectively. The slope of this ln dose effect curve was 0.1132 ± 0.0176 (R^2 0.55). The ED50 was 9.2 {6.4, 13.6} mg. The slope of the offset time effect curve was -0.00355 ± 0.00036 (R^2 0.54). The $t_{\frac{1}{2}}$ of the drug at its site of action after inhalation ≈ 25 min.

We thank Sandoz Ltd. for the supply of AH 21-132 and financial support & Dr T.B. Stretton, Manchester Royal Infirmary, for the use of pulmonary function laboratory facilities.

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194P POTASSIUM CHANNEL BLOCKADE: EFFECTS ON CROMAKALIM-INDUCED RELAXATION OF THE GUINEA-PIG TRACHEAL CHAIN

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The K^+-channel activator, cromakalim (CROM) has been previously shown to inhibit or relax the spontaneous tone of a variety of isolated tissues including guinea-pig trachealis (Allen et al., 1986). Moreover, the CROM-induced efflux of $^{86}Rb^+$ in this tissue is much lower than would be predicted from studies in other tissues such as the portal vein (Foster et al., 1989). It was decided therefore, to investigate the effects of a variety of K^+-channel blockers on the relaxant effects of CROM in the guinea-pig isolated tracheal chain in an attempt to further characterise the K^+-channels in this preparation.

Tracheal rings were prepared and set up in Krebs solution (37°C, 95% O_2/5% CO_2) under a resting load of 200mg for isotonic recording of length changes and left to generate spontaneous tone. Concentration-responses to CROM were carried out (0.1-100uM) and compared to the maximal relaxation produced by isoprenaline (1uM). The effects of glibenclamide (GLIB 1-10uM), procaine (PRO 0.1-10mM), tetraethylammonium (TEA 0.1-10mM), apamin (APA 0.1uM) and phentolamine (PHEN 10uM) were assessed against the relaxation produced by CROM.

CROM produced concentration-related relaxation of the tissue (EC_{50} 2.73±0.52μM) and was antagonised by GLIB and PHEN (concentration-ratios 8.89±2.36 & 22.33±7.06 at 1 and 10 μM respectively). The rightward shift produced by GLIB appeared to be parallel whereas that produced by PHEN was not. GLIB at concentrations above 5uM produced graded relaxation of the tissues. This effect was insensitive to either nitrendipine or propranolol (0.5 & 1uM respectively). PRO, TEA (0.1mM) & APA (0.1uM) caused no significant antagonism of the CROM-induced responses. Indeed, PRO and TEA produced marked contraction followed by supramaximal relaxation of the tissues at concentrations above 0.1mM, making antagonist activity difficult to interpret.

The antagonism of CROM by GLIB and PHEN in vascular tissues has been previously reported by Wilson (1989) and McPherson & Angus (1989) respectively and the results obtained in these current studies are in agreement with these previous findings. However, these current results have revealed differences in the way K^+-channels are operated in the guinea-pig trachea and lead us to conclude that either GLIB possesses true partial agonist activity at a K^+-channel or that it activates some mechanism peculiar to trachea.

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195P EFFECT OF CROMAKALIM ON REGIONAL BLOOD FLOW THROUGH THE KIDNEY

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The kidney has two vascular beds; the cortex, made up of afferent and efferent arterioles, glomerular and peritubular capillaries; and the papilla, in which the efferent arterioles give rise to the vasa recta. The aim of this study was to examine whether cromakalim, the potassium channel activator (Hamilton & Weston, 1989), had any differential effect on the perfusion of these two regions.

Male Sprague-Dawley rats (110-140 g) were anaesthetised with sodium pentobarbitone ip. The right carotid artery was cannulated for blood pressure measurements and the left jugular vein for administration of saline (2 ml/h) and drugs. The kidney was exposed, placed dorsal side up in a moulded cup and the papilla displayed. Cortical and papillary perfusions (red cell flux) was measured using a Periflux PF3 laser-Doppler flowmeter with a PF303 probe. Flow signals were recorded for 10 s from 10 positions on the cortex, while a single reading of 1 min was taken from the papilla. Cromakalim was given, as bolus doses, and measurements taken 15 min later.

	BP	LDU_C	LDU_P	C/P	R_C	R_P
Control	100 ± 2	240 ± 9	204 ± 20	1.25 ± 0.14	0.42 ± 0.03	0.52 ±0.06
50 μg/kg	93 ± 3	221 ± 12	206 ± 14	1.12 ± 0.12	0.43 ± 0.02	0.47 ± 0.04
100 μg/kg	88 ± 3[1]	210 ± 16	206 ± 12	1.06 ± 0.13	0.43 ± 0.03	0.44 ± 0.03
300 μg/kg	71 ± 5[3]	175 ± 18[3]	196 ± 12	0.91 ± 0.11	0.42 ± 0.03	0.38 ± 0.03[1]
500 μg/kg	64 ± 4[3]	158 ± 20[3]	197 ± 14	0.83 ± 0.12[1]	0.44 ± 0.05	0.34 ± 0.03[1]

BP = blood pressure, mmHg; LDU_C - laser-Doppler flux units, cortex; LDU_P = laser-Doppler flux units, papilla; C/P = cortex to papillary ratio; R_C = resistance in cortex taken as BP/LDU_C; R_P = resistance in papilla, taken as BP/LDU_P. [1] = $P<0.05$; [2] = $P<0.01$; [3] = $P<0.001$ (Student's 't' test). Number of animals = 6.

The marked suppression of blood pressure caused by cromakalim was probably due to its hyperpolarising action on vascular smooth muscle. Cortical perfusion appeared relatively maintained to 88 mmHg, suggesting autoregulation, but below this blood pressure, cortical perfusion fell markedly. However, perfusion of the papilla was well maintained, with a fall in resistance occurring as blood pressure was reduced over the whole range. Whether this fall in resistance was a myogenic response to pressure reduction or a direct action of the cromakalim on the papillary vasculature remains to be determined.

Cromakalim and funding were generously provided by Beecham.

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196P CROMAKALIM INCREASES THE MEMBRANE PERMEABILITY OF FROG SKELETAL MUSCLE *IN VITRO*

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Cromakalim belongs to the newly recognized class of drugs referred to as 'potassium channel openers', which increase potassium conductance in smooth muscle (as well as other tissues) leading to membrane hyperpolarization and inhibition of contractile activity. Various findings, for example, inhibition of cromakalim's action in smooth muscle by sulphonylureas (e.g. Quast & Cook, 1989), now suggest that it is the ATP-sensitive K^+ channel which is opened by cromakalim. Skeletal muscle also possesses ATP-sensitive K^+ channels (Spruce *et al.*, 1985) which may be activated by metabolic exhaustion (Castle & Haylett, 1987). The present study examines the ability of cromakalim to increase K^+ permeability in frog skeletal muscle.

Experiments were performed on sartorius muscles of *Rana temporaria* at room temperature (20-25°C). Muscles were loaded for 90 min in frog Ringer containing $^{86}Rb^+$ (as a tracer for K^+) and efflux into non-radioactive Ringer was followed. Cromakalim (30-300μM) applied for five 2 min periods, after washout for 40 min caused an increase in $^{86}Rb^+$ efflux. With 100μM the increase was 88±12% (mean±s.e.m.; n=30) and with 300μM was 172±40% (n=5). The response was potently inhibited by glibenclamide, the response to 100μM cromakalim being blocked with an IC_{50} between 10 and 30 nM. (Diazoxide which increases the opening of ATP-sensitive K^+ channels in pancreatic β-cells, had no clear effect on $^{86}Rb^+$ efflux at 600μM (n=3)).

The action of cromakalim was further explored using intracellular recording. In Ringer solution in which Cl^- had been replaced by isethionate, 200μM cromakalim produced a clear hyperpolarization (by up to 10mV) and an accompanying reduction in the input resistance of about 50% (as measured by current injection from a second microelectrode). These effects could also be seen in normal Ringer but were smaller, presumably because of the shunting effect of the Cl^- conductance. The effects of cromakalim on membrane potential and conductance were inhibited by both glibenclamide (1μM) and tolbutamide (300μM).

Further studies are needed to see whether these actions of cromakalim can indeed be attributed to an action on ATP-sensitive K^+ channels. It may be noted that the potency of glibenclamide in the present work was considerably greater (100X) than found in the studies with poisoned muscle (Castle & Haylett, 1987). In parallel studies Spuler *et al.* (1989) have shown that cromakalim can increase K^+ conductance in human skeletal muscle.

Supported by the Wellcome Trust. We thank Beecham Research Laboratories for the supply of cromakalim.

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197P LOCALISATION OF MRNAS ENCODING SUBUNITS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR TO DISTINCT CELL GROUPS IN THE RAT SPINAL CORD

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The recent determination of the nucleotide sequences of the mRNAs encoding different subunits of the nicotinic acetylcholine receptor (NAChR) allows the sites of synthesis of these subunits to be identified in the rat CNS. We have used selective 45mer oligonucleotides, labelled with ^{35}S-dATP using the enzyme terminal transferase, to localise the *a*2, *a*3, *a*4 and ß2 subunit mRNAs in rat spinal cord by in-situ hybridisation histochemistry.

Cryostat sections of rat lumbar spinal cord were hybridised overnight with each of the oligonucleotides in buffer containing 50% formamide at 42°C, as detailed elsewhere (Wisden et al., 1988; Morris, 1989). After stringent washing, the sections were exposed to x-ray film or dipped in photographic emulsion. Sections were obtained from four different animals. A non-complementary control 45mer probe labelled to the same specific activity showed no hybridisation signal.

The *a*2-subunit probe gave a hybridisation signal only in small (<12µm) and medium-sized (12-20µm) cells in the ventral horn. The *a*3-subunit produced a signal in laminae II, IV and X, while some motorneurones also contained the *a*3 transcript. The *a*4 transcript was found in many cells in all laminae, as was the ß2 transcript. The majority of motorneurones were found to contain the *a*4 and ß2 transcripts.

The results suggest that nAChRs in different cell populations of the rat spinal cord may have different subunit structures, therefore implying the existence of subtypes of nAChR in this tissue.

B J M holds an Ian Henderson Fellowship from the Mental Health Foundation

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198P DIRECT RELAXATION OF RABBIT PULMONARY ARTERIAL STRIPS BY FLASH PHOTOLYSIS OF CAGED CYCLIC GMP

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The relaxant effects of nitrovasodilators and endothelium-derived factors (EDRF) on blood vessels are thought to be mediated by cyclic GMP (cGMP). Although they are mimicked by 8-bromo-cGMP, high concentrations (>1 mM) are required and relaxation is slow in onset and poorly reversible. However, rapid elevation of intracellular cGMP can be achieved using a photolabile precursor of cGMP, o-nitrobenzyl cGMP (caged cGMP; Nerbonne et al, 1984). Being membrane permeant this easily enters cells from the perfusing solution. A brief flash of light, from a flashlamp focussed onto the strip, then releases free cGMP with ~2-5% photolysis per flash. The effects of cGMP were compared on contractions induced either by noradrenaline (NA; 1-6 uM) or by raising the extracellular K^+ concentration (20-100 mM). Isometric tension was measured in srips held under 1.2 g of tension. Since light is known to relax vascular smooth muscle directly, probably by activating guanylate cyclase (Karlsson et al, 1984), we first investigated the effects of flashes without the probe. Flashes had little effect on basal tension, but in strips precontracted with NA, a single flash often induced rapid relaxations (up to 30%). These persisted after removing the endothelium, but were abolished by exposure to haemoglobin (1-5 uM), which inhibits cyclase activation. When caged cGMP (1-100 uM) was present with haemoglobin, flashes produced large, rapid relaxations. They peaked within a few seconds, reversed within 5-10 min and were observed within 1-2 min exposure to the probe.

Elevation of cGMP following a flash was always more effective at relaxing tissue precontracted with NA than with high K^+. Flashes regularly relaxed strips by up to 50% of the NA-induced contraction, but by <10% of comparable K^+-induced (50 mM) contractions. Preliminary experiments suggest that the percent relaxation induced by a flash is unaffected by inhibiting Ca^{2+} influx, but suppressed in the presence of procaine (2 mM), an inhibitor of intracellular Ca^{2+} release. These observations are consistent with a role for cGMP in regulating intracellular Ca^{2+} mobilisation, and with effects of cGMP and sodium nitroprusside in isolated pulmonary arterial cells (Clapp & Gurney, 1989).

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199P EFFECT OF NEUROPEPTIDE Y ON THE MESENTERIC ARTERIAL BED OF THE PITHED RAT: EFFECT OF LOW VENTILATION VOLUME

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NPY induces vasoconstriction in the perfused mesenteric arterial bed of the rat (Westfall *et al.*, 1987). We have previously shown that another vasoactive peptide, endothelin-1 (ET-1) is a potent mesenteric arterial vasoconstrictor both *in situ* and *in vivo* in the pithed rat (MacLean *et al.*, 1989). The potency of ET-1 on the *in situ* mesenteric bed is increased by lowering the ventilation volume of the pithed rat preparation (MacLean *et al.*, 1989). Here, for comparison, the effects of NPY on the mesenteric arterial bed was investigated in the pithed rat both *in situ* and *in vivo*, and the effect of lowering ventilation volume investigated *in situ*.

Male Wistar rats (250-300g) were anaesthetized with 120 mg kg^{-1} i.p. sodium thiopentone, pithed and respired with air through a tracheal cannula with a respiratory pump operating at 54 cycles min^{-1} with a volume of either 20 ml kg^{-1} (control) or 10 ml kg^{-1}. The *in situ* blood perfused mesenteric bed preparation used was a modification of that described by Jackson & Campbell (1980) (MacLean *et al.*, 1989). NPY (50 pmol - 2 nmol) was given as bolus injections (0.1-0.3 ml) and dose-response curves were constructed. In two groups of 8 rats, ventilated at 20 ml kg^{-1}, the effects of a pressor dose of NPY (500 pmol bolus injection followed by a 200 pmol min^{-1} infusion) and saline (0.5 ml bolus and 0.1 ml min^{-1} infusion) on the vascular resistance of the mesenteric bed of the pithed rat *in vivo* were determined using tracer microspheres as described previously (see MacLean *et al.*, 1989).

In 6 pithed rats, lowering the ventilation volume induced moderate blood acidosis (pH: 7.42 ± 0.25 to 7.23 ± 0.15), hypoxia ($P\ O_2$ (mmHg): 83.3 ± 2.4 to 60.1 ± 2.9) and hypercapnia ($P\ CO_2$ (mmHg): 38.9 ± 2.0 to 58.2 ± 3.5). There was no significant difference in mean arterial blood pressure (MAP), heart rate or mesenteric perfusion pressure between the rats ventilated at 20 ml kg^{-1} (49.2 ± 1.8 mmHg, 332 ± 16 beats min^{-1}, 64.2 ± 5.4 mmHg respectively) and those ventilated at 10 ml kg^{-1} (46.7 ± 2.1 mmHg, 361 ± 14 beats min^{-1}, 62.7 ± 4.8 mmHg respectively). In all animals NPY induced a dose-dependent pressor response which was maximal at a 400 pmol dose. At 200, 400 and 750 pmol doses of NPY, the pressor responses to NPY in the low-ventilated animals (13.3 ± 4.2, 35.8 ± 3.5, 21.7 ± 3.6 mmHg respectively) were significantly greater than those in the control animals (24.2 ± 3.5, 52.5 ± 6.8, 42.5 ± 7.5 mmHg respectively; $p<0.05$; Student's unpaired t-test). *In vivo*, in the NPY treated rats, NPY increased MAP from 37.5 ± 2.5 mmHg by 29.4 ± 2.5 mmHg. NPY increased mesenteric vascular resistance from 90.1 ± 11.1 mmHg ml^{-1}min g (saline treated group) to 366 ± 57 mmHg ml^{-1}min g (NPY-treated group) [$p<0.001$; one-way analysis of variance].

The study shows that NPY is a potent vasoconstrictor in the mesenteric bed of the pithed rat *in vivo* and *in situ* and, like ET-1, reducing ventilation volume increases its potency on this vascular bed.

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200P EFFECT OF BRADYKININ B_1 RECEPTOR SELECTIVE PEPTIDES ON THYMIDINE UPTAKE BY RABBIT COELIAC ARTERY SMOOTH MUSCLE CELLS

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Several vasoactive mediators cause cellular proliferation, in addition to their more rapid actions on vascular smooth muscle tone. Proliferation of vascular cells may underlie remodelling of vascular architecture. Such remodelling is believed to be important in the pathophysiology of arterial hypertension and other vascular disorders (e.g. Lever, 1988), although this is incompletely understood. Bradykinin causes fibroblast proliferation (Goldstein & Wall, 1983), and relaxes freshly prepared strips of rabbit coeliac artery by an endothelium independent prostaglandin-mediated action on B_1 receptors (Ritter *et al.*, 1989). We have established a rabbit coeliac artery smooth muscle cell line and used this to investigate the effects of bradykinin receptor stimulation on vascular smooth muscle cell proliferation. Female New Zealand white rabbits (3.4-4.5 kg) were used to prepare the primary isolates, using the method of Ives *et al.* (1978) modified by the addition of an overnight incubation at 4 °C of chopped coeliac artery with the enzyme mixture before incubation at 37 °C and dispersal. Cells were grown at 37 °C in a 5% CO_2/air atmosphere using medium E199 (Flow Laboratories) supplemented with 10% foetal calf serum (FCS), glutamine, insulin, transferrin, selenium and antibiotics. Immunocytochemistry was positive for fibronectin, desmin, vimentin, myosin and α–smooth muscle actin and negative for epithelial and endothelial cell markers (cytokeratins and factor VIII). Cells were subcultured using trypsin/EDTA and the effect of bradykinin-related peptides on ^{3}H-thymidine incorporation was studied at passage 2-6 using subconfluent cells in 96-well plates. Cultures were rendered quiescent by a three day incubation with medium containing 0.5% FCS. Peptides or known mitogens were added and cells incubated 40 h and ^{3}H-thymidine (Du Pont) then added to give a final activity of 5 μCi/ml and incubated for 3 h. Supernatants were discarded and cells washed twice with phosphate buffered saline, and fixed in methanol/acetic acid/water at 4 °C for 1 h. DNA was extracted using 1% sodium dodecyl sulphate in NaOH (0.1M) overnight at 37 °C, and tritium counted in a liquid scintillation counter (Pharmacia LKB). Bradykinin with 0.5% FCS caused a small dose related increase in ^{3}H-thymidine uptake; the effect was more marked in the presence of 2% FCS, the highest dose of bradykinin causing approximately a doubling of uptake. Des Arg^{10}-kallidin, a selective B_1 receptor agonist (Regoli & Barabé, 1980) was much more potent than bradykinin, causing approximately a 15 fold increase in uptake at 10^{-9} M. Its dose response curve was bell-shaped. The effects of low concentrations of des Arg^{10}-kallidin (10^{-9}-10^{-7} M) were antagonised > 50% by a selective B_1 antagonist [Leu^9]-des Arg^{10}-kallidin (5 x 10^{-7} M). Higher concentrations of des Arg^{10}-kallidin (10^{-6}-10^{-4} M) caused smaller effects than lower concentrations and were not significantly antagonised by [Leu^9]-des Arg^{10}-kallidin, 5 x 10^{-7} M. We conclude that kinins stimulate proliferation of rabbit coeliac artery smooth muscle cells, and that B_1 receptor activation is particularly effective in this regard.

We are grateful to Dr S.N. Emancipator and M.S. Simonson (Case Western Reserve University, Cleveland, Ohio 44106, USA) for some of the immunocytochemistry and for advice on cell proliferation measurements respectively.

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201P RHYTHMICAL CONTRACTILITY *IN VITRO* OF HUMAN UTERINE BLOOD VESSEL CIRCULAR MUSCLE STRIPS

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During studies utilising human isolated uterine vessels (Leathard, 1989; Yeats & Leathard, 1989) it was recognized that vein strips usually contracted rhythmically whereas artery strips were usually quiescent. The incidence and characteristics of rhythmical activity in 204 vein and 314 artery strips from 93 and 85 patients respectively are now compared.

Macroscopically-normal vessels, fresh from surgical hysterectomies, were cut into helical strips (30-40mm x 2-4mm) and suspended (for recording isotonic contractility against a 0.5g or 1g load) in isolated organ baths at 37°C, containing Krebs' solution bubbled with 5% CO_2 in O_2 (pH 7.4). Amplitude was calculated as a percentage of the maximum noradrenaline (NA, $3x10^{-6}$M)-evoked contraction of each strip, and frequency as the number of contractions in 10min. Statistical comparisons utilized Students' 't' or Mann-Whitney 'U' tests.

After 1-2 h equilibration and preliminary monitoring of NA-evoked contractions, rhythmical contractions were recorded from 95% of the 204 vein strips (frequency range 3-54; amplitudes 4-92%) but in only 22% of the 314 artery strips (frequency range 1-24; amplitudes 4-56%). Statistical comparisons of 96 artery and 91 vein strips from 30 cases that yielded both confirmed that vein contractions were larger and at higher frequencies than those of arteries ($p<0.002$ for both parameters). Taking only those cases in which hysterectomy was performed at a stage of the menstrual cycle which could be determined with confidence from the patients' notes, there was no evidence of variation in the activity of the vein strips between follicular (days 7-15, n=8, amplitude 28±13%, frequency 13±4 per 10min) and luteal phases (days 16-28+, n=9, amplitude 26±14%, frequency 16±6 per 10min), but amplitudes of artery rhythmical contractions were greater in the luteal (12±13%) than in the follicular phase (3±5%, $p<0.05$), although their frequencies did not differ (follicular 1±2, luteal 2±2 per 10min). Rhythmical activity of vein strips was unaffected by phentolamine ($2.7x10^{-6}$M), or by tetrodotoxin ($3x10^{-7}$M), (n=4 in each experiment) indicating that rhythmical contractions were independent of adrenergic innervation.

Thus, human uterine venous muscle in vitro contracts rhythmically. In vivo this may aid propulsion of blood, as postulated for rat portal vein (Keakinge, 1979). The less pronounced contractility of uterine artery appears to vary during the menstrual cycle, and may therefore be modulated by ovarian steroids.

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202P RABBIT JUGULAR VEIN CONTAINS THREE DIFFERENT RELAXANT PROSTAGLANDIN RECEPTORS

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Recent reports (Giles *et al* 1989, Lawrence *et al* 1989) have demonstrated the presence of a putative EP_2-receptor which mediates relaxation of rabbit jugular vein (RJV). This tissue also possesses contractile TP-receptors and relaxatory DP-receptors (Giles et al 1989). This study examines the utility of RJV as an EP_2-receptor assay.
Preparation of RJV rings and human washed platelet (HWP) aggregation assays have been described previously (Giles *et al* 1989). HWP were aggregated with 50μM ADP and RJV was precontracted with 1μM histamine. 1-5μg ml^{-1} indomethacin was present in both assays; the TP-receptor antagonist BM13.177 (30μM) was included in the RJV assay.

Prostacyclin and the IP-receptor analogues iloprost, carbacyclin and 9β-methyl carbacyclin caused relaxation of RJV and inhibition of platelet aggregation. All were full agonists and their relative potency was similar in both assays, the compounds being approximately 10-fold less potent in RJV (Table 1). All concentration-effect (E/[A]) curves were monophasic and were unaffected by the selective DP-receptor antagonist BW A868C (1μM).

Table 1. Potencies of IP analogues.

values are -log EC_{50} ±s.e. mean
( ) = potency relative to prostacyclin

	HWP(n=3-4)	RJV(n=6-7)
Iloprost:	8.85±0.09 (0.5)	7.42±0.06 (0.9)
Prostacyclin	8.53±0.04 (1)	7.36±0.10 (1)
Carbacyclin	7.72±0.08 (6.6)	6.57±0.09 (6.2)
9β-methyl carbacyclin	7.33±0.26 (15.9)	6.47±0.13 (7.8)

PGE_1(0.03nM-3μM) and 6-keto-PGE_1(1nM-3μM) caused relaxation of RJV. E/[A] curves were consistently flat and, on many occasions, clearly biphasic. In HWP both agonists were considerably less potent than the first phase of agonism in RJV but 10-fold more potent than the second phase. Responses were unaffected by 1μM BW A868C. Such a result is consistent with agonist action through two different receptors in the RJV, the first through a receptor (?EP_2) which is not present in significant concentrations on HWP, and the second phase through the IP-receptor. PGE_2 (0.1-30nM) also potently relaxed RJV, presumably via the EP-receptor since it was unaffected by BW A868C, and did not inhibit platelet aggregation. Fluprostenol (1-10μM), an FP-receptor agonist, was without significant effect in RJV, and the EP_1 receptor agonist sulprostone (1-30μM) elicited small contractions which were antagonised by BM13.177; this data suggests that RJV does not possess significant densities of EP_1 or FP-receptors.

RJV contains IP-, DP, ?EP_2 and TP-receptors. Clearly, such receptor heterogeneity means that agonist action at a single receptor cannot be guaranteed and emphasises the need for caution when using this assay for classification purposes.

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203P ANGIOTENSIN II-INDUCED INCREASES IN $^{45}Ca^{2+}$ UPTAKE AND CONTRACTILE RESPONSES IN GUINEA-PIG AORTA

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An approach to gain information about the sources of Ca^{++} triggering contraction upon receptor stimulation is to investigate both contractile responses and stimulated $^{45}Ca^{++}$ uptake in intact vessels, using different compounds which interfere with either Ca^{++} influx or intracellular processes.

For the measurement of slowly exchanging $^{45}Ca^{++}$ (essentially as described by Wermelskirchen et al., 1988), guinea-pig aortic strips were suspended in a gassed (95% O_2 + 5% CO_2) Krebs-Henseleit (K-H) solution (1.25 mM Ca^{++}, 37°C, pH=7.3) under a resting tension of 0.33 g for 30-45 minutes. Subsequently the strips were treated with the antagonists for 45 minutes and incubated in a $^{45}Ca^{++}$-containing K-H solution (1 µCi/ml) without or with angiotensin II (A II) and/or the antagonists for 10 minutes. Thereafter the strips were washed (45 min, 4°C), dried, weighed and dissolved. The residual $^{45}Ca^{++}$-tissue content was measured by means of liquid scintillation counting. For contraction studies helically cut strips were suspended in the K-H solution under a resting tension of 2 g. Strips were pretreated with the antagonists for 30 minutes or incubated in a Ca^{++} "free" medium for 10 or 45 minutes.

A II was virtually unable to stimulate $^{45}Ca^{++}$ uptake in the lower part of the guinea-pig aorta. In the upper parts A II elicited a concentration-dependent increase in slowly exchanging $^{45}Ca^{++}$ by maximally 139.2 $\pm$ 6.8% of the basal uptake (=100%). Incubation of the strips in a Ca^{++} entry blocker (nifedipine, verapamil or diltiazem)-containing solution led to a concentration-dependent and finally complete inhibition of the A II-stimulated $^{45}Ca^{++}$ uptake. $CoCl_2$ ($3x10^{-4}$ M), TMB-8 (10^{-4} M) and chlorpromazine (10^{-4} M) also entirely inhibited the increase in slowly exchanging $^{45}Ca^{++}$. Basal $^{45}Ca^{++}$ uptake was not influenced by the abovementioned compounds, with the exception of $CoCl_2$ which slightly reduced basal uptake. Functional experiments showed concentration-dependent contractile responses to A II. The maximal response was partly (1/3) inhibited by the organic Ca^{++} entry blockers. Comparable results were found when the strips were incubated in a Ca^{++} "free" solution for 10 minutes. The contraction to A II after pretreatment with $CoCl_2$ was reduced by about 2/3 and a similar contractile response could be observed after an incubation period of 45 minutes in Ca^{++} "free" medium. Complete suppression of the contraction was observed after treatment with TMB-8 and chlorpromazine.
The results indicate that the contraction to A II in the guinea-pig aorta may partly (1/3) be the result of Ca^{++} entering the cell through Ca^{++} entry blocker-sensitive channels. It is likely that the inhibitor of intracellular Ca^{++} release TMB-8 and the calmodulin antagonist chlorpromazine also possess Ca^{++} entry blocking properties. The remaining contraction seems to be the result of the release of Ca^{++} from intracellular pools, one of which (probably membrane bound) can easily be washed out. The stronger inhibitory effect of $CoCl_2$ can be explained by its ability to replace Ca^{++} at its binding sites in the plasmalemma, thereby excluding a certain fraction of Ca^{++} essential for tension development.

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204P CHARACTERISATION AND MODULATION OF VASCULAR α-ADRENOCEPTOR RESPONSES IN RAT KIDNEY

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The characterization of adrenoceptors and modulation of their effector systems is an area of continuing research interest. In the present experiments we have characterized vascular alpha-adrenoceptors in the isolated perfused rat kidney and examined the influence of angiotensin II (Ag II) and calcium upon noradrenaline (NA)-induced vasoconstriction.

Kidneys were perfused with Krebs solution containing cocaine (30 µM), corticosterone (30 µM), propranolol (1 µM) and indomethacin (10 µM) as described previously (Bond et al., 1989). Vasoconstrictor responses to bolus injections and infusions of NA were measured. Only bolus injections of UK 14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline), a selective alpha$_2$-adrenoceptor agonist, were administered. Prazosin (1-30 nM), Ag II (0.25 & 0.5 µM), and nitrendipine (1 µM) were perfused for 1h prior to testing whereas phenoxybenzamine (PBZ; 0.01-0.1 µM) was perfused for 15 min, followed by washout for 45 min.

Prazosin evoked parallel dextral shifts in the concentration-effect curve to NA with no change in maximum response. However, with bolus injection of NA, the slope of the Schild regression differed significantly from 1 (slope = 1.33; 95% CL: 1.09-1.56). This deviation resulted because of the difficulty in attaining equilibrium conditions with bolus injections of NA in a perfused system. The kinetics of the interaction also explains the production of biphasic responses to NA (phasic and tonic components) in the presence of prazosin. Infusion of NA gave a Schild regression with a slope not significantly different from 1 (slope = 1.05; 95% CL: 0.91-1.22) and a pA_2 value of 9.6. Unlike NA, UK 14,304 was virtually inactive as an agonist (intrinsic activity relative to NA = 0.07). Receptor inactivation with PBZ revealed a spare receptor population of 70% for NA and an equilibrium dissociation constant of 2.1 µM.

Dunn et al. (1989) reported that Ag II can 'disclose' previously quiescent postjunctional alpha$_2$-adrenoceptors in the distal saphenous artery of rabbits. However, in the present experiments, infusion of Ag II did not influence responses to NA or UK 14,304, even after removal of endothelial cells with saponin. Vasoconstrictor responses to NA were resistant to inhibition by nitrendipine (3-fold dextral shift) but were abolished rapidly (< 2.5 min) by perfusion with calcium-free Krebs solution containing EGTA (2 mM), an effect fully reversed (< 0.5 min) by calcium-containing Krebs solution.

The results suggest a homogeneous population of alpha$_1$-adrenoceptors in the renal vasculature as proposed originally by Shmitz et al. (1981). Furthermore, these receptors appear to signal information via a mechanism critically dependent upon extra-cellular calcium. The signaling process is largely nitrendipine resistant and unaffected by Ag II.

This work was supported by NIH Grant GM 39621.

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205P ENANTIOMERS OF NIGULDIPINE DIFFERENTIALLY INHIBIT α_1-ADRENOCEPTOR-MEDIATED HYDROLYSIS OF PHOSPHOINOSITIDES AND CONTRACTION IN RAT AND GUINEA-PIG VASCULAR TISSUE

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We have studied the effect of the enantiomers of the novel dihydropyridine (DHP) niguldipine on noradrenaline (NA) induced vasoconstriction and hydrolysis of phosphoinositides (HPI) in rat aorta (RA), guinea-pig aorta (GPA) and rat perfused hindquarters (RPH). In the RPH KCl induced vasoconstriction was also studied. In RA α_{1A}- and α_{1B}-adrenoceptors coexist, in GPA and RPH α_{1B}-adrenoceptors mediate vasoconstriction insensitive to calcium antagonists (Beckeringh & Brodde, 1989; Beckeringh et al., 1984; Korstanje & Van Zwieten, 1987). In ring segments of RA and GPA concentration-response (CR) curves were constructed as described by Beckeringh et al. (1984). Contraction was expressed as percentage of the effect by 100µM NA (Emax). In the RPH vasoconstriction to NA and KCl was measured as described by Korstanje & Van Zwieten (1987). In RA and GPA HPI to NA was measured according to the method of Minneman & Johnson (1984). Data are means ± S.E.M. (n=3-6).
In GPA (+)-niguldipine fully inhibited NA (100µM) induced HPI (K_i: 27.1 ± 1.6 nM). (-)-Niguldipine was 100-fold less potent. In RA (-)-niguldipine did not inhibit NA induced HPI, but at higher concentrations (+)-niguldipine inhibited HPI by about 30%. In RA and GPA prazosin fully inhibited HPI (K_i: 0.06 ± 0.01 and 3.4 ± 0.3 nM). In RA the pseudo-Hill coefficient was lower than 1. In RA, similar to darodipine (1 µM), (+)-niguldipine (1 µM) non-competitively inhibited contraction by NA and reduced Emax by about 30%. (-)-Niguldipine was less effective than (+)-niguldipine. In GPA and RPH (-)-niguldipine (1 µM) displaced the CR curve to NA in a parallel manner to the right (K_B: 6.39 ± 0.11 and "K_B": 7.78 ± 0.09). (+)-Niguldipine (1 µM) inhibited NA induced vasoconstriction in a non-competitive manner and decreased Emax by 49 ± 4 and 72 ± 4%, respectively. In GPA and RPH darodipine and nitrendipine lacked inhibitory effect. In RPH niguldipine (1 µM) and nitrendipine completely blocked KCl induced vasoconstriction.
In contrast to radioligand binding studies (Boer et al., 1989), in functional experiments (+)-niguldipine did not display α_{1A}-adrenoceptor antagonism, but inhibited α_{1A}-adrenoceptor (RA) and KCl (RPH) induced calcium influx. In GPA and RPH (-)-niguldipine displayed α_{1B}-adrenoceptor antagonism. The effect of (+)-niguldipine at α_{1B}-adrenoceptors suggests the involvement of an additional inhibitary component. The differential results from radioligand binding and functional studies suggest that a DHP sensitive calcium channel is an integral part of α_{1A}-adrenoceptors. This idea is strongly supported by the complete dependance of contraction to the full agonist indanidine, which does not activate HPI, on extracellular calcium in RA (Chiu et al., 1987).

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206P NITROVASODILATORS MODULATE BLOOD FIBRINOLYTIC ACTIVITY

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Platelets, in addition to their procoagulant role, have an inhibitory effect on fibrinolysis, which is mainly due to the release of a plasminogen activator inhibitor (PAI-1, Erickson et al., 1984). Many of the nitrovasodilators, including sodium nitroprusside (SNP) and nitric oxide (NO/EDRF) itself have been found to inhibit platelet aggregation and adhesion (Radomski et al., 1987). To test whether these anti-platelet properties mediate changes in the thrombolytic system we assessed the fibrinolytic activity of SNP and glyceryl trinitrate (GTN) in vitro and ex vivo in rabbits. SNP (30 µg/kg) or GTN (30 µg/kg) was injected intravenously, and blood was sampled at 1, 5, 15, 30 and 60 min after injection. The fibrinolytic activity was determined using the euglobulin clot lysis time (ECLT, von Kaulla and Schultz, 1958), tissue plasminogen activator (t-PA) activity and PAI-1 activity (Biopool, Sweden). In addition, t-PA antigen levels were measured ex vivo by enzyme-linked immunosorbent assay (Biopool). In vitro, SNP (0.1-30 µg/ml) was instilled directly onto euglobulin clots or added to whole blood measuring ECLT and t-PA/PAI-1 activity. In addition, the effect of SNP on the fibrinolytic activity of exogenous t-PA was studied in vitro in whole blood, PRP or PPP, incubated at 37°C for 60 min.

Ex vivo, SNP (30 µg/kg) but not GTN (up to 30 µg/kg) shortened ECLT at 30 min after administration by 34 ± 5 % (mean ± SEM, n = 3). Assays of t-PA and t-PA antigen activity showed that this activation of fibrinolysis was not due to extra t-PA release but rather to the prolongation of its effects. SNP did not activate fibrinolysis in vitro, but it prevented the rapid disappearance of activity of exogenous t-PA incubated in PRP and whole blood, but not in PPP. Furthermore, SNP at concentrations with anti-platelet potency (1-30 µg/ml) inhibited the spontaneous release of PAI-1 activity from platelets in whole blood incubated at 37°C for 60 min.

Thus, anti-platelet nitrovasodilators exhibit fibrinolytic activity through inhibition of the release of PAI-1 from platelets. The administration of anti-platelet nitrovasodilators along with t-PA may have important therapeutic consequences because of the prolongation of the efficacy of t-PA, perhaps allowing a reduction in the total administered dose.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Ltd. CT is supported by a research grant of the Fritz Thyssen Stiftung, Koln, FRG.

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207P RADIOIMMUNOASSAY FOR ENDOTHELIN-1: ANTIBODY CROSS-REACTIVITY WITH ENDOTHELINS, SOME ANALOGUES OF ENDOTHELIN-1 AND SARAFOTOXIN S6B

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In the family of the vasoactive endothelins-1, -2 and -3 (ET-1, ET-2 and ET-3 respectively), ET-2 and ET-3 correspond to [Trp^6,Leu^7]- and [Thr^2,Phe^4,Thr^5,Tyr^6,Lys^7,Tyr^{14}]-ET-1 respectively (Inoue *et al.*, 1989). Radioimmunoassay methods have been used to detect the presence of ET-1 in tissues and cell culture mediums and it is of interest to have some idea of the antigenic determinants of this peptide.

The cross-reactivity of a rabbit anti-ET-1 antibody with the various endothelins and some ET-1 analogues, [$Ala^{1,3,11,15}$]-, [$Ala^{1,15}$]-, [$Cys(Acm)^{3,11}$]-ET-1 and sarafotoxin S6b (S6b; [Lys^4,Asp^5,Met^6,Thr^7, Leu^{12},Gln^{17},Val^{19}]-ET-1) having a high affinity for specific [^{125}I]ET-1 binding sites in rat tissues (Jones *et al.*, 1989; and unpublished observations), has been tested. Assays were carried out using a radioimmunoassay kit (Peninsula). The method described in the kit was used, except that the analogues replaced unlabelled ET-1 during the first incubation period when necessary. All values were determined in duplicate.

Table 1. The IC_{50} values (concentrations of peptides which displaced 50% of [^{125}I]ET-1 bound to the antibody) and the derived degree of cross reactivity expressed as a percent (%).

Peptide	IC_{50} (pg)	% cross reactivity
ET-1	20	100
ET-2	150	13
ET-3	200	10
[$Ala^{1,3,11,15}$]ET-1	200	10
[$Ala^{1,15}$]ET-1	150	13
[$Cys(Acm)^{3,11}$]ET-1	200	10
S6b	5000	0.4

The results (shown in Table 1) demonstrate that even though there is a lot of homology between the sequences of the different endothelins, their affinities for the antibody were decreased by a factor of about 10 for ET-2 and -3 compared to that of ET-1. The replacement of 2 or 4 of the cysteine residues by alanines or the inhibition of disulphide bond formation by substitution of Acm groups on cysteines 3 and 11 also reduced affinity for the antibody by a similar factor. S6b had a very low affinity for this antibody. The results suggest that the important amino acids for the recognition of the antibody may be located between the residues 4 to 7. A similar conclusion has been reached using another antibody (Fleminger *et al.*, 1989), perhaps indicating that this region is determinant for antigenicity.

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208P RAT AORTIC RINGS DENUDED OF ENDOTHELIUM ARE MORE SENSITIVE TO SODIUM NITROPRUSSIDE AFTER PRECONTRACTION BY SARAFOTOXIN S6B THAN BY ENDOTHELIN-1

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Endothelin-1 (ET-1), a peptide isolated from endothelial cell cultures (Yanagisawa *et al.*, 1988) and the the analogous peptide sarafotoxin S6b from Atractaspis engaddensis venom (Lee & Chiappinelli, 1988) are both potent spasmogens in isolated rat aorta. Sodium nitroprusside (SNP) relaxes aortic smooth muscle via an increase in the intracellular concentration of cyclic GMP (Waldman & Murad, 1987). In the present study, the relaxation and the rise in tissue cyclic GMP caused by SNP were examined in rat aortic rings precontracted with ET-1 or sarafotoxin S6b.

The thoracic aortae from male Sprague-Dawley rats were carefully dissected, cut into four pieces, denuded of endothelium by rubbing with forceps and equilibrated in a gassed Krebs solution for a total of 1 hour. Where smooth muscle contraction was monitored, it was measured isometrically. All data are expressed as means ± s.e.mean. Rings were contracted with 10 nM ET-1 or 10 nM sarafotoxin S6b, concentrations which in both cases gave similar just-maximal responses (2.11 ± 0.15 and 1.99 ± 0.13 g, respectively). After 20 minutes preincubation, when contraction was maximal, concentration-response curves were obtained by cumulative addition of SNP. For cyclic GMP measurements, 300 nM SNP was added to rings preincubated as above. After 1 minute, rings were frozen using clamps precooled in liquid N_2. The tissues were subsequently homogenized in 0.6 M trichloroacetic acid followed by extraction of the acid supernatant with diethyl ether. Extracts were assayed for cyclic GMP by radioimmunoassay using an Amerlex kit (Amersham, UK). Protein was assayed by the Lowry method (1951). All comparisons were made using Student's t-test.

SNP elicited concentration-dependent relaxations of aortic rings. The EC_{50} for SNP-induced relaxations was 430 ± 180 nM (n = 7) or 34.6 ± 18.8 nM (n = 8) when ET-1 or sarafotoxin S6b were used as spasmogens, respectively. The EC_{50} for ET-1 was significantly higher ($P < 0.05$). Measurement of cyclic GMP gave the results shown in Table 1. The results of cyclic GMP measurements show that whilst in all cases SNP increased tissue cyclic GMP content, neither ET-1, nor sarafotoxin S6b significantly altered the effect caused by SNP alone ($P > 0.05$). In conclusion, ET-1 or sarafotoxin S6b modifies the relaxant response of rat aorta to SNP, but does not modify the stimulation of guanylyl cyclase by SNP. Hence, ET-1 and sarafotoxin probably differentially modifies some process involved in SNP-induced relaxation subsequent to the activation of guanylyl cyclase.

Table 1. Effect of 300 nM SNP on tissue cyclic GMP levels in rat aortae contracted by ET-1 or sarafotoxin S6b

Spasmogen	Relaxant	Cyclic GMP pmol/mg protein	n
None	None	1.28 ± 0.02	8
None	SNP	28.65 ± 3.91*	7
ET-1	SNP	32.69 ± 4.07*	7
Sarafotoxin	SNP	24.65 ± 2.24*	6

*Significantly different to control (no SNP; $P < 0.05$).

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209P EFFECTS OF ENDOTHELIN-1 AND SARAFOTOXIN S6B ON CARDIAC OUTPUT IN CONSCIOUS RATS

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We have compared the central haemodynamic effects of endothelin-1 (Et-1) and sarafotoxin S6b (S6b) in the same conscious rats. Male, Long Evans rats (350-450g) were anaesthetized (sodium methohexitone 60 $mgkg^{-1}$ i.p., supplemented as necessary) and had an electromagnetic flow probe (Skalar MDL 1401) implanted around the ascending aorta. At least 7 days later animals were briefly re-anaesthetized (sodium methohexitone, 40 $mgkg^{-1}$) and had intravascular catheters implanted. The following day continuous recordings were made of ascending thoracic aortic flow (i.e. cardiac output), central venous and systemic arterial pressures. These signals were also fed into a custom-built microprocessor (Department of Instrument Services, University of Limburg) interfaced with a Tandon 386 microcomputer; this system provided values for cardiac output, heart rate, stroke volume, peak and maximum positive slope of the thoracic aortic flow signal, central venous pressure, mean arterial pressure, total peripheral conductance and heart rate averaged over 2 sec epochs. Animals received Et-1 and S6b (4 and 40 pmols i.v.) in random order, but with the low dose given before the high dose. Peptides were given in 0.1 ml (dissolved in isotonic saline containing 1% bovine serum albumin) and high doses were separated by at least 60 min. The low bolus dose of Et-1 and S6b increased cardiac output (7±2 and 7±2 ml min^{-1}, respectively, at 0.5 min post injection; $P<0.05$ (Friedman's test)). The table summarizes some of the results following the high dose of the peptides.

Table 1. Cardiovascular changes after Et-1 and S6b (40pmol) in conscious, Long Evans rats (n=8); values are mean (s.e.m.). * $P<0.05$ versus baseline (Friedman's Test)

	Et-1		S6b	
Time after injection (min)	0.5	2.0	0.5	2.0
Heart rate ($bmin^{-1}$)	20(8)*	-30(5)*	20(11)*	-38(14)*
Mean arterial pressure (mmHg)	-14(2)*	20(3)*	-8(2)*	17(3)*
Cardiac output (ml min^{-1})	13(3)*	-17(2)*	13(3)*	-16(2)*
Total peripheral conductance ($\mu l\ min^{-1}\ mmHg^{-1}$)	270(56)	-310(33)*	230(44)	-290(31)*

The cardiovascular effects of Et-1 and S6b were indistinguishable, consistent with these peptides having the same site and mode of action (see Kloog & Sokolowsky, 1989).

The techniques used were set up with the help of Drs Schoemaker, Geilen and Smits (University of Limburg). PAK is supported by a grant from Celltech Ltd.

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210P ENDOTHELIN RECEPTORS ON HUMAN AND RAT CARDIAC MYOCYTES: A COMPARISON WITH ISOLATED SMOOTH MUSCLE CELLS

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Low and high resolution autoradiography of endothelin receptors was performed on intact rat myocardium and samples of human ventricle obtained from explanted hearts at the time of transplant. Slide-mounted sections of myocardium were incubated in 20 to 50pM [^{125}I]-endothelin, the degree of non-specific binding being established by incubating paired sections in the presence of 500nM unlabelled endothelin. In addition to specific binding to the smooth muscle of the blood vessel lumen, there was considerable binding associated with cardiac myocytes. To discover whether there was any functional correlate for this binding, muscle cells were isolated enzymatically from human and rat ventricle, and from rat femoral artery, and their contractile characteristics studied. Single cardiac cells were superfused with physiological saline containing 1.3 mM calcium, and their length change monitored continuously using video microscopy. Cardiac myocytes were electrically stimulated at 0.5Hz (rat) or 0.2Hz (human), 32°C. Endothelin had a pronounced positive inotropic effect on both rat and human myocytes. The contraction amplitude was approximately doubled in both cases, from 4.1 ± 0.8% cell length to 8.1 ± 1.3% for rat (mean ± sem, n=9, $p<0.001$), and from 2.1 ± 0.5% to 4.0 ± 0.5% in human (n=10, $p<0.001$). In rat, the magnitude of the effect was comparable to that of the alpha-adrenoceptor agonist phenylephrine, which raised the amplitude from 2.6 ± 0.5% to 5.0 ± 1.0% (n=7, $p<0.001$) in the presence of propranolol. The maximum contraction amplitude of the human cells, produced by raising extracellular calcium to >10mM, was 11.4 ± 1.1% cell length (n=9), significantly greater than endothelin ($p<0.001$). The threshold for the effect of endothelin was around 0.3nM and maximum effects were attained at 30nM, compared with 1 and 100uM respectively for the alpha-adrenoceptor effect. Endothelin had a potent action on single vascular smooth muscle cells. The EC50 for endothelin was 36 ± 4 pM (n=8) compared with 3.0 ± 1.6 uM (n=7) for phenylephrine. The maximum decrease in resting length with endothelin was 15 ± 3% (n=12). Maximum contraction was attained by depolarisation with 80mM KCl, and was 26 ± 3% (n=11) of resting length. The response to endothelin in single smooth muscle cells was quantitatively similar to phenylephrine (85 ± 9% of KCl contracture, n=12 v 83 ± 7%, n=7 for phenylephrine). We conclude that endothelin binding has a significant functional correlate in the direct effects on cardiac myocyte contraction for both rat and man. Endothelin affects rat heart cells at higher concentrations than it does smooth muscle cells, whereas the effective range for phenylephrine is similar for both tissues.

211P EFFECT OF ENDOTHELIN-1 ON PUPIL DIAMETER FOLLOWING INJECTION INTO THE ANTERIOR PRETECTAL NUCLEUS OF THE RAT

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The presence of high affinity binding sites for [^{125}I]endothelin-1 in the CNS of rats (Jones *et al.*, 1989) suggests that endothelin may play a neuromodulatory or neurotransmitter role. Brain areas which show a particularly high density of [^{125}I]endothelin-1 binding sites are the pretectal nuclei (Jones *et al.*, 1989). As these areas are involved in the pupillary light reflex it was of interest to examine the effects of endothelin-1 on pupil diameter following injection into the anterior pretectal nucleus (APT) of the rat.

Male Sprague-Dawley rats (Charles River, France) were prepared with unilateral indwelling guide cannulae using standard stereotaxic procedures. These allowed the placement of an injection cannula into the right APT at the coordinates AP -5.3 mm, L +2.1 mm from the bregma and 5.5 mm below the skull surface (Paxinos & Watson, 1982). Rats were allowed 7 days to recover. Endothelin-1 was dissolved in sterile saline and injected in a volume of 0.5 µl. Pupil diameter was measured with a scale fitted to the viewfinder of a 35 mm camera with 105 mm macro lens and 56 mm extension tube. Rats received only one injection of endothelin-1 except as described below.

Endothelin-1 dose-dependently increased pupil diameter in both eyes (Figure 1). The effect was rapid in onset and appeared to be slightly more marked on the contralateral side. A second injection of endothelin-1 (500 fmoles rat^{-1}) 4 - 6 days later, to a rat that had already responded, failed to increase pupil diameter significantly (maximum diameter: 2.5 ± 0.3 units, n = 4).

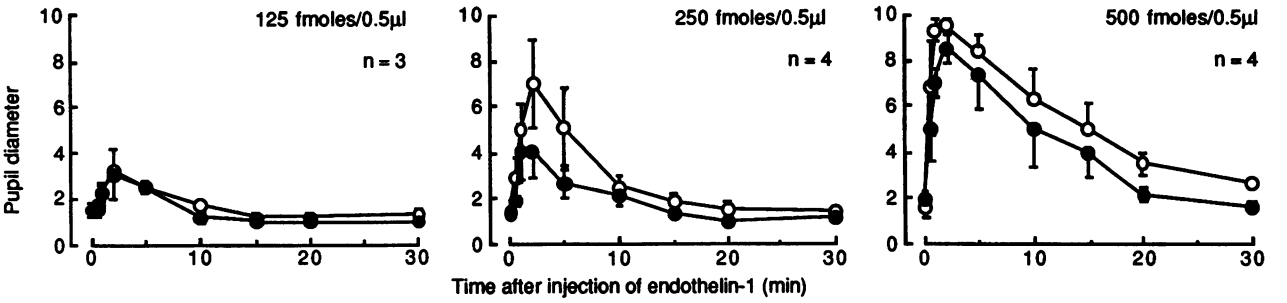

Figure 1. The effect of endothelin-1 injected into the right APT on pupil diameter. Pupil diameter was measured in arbitrary units (2.5 units = approximately 1mm). Left eye (open symbols); right eye (filled symbols). All values are mean ± s.e. mean.

These results demonstrate that small quantities of endothelin-1 injected into the APT, a brain area with a high density of [^{125}I]endothelin-1 binding sites, can produce a physiological change associated with that area, in this case pupil dilatation. This effect may therefore represent stimulation of functional receptors. However, the observation that animals respond only once to endothelin-1 remains to be explained.

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212P ENDOTHELIN-INDUCED CONTRACTION OF HUMAN OMENTAL ARTERIES

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Whether the pharmacological intervention of endothelin observed in various species can be extrapolated to human vessels remains to be determined. The present experiments were designed to elucidate the effects of endothelin on isolated human omental arteries with special emphasis on endothelium dependent responses as well as extracellular calcium requirements.

Arterial segments were taken from portions of human omentum during the course of abdominal operations (15 patients, 7 men and 8 women, aged 30-79 years). Cylindrical segments (3-4 mm in length and 400-800 µm in outside diameter) were cut for isometric recording of tension and set up in a 4 ml bath containing Krebs-Henseleit solution. The preparations were equilibrated at a passive tension of 1 g for 2 hours. In some experiments the endothelium was rubbed by inserting a roughened wire into the lumen. After each experiment the arteries were opened flat and stained with $AgNO_3$ to visualize the endothelium (Caplan and Schwartz, 1973). Functional integrity of the endothelium was demonstrated by the presence of relaxation induced by acetylcholine (10^{-6}M).

Cumulative applications of endothelin (10^{-10}-$3x10^{-8}$, Endothelin-1-Human, Scientific Marketing Associates, London) produced a dose-dependent constrictor response. Maximal contractile response was about 245% of the maximal contraction induced by potassium chloride and EC_{50} value was 5.4 x 10^{-9}M. Removal of endothelium did not affect these responses. Removal of extracellular calcium or addition of the calcium antagonist nicardipine (10^{-6}M) reduced maximal response of endothelin to about 50% without significant changes in EC_{50}.

These experiments show that the endothelin-induced contraction in human omental arteries is not linked to the presence of intact endothelial cells. Our data also show, in agreement with previous observations of Hughes et al. (1989), that the contractile effects of endothelin cannot be explained solely by voltage dependent calcium channels.

This work was supported by Ministerio de Sanidad.

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213P COMPARISON OF THE RELAXANT PROFILES OF LEMAKALIM (BRL 38227) AND DAZODIPINE IN RAT ISOLATED AORTA PRECONTRACTED BY ENDOTHELIN

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The dependence of endothelin (ET) contractions on Ca^{2+} influx through voltage operated Ca^{2+} channels (VOC) is equivocal since some workers (Yanagisawa et al, 1988), but not others (D'Orleans-Juste et al, 1989), have shown that Ca^{2+} antagonists inhibit ET-induced contractile responses in isolated blood vessels. We have examined the ability of the K^+ channel activator, lemakalim (the active (-) enantiomer of cromakalim) which indirectly influences Ca^{2+} entry through receptor operated Ca^{2+} channels (ROC), in comparison to dazodipine (DAZ, an antagonist of Ca^{2+} entry through VOC) as relaxants of ET-induced contractions.
Isometric tension was recorded from rings of rat isolated aorta (RIA), denuded of endothelium (n=5 per group) suspended in Krebs-Henseleit solution at 37°C. Tissues were contracted once with either ET (0.1μM), noradrenaline (NA, 0.1mM) or KCl (90mM) and relaxed by cumulative addition of lemakalim, DAZ or BRL 38226 (the (+) enantiomer of cromakalim, Buckingham et al, 1986). In another group, tissues were pre-incubated with either glibenclamide (GLIB; 3μM) or DMSO vehicle 15 min before cumulative addition of lemakalim. In separate experiments, tissues were exposed to normal or Ca^{2+} deplete conditions in which Ca^{2+} was replaced by equimolar Mg^{2+} + DAZ (50nM). Contraction to KCl (30mM) was abolished by this treatment but ET (0.1μM) elicited a slowly developing and well maintained contraction.
In endothelium-denuded RIA, lemakalim (0.3-30μM) evoked concentration dependent relaxation of ET and NA contractions (IC_{50} 5.3μM and 4.3μM respectively). DAZ was a potent relaxant of KCl-induced (90mM) contraction (IC_{50} 13nM) but was less potent as a relaxant of ET (-28±9% at 1μM). BRL 38226 had weak relaxant activity (-27±14% at 100μM) against ET-induced contractions. GLIB (3μM) evoked an approximate 30 fold rightward displacement of lemakalim's relaxant activity against ET. In Ca^{2+} deplete conditions, the maximum contractile response to ET (0.1μM) was significantly less (0.89±0.04g) than in normal conditions (3.08±0.44g); readdition of Ca^{2+} (2.5mM) to the Ca^{2+} deplete solution produced a contractile response (2.35±0.23g) despite the presence of DAZ (50nM). In Ca^{2+} deplete conditions, lemakalim (0.1 to 30μM) had no relaxant activity against ET.
In summary, lemakalim relaxed RIA pre-contracted by ET, by a stereospecific, GLIB sensitive mechanism and displayed similar potency as a relaxant of NA-induced contraction. In contrast, DAZ was a weak inhibitor of ET contractions compared to its potency against KCl. ET contractions are partly dependent on Ca^{2+} influx through VOC but other mechanisms sensitive to inhibition by K^+ channel activator drugs are also involved. However, the component of ET contractions persisting in Ca^{2+} deplete conditions was insensitive to lemakalim, suggesting that the Ca^{2+} store involved in the ET response is different from the cromakalim-sensitive store responsible for caffeine-induced contractions (Wilson, 1988).

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214P LACK OF EFFECT WITH ICI 170809 AT THE 5-HT_3 RECEPTOR IN THE ISOLATED RABBIT HEART

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We have previously shown that ICI 170809 possesses potent 5-HT_2 antagonist activity (Blackburn et al., 1988a) has low affinity for the 5-HT receptor in the dog basilar artery and little or no effect for the 5-HT_1-like receptor in the dog saphenous vein (Blackburn et al., 1988b). However, we have not previously reported the effects of this compound at the 5-HT_3 receptor mediating release of noradrenaline in the rabbit heart. We therefore decided to evaluate the effects of ICI 170809, together with a number of known standard compounds, on the 5-HT induced tachycardia in the isolated perfused rabbit heart - a system demonstrated to be sensitive to the effects of 5-HT_3 antagonists (Humphrey 1984).

The isolated perfused rabbit heart was set up as described by Fozard and Mwaluko (1976). 5-HT-induced tachycardia was evaluated in the absence and presence of ICI 170809 (10^{-7}M), ketanserin (10^{-7} to 10^{-6}M), methiothepin (5x 10^{-8}M), MDL 72222 and GR 38032F (5x 10^{-10} to 5x 10^{-8}M). All compounds were allowed a 15 min contact time prior to addition of 5-HT.

MDL 72222 and GR 38032F (5x10^{-8}M) produced insurmountable antagonism of the 5-HT-induced tachycardia, making calculation of concentration ratio's not possible. However, in the caudal artery and dog saphenous vein (5-HT_2 and 5-HT_1 respectively) these compounds had no significant antagonist effects at 5x10^{-8}M, (MDL 72222 concentration ratio's = 1.15 and 1.52 respectively; GR38032F concentration ratio's = 1.40 and 1.68 respectively). ICI 170809 (1x10^{-7}M) was found to have no significant effect on the 5-HT-induced tachycardia in the isolated rabbit heart (concentration ratio = 1.20±0.29). However, in the caudal artery preparation this compound has potent antagonist activity (pA_2 value = 9.78). Ketanserin (10^{-7}M) had no significant effect on the 5-HT response in the isolated heart. Methiothepin was also found to be inactive in the isolated heart preparation at 5x10^{-8}M (concentration ratio = 1.00).

These results demonstrate that ICI 170809 possessed no significant 5-HT_3 antagonist activity in the rabbit heart and thus could prove to be an invaluable tool for probing functional 5-HT receptors.

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215P 5-METHOXYTRYPTAMINE AND 2-METHYL-5-HYDROXYTRYPTAMINE AS DISCRIMINATIVE TOOLS FOR EXCITATORY NEURONAL RECEPTORS (5-HT_3 AND NOVEL SITE) IN GUINEA-PIG ILEUM

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Recently, Bockaert and colleagues described a novel receptor for 5-hydroxytryptamine (5-HT) in brain which they termed the 5-HT_4 receptor (Dumuis *et al.*, 1988, 1989). A similar receptor exists in guinea-pig ileum where it mediates the first, high potency phase of the neuronally-mediated biphasic concentration-effect curve to 5-HT (Craig & Clarke, 1989; Clarke *et al.*, 1989). The second, low potency phase is mediated by 5-HT_3 receptors. We now report that the two receptor sites in ileum can be discriminated and each 'isolated' for pharmacological analyses by exposure to selective agonists. Responses mediated by the novel site can be abolished by incubation with 5-methoxytryptamine (5-MOT). This manoevre was taken from the original observation by Fozard (1985). 5-HT_3-mediated responses were abolished by prior exposure to 2-methyl-5-HT (2-M-5-HT).

Longitudinal muscle strips of ileum were prepared as described previously and stimulated electrically to evoke a submaximal cholinergic 'twitch' response (Craig & Clarke, 1989). Cumulative concentration-effect curves to 5-HT ($1x10^{-10}$ to $3x10^{-5}$ M) or renzapride (BRL 24924; $3x10^{-9}$ to $3x10^{-6}$ M) were constructed by adding the compounds at 1 min intervals in 0.5 log M increments. Responses were measured as the algebraic sum of the increase in 'twitch' height and base-line contraction and were expressed relative to a bolus challenge with 5-HT ($1x10^{-5}$ M) given 1.5 h previously. Tissues were incubated with 5-MOT ($1x10^{-5}$ M) or 2-M-5-HT ($1x10^{-5}$ M) or both for 30 min before testing and, unless otherwise stated, were retained in the organ bath during the construction of concentration-effect curves.

5-MOT and 2-M-5-HT contracted the ileum, but the response exhibited fade and the original base-line tension was recovered in 2-5 min. 'Twitch' height often remained elevated after 5-MOT, but not 2-M-5-HT. 5-MOT abolished selectively the first phase of the concentration-effect curve to 5-HT, whereas 2-M-5-HT abolished selectively the second phase. 5-HT was totally inactive in preparations pretreated with both 5-MOT and 2-M-5-HT. The effect of 5-MOT and 2-M-5-HT showed selectivity as incubation with both agonists failed to alter responses to DMPP and carbachol. Furthermore, 5-MOT failed to alter the affinity of ICS 205-930 for the 5-HT_3 receptor ($pA_2 = 8.0$) and 2-M-5-HT did not alter the affinity of ICS 205-930 for the novel 5-HT receptor ($pA_2 = 6.6$; Craig & Clarke, 1989). Interestingly, 5-MOT, but not 2-M-5-HT, abolished completely the concentration-effect curve to renzapride. This effect was reversed completely after washing out 5-MOT.

The present experiments demonstrate that 5-MOT and 2-M-5-HT can be used as tools to discriminate the novel 5-HT receptor in the ileum from the 5-HT_3 site. This finding is of importance as no selective antagonist exists for the novel site (Clarke *et al.*, 1989). Finally, the result obtained with renzapride forges a link between the novel site in the ileum and the 5-HT_4 receptor described by Dumius *et al.* (1989).

This work was supported by NIH Grant NS24871.

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216P CHARACTERISATION OF 5-HT RECEPTORS MEDIATING CONTRACTIONS OF GUINEA-PIG ILEUM, *IN VITRO*

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The concentration-response curve to 5-hydroxytryptamine (5-HT) in the isolated guinea-pig ileum is biphasic (Buchheit et al. 1985). The second phase is due to stimulation of 5-HT_3 receptors whilst the receptors mediating the initial phase remain to be characterised (Buchheit et al. 1985).

Isolated portions (1.5 cm) of proximal ileum from male guinea-pig (Dunkin-Hartley, 300-350 g) were suspended under 1.0 g tension in Tyrode solution (pH 7.4, 37°C) containing 1 μM methysergide (to antagonise 5-HT_1-like and 5-HT_2 receptors) and 1 μM GR 38032F (to antagonise 5-HT_3 receptors). Concentration-response curves to agonists were constructed in a non-cumulative fashion (30 s exposure on a 5 min dose-cycle), and antagonist equilibration periods were 60 min. All values quoted are mean ± s.e. mean from 6-10 preparations. Concentration-dependent contractions were observed to 5-HT, 5-methoxytryptamine, BR 24924, and zacopride (-log EC_{50} values were 7.5 ± 0.8, 7.0 ± 0.03, 7.0 ± 0.08 and 6.3 ± 0.12, respectively). Zacopride acted as a partial agonist (intrinsic activity = 0.82) with respect to 5-HT and the dissociation constant (-log K_A) of 6.1 ± 0.08. 2-Methyl-5-HT, GR 43175 and 8 OHDPAT were inactive either as agonists or antagonists at the concentrations studied (10 nM - 10 μM) and no agonist responses were observed with BRL 43694, ICS 205-930 or GR 38032F (1 nM - 10 μM). The responses to 5-HT, 5-methoxytryptamine and zacopride were abolished in the presence of atropine (1 μM) or tetrodotoxin (0.1 μM) and were enhanced in terms of maximal response, but not the potency, in the presence of 0.1 μM physostigmine. The responses to all three agonists were unaffected in the presence of 1 μM GR 38032F, BRL 43694, quipazine or n-methylquipazine. ICS 205-930 (3 μM) antagonised responses to 5-HT, 5-methoxytryptamine and zacopride (-log K_B values = 6.3 ± 0.12, 6.0 ± 0.09 and 6.5 ± 0.14, respectively). ICS 205-930 (10 μM) did not affect responses to carbachol either in terms of potency (control -log EC_{50} = 6.5 ± 0.04; ICS 205-930 = 6.6 ± 0.06) or maxima obtained.

In conclusion, the above results appear to involve 5-HT receptors distinct from 5-HT_1-like, 5-HT_2 or 5-HT_3 subtypes which, when stimulated, elicit acetylcholine release. Pharmacologically, the receptors appear similar to those mediating enhancement of 'twitch' response in the field stimulated ileum (Craig and Clarke, 1989) or adenylate cyclase activation in the CNS (Dumius et al., 1988a,b) and may be a peripheral example of a 5-HT_4 receptor.

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217P ICI 170809: APPARENT ANTAGONIST AFFINITY DIFFERENCES FOR 5-HT BETWEEN RABBIT AORTA, RAT CAUDAL ARTERY AND PIG CORONARY ARTERY

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We have previously shown that the novel 5-HT antagonist - ICI 170809 -possesses high affinity for the 5-HT_2 receptor in vitro in isolated tissues and radioligand binding studies (Blackburn et al 1988). We now have evidence that ICI 170809 can reveal different apparent antagonist affinities between tissues previously thought to contain a homogenous population of 5-HT receptors.

Our studies have investigated the 5-HT-antagonist effects of ICI 170809 on pig coronary artery rings, rabbit aortae and rat caudal artery strips. The tissues were set up in organ baths under resting loads of between 0.5 and 2.0g and bathed in Krebs solution at 37°C gassed with 95% oxygen and 5% CO_2. After obtaining control concentration-response curves to 5-HT, ICI 170809, $1x10^{-9}$-$1x10^{-7}$M, was incubated with the tissues for 30 min. after which time the responses to 5-HT were repeated. Antagonist activity was then calculated from the concentration-ratios obtained at each concentration of ICI 170809 and affinities for the 5-HT receptor calculated by construction of a Schild plot and determination of the pA_2 values.

In the rat caudal artery ICI 170809 produced potent antagonism of the 5-HT-induced contractions, the Schild-plot analysis yielding a pA_2 value of 9.78±0.12 and although the slope of the line was not different from unity it was low (0.73). In the pig coronary artery and rabbit aorta ICI 170809 appeared to be significantly weaker ($p<0.05$) as an antagonist of 5-HT-induced contractions. The Schild-plot analysis in these instances yielded pA_2 values of 8.11±0.29 and 8.18±0.04 respectively. However, although again the slopes of the lines were not significantly different from 1.00, in the case of the coronary artery the slope was unusually high (1.33).

The apparent differences in antagonist affinities that ICI 170809 has revealed between these tissues could indicate heterogenous populations of 5-HT receptors. Indeed, this was the conclusion reached by Bradley et al (1986) in describing differences in activity between different tissues with trazadone. However, erring on the side of caution these authors suggested that the involvement of complicating experimental factors might account for the differences. Indeed, the slopes of the Schild plots obtained in our experiments could indicate mechanisms being involved other than simple competition.

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218P 5-HT STIMULATES DIACYLGLYCEROL PRODUCTION IN RABBIT ISOLATED CEREBRAL ARTERIES

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5-Hydroxytryptamine (5-HT) contracts the rabbit basilar artery by mechanisms largely independent of a change in the smooth muscle membrane potential (Garland, 1987). The contractions are blocked by the protein kinase C inhibitors H7 and polymyxin B, inferring a role for this enzyme in the contractile response of the basilar artery to 5-HT (Clark & Garland, 1989). Physiologically, protein kinase C is activated by membrane 1,2 diacyl-sn-glycerol (DG) which is normally formed by the hydrolysis of polyphosphoinositides (Berridge, 1984).

We have now measured directly the membrane levels of DG in the basilar artery, after conversion to phosphatidic acid with E. coli DG kinase (Amersham International DG Kit) in the presence of ^{32}P- -ATP. Artery segments were stimulated with 5-HT (10^{-8}-10^{-4} M) for periods of between 30 seconds to 5 minutes. Time and concentration-dependent increases in DG concentrations were obtained in response to 5-HT. Maximal accumulation of DG occurred after 30 seconds, when significant ($P<0.01$) increases ranging from 19% to 73% above basal values were obtained (n=6-16). The maximum response to 5-HT was obtained with 10^{-6} M. Increases in response to 5-HT were not as marked after longer periods of exposure. 10^{-6} M 5-HT increased DG concentrations by 58% after 30 seconds, whereas after 2 and 5 minutes the increases were only 37% and 24%, respectively (n=17). The 5-HT-stimulated increase in DG was completely blocked by the putative phospholipase C inhibitor 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate ($7x10^{-5}$ M), which inhibits phosphoinositide hydrolysis in vascular smooth muscle cells (Nakaki et al, 1985).

The results show that 5-HT can produce DG in the basilar artery, which probably reflects the activation of a membrane phospholipase C. Together with the observed inhibition of 5-HT-induced contraction in this artery with protein kinase inhibitors, they suggest an important contribution from protein kinase C in the contractile response of cerebral arteries to 5-HT.

This work was supported by the Wellcome Trust.

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219P EFFECTS OF 5-HT, METITEPINE AND KETANSERIN ON THE RELEASE OF ENDOGENOUS NORADRENALINE (NA) FROM THE RAT TRACHEA *IN VITRO*

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A sympathetic innervation of the airway smooth muscles has been demonstrated in several mammalian species (see Gabella, 1987) and functional studies showed inhibitory sympathetic effects on the airway smooth muscle tone. However, little is know about a possible presynaptic regulation of the release of NA in the airways. Recently we described that the impulse-induced release of endogenous NA can be measured from the isolated rat trachea incubated in vitro (Racké et al., 1989a).

The rat trachea was isolated, cut open at the ventral side and incubated in 1 ml Krebs-HEPES solution which contained the neuronal uptake inhibitor desipramine (1 µM) and, in addition, yohimbine (1 µM), in order to prevent the autoinhibition of NA release, and tyrosine (10 µM), in order to allow a sufficient de novo synthesis of NA (Racké et al., 1989a). The medium was changed every 10 min and NA released into the medium was determined by HPLC with electrochemical detection (Racké et al., 1989b). Two periods of electrical stimulation (S1 and S2, each 3 Hz 3 times for 1 min with 1 min intervals) were caried out, S1 after 50 min and S2 after 100 min of incubation.

The spontaneous outflow of NA determined immediately before S1 was about 5 pmol/g/10 min. S1 induced the release of 49 $\pm$ 3.5 pmol/g (n=53). In control experiments the ratio S2/S1 was 0.94 $\pm$ 0.04 (n=10). 5-HT, added 20 min before S2, reduced the evoked release of NA by 30 and 45 % at 1 and 10 µM, respectively. Metitepine (1 µM) alone had no effect on the release of NA, but shifted the concentration response curve for 5-HT to the right (apparent pA_2-value of 7.7). Ketanserin, added 20 min before S2, reduced the evoked release of NA by 30 and 36 % at 0.1 and 1 µM, respectively. 0.1 µM ketanserin, present 40 min before S2, reduced the release of NA by 45 %. When 0.1 µM ketanserin was present from the onset of incubation, the release of NA evoked by S1 was not affected, but that evoked by S2 was reduced by 40 %.

Ketanserin reduced the release of endogenous NA from the isolated vena cava inferior, incubated and stimulated electrically as described above for the trachea, in a similar manner.

In conclusion the release of endogenous NA from the rat trachea is inhibited by presynaptic 5-HT receptors. Ketanserin inhibits the impulse-induced release of endogenous NA by a mechanism unrelated to 5-HT receptors, possibly be interferring with the de novo synthesis of NA or with the refilling of the NA stores with newly synthesized NA. This direct reduction of the sympathetic neurotransmission may contribute to the - up to the present not fully understood - antihypertensive effect of ketanserin.

Supported by NMFZ University of Mainz and the Deutsche Forschungsgemeinschaft

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220P THE EFFECT OF LDL ON THE CONTRACTILE RESPONSE TO 5-HT IN RABBIT AORTA

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Individuals with modest atherosclerotic lesions are often prone to vasospasm. A potentiation of the contractile responses of 5HT have been observed in animal models of the disease (Verbeuren et.al., 1986; Lopez,et.al., 1989). As low-density lipoproteins (LDL) and their oxidised products are known to have vasoactive effects and are present in atherosclerotic lesions, we have examined the possibility that native or oxidized LDL might enhance the contractile response to 5HT.

LDL (density 1.019-1.063g/ml) were prepared from fresh human plasma by discontinuous gradient ultracentrifugation in the presence of 0.3mM EDTA. Oxidative modification was carried out by incubation of LDL with 5 M Cu2+ for 24hr. (Parthasarathy et al. 1985) followed by extensive dialysis against Tyrode's buffer. The contractile response to 5-HT and endothelium-dependent relaxation were assayed using rings from isolated thoracic aortae of 6 month-old New Zealand White rabbits (Andrews et al. 1987). Rings were contracted to cumulative doses of 5-HT in the presence of native LDL or oxidized LDL at 2 mg protein/ml or Tyrode's buffer (control). Tissues were then washed and contractions repeated to determine the reversibility of the effect.

Contractile responses to 5HT in both intact and endothelium-denuded rings were unaltered by the presence of native LDL whereas those to noradrenaline were attenuated (Andrews et al. 1988). Oxidized LDL from all the donors tried, on the other hand, caused an immediate reversible increase in sensitivity to 5-HT in endothelium-denuded rings as shown by the ten-fold leftward shift in the dose response curve. This effect was less if endothelium was present.

We have shown that oxidized LDL causes a reversible enhancement of 5-HT responses whereas native LDL inhibits responses to NA. These effects mimic similar changes in reactivity found in isolated atherosclerotic aorta from cholesterol-fed rabbits (Verbeuren et al. 1986).

Supported by British Heart Foundation.

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221P COMPARISON OF THE EFFECTS OF METHYSERGIDE AND METHYLERGOMETRINE WITH GR43175 ON FELINE CAROTID BLOOD FLOW DISTRIBUTION

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The novel anti-migraine agent GR43175 acts at 5-HT_1-like receptors to decrease the shunting of blood through feline carotid arteriovenous-anastomoses (AVAs) (Perren *et al*, 1988). Methysergide, however, which is useful only in the prophylactic treatment of migraine, not only reduces AVA shunting but increases nutrient (non-AVA) blood flow in pigs (Saxena and Verdouw, 1984). Since it is now believed that methysergide's metabolite, methylergometrine is the active principle and, like GR43175, can abort migraine headache acutely (Bianchine and Eade, 1969; Doenicke *et al*, 1988), we have compared the effects of these agents on the partitioning of feline carotid blood flow.

Male cats (2.6-3.3kg) were anaesthetised using chloralose (60mg/kg) and pentobarbitone sodium (20mg/kg), and artificially ventilated. Left common carotid blood flow distribution was measured by injection of radiolabelled microspheres into the carotid circulation via the lingual artery (Spierings and Saxena, 1980). Animals (n=5 each group) received (iv bolus) either saline, methysergide (30-1000ug/kg), methylergometrine (3-100ug/kg), or GR43175 (30-1000ug/kg).

Prior to drug administration 51.4±2.4% (mean±s.e.mean, n=20) of carotid blood flow (23.6±1.1 ml min^{-1}) was shunted through AVAs whilst 34.0±1.4% and 13.4±1.0% was distributed to extracerebral and cerebral tissues respectively. In the saline control group, 5 estimates of AVA, cerebral and extracerebral conductance were consistent over 1 hour. GR43175 caused a dose-related fall in AVA conductance (max: 92.2±2.1%) but without any change in extracerebral or cerebral conductance. Unlike this selective action of GR43175, methysergide (M) and methylergometrine (ME) had a dual effect: each caused a dose-related fall in AVA conductance (max: 90.4±2.0%, M; 89.1±4.2%, ME) and at higher concentrations an increase in both cerebral (max: 118±51.5%, M; 75.5±29.4%, ME) and extracerebral conductance (max: 104.8±23.4%, M; 95.2±52.4%, ME). This increase in conductance was associated with a dose-dependent fall in blood pressure (BP) (Δ diastolic BP: 49±3mmHg, M; 48±10mmHg, ME).

Therefore, methysergide and methylergometrine reduce AVA shunting, presumably via 5-HT_1-like receptor activation (MacLennan *et al*, 1988), but also increase nutrient blood flow by an unknown mechanism. In contrast, our results confirm that GR43175 selectively reduces AVA blood flow (Perren *et al*, 1988). Evidently, the different effects of these agents on feline carotid blood flow distribution do not explain their different clinical applications in the therapy of migraine.

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222P INVOLVEMENT OF CAPSAICIN-SENSITIVE AFFERENT NEURONS IN THE ACID SECRETORY RESPONSES OF THE RAT STOMACH

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Afferent neurons, sensitive to the selective neurotoxin capsaicin, are present in the stomach (Sharkey et al., 1984). There is evidence that these neurons participate in various aspects of the gastric physiology such as mucosal protection or regulation of blood flow (Lippe et al., 1989; Esplugues et al., 1989). We have now studied the effects of capsaicin pretreatment on the acid secretory responses of the rat stomach following either direct stimulation of the oxyntic cell with carbachol (4 µg/kg i.p.) or vagally mediated responses induced by hypoglycemia (insulin 0.3 I.U./kg i.p.) or gastric distention (20 cm H_2O).

Capsaicin (50 mg/kg) or vehicle (10% ethanol, 10% Tween 80, 80% saline) was administered s.c. in 2 day old rats under ether anaesthesia and the animals used 3-4 months later. After fasting for 24 h rats were anaesthetized with urethane (1.5 g/kg i.p.), and two polyethylene cannulae inserted into the gastric lumen through the oesophagus and duodenum. The stomach was continuously perfused with saline (0.9 ml/min) and acid output was determined by automatic titration of the perfusate to pH 7 with 0.01 N NaOH. Acid secretion was stimulated once the acid output had remained constant for 60 min (basal). In some experiments, the vagus was cut bilaterally or the celiac mesenteric ganglion complex removed.

Basal acid secretion (3.5 ± 0.9 µEq/100g/20min, mean±s.e.mean, n=21) was not affected by capsaicin pretreatment (2.9±0.7 µEq/100g/20min, n=13). After administration of carbachol (n=7) or insulin (n=7) acid output peaked (35±3.7 and 21.8±7.2 µEq/100g/20min respectively) 60 or 100 min later. These values were not significantly influenced by capsaicin pretreatment (34.5±6.8 and 30±6.9 µEq/100g/20min, n=3 and n=5 respectively) or celiac ganglionectomy (31.3±5.9 and 26±6.3 µEq/100g/20min, n=3). Vagotomy abolished the acid response to insulin. Distention induced an increase in acid secretion which peaked 140 min later (24 ± 5.7 µEq/100g/20min, n=7). Capsaicin pretreatment, celiac ganglionectomy or vagotomy abolished the acid-response to gastric distention.

These findings suggests that capsaicin-sensitive afferent neurons, located in the vagus and/or in the celiac ganglia, are involved in the nervous reflex mediating the acid secretory responses to gastric distention.

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223P EFFECT OF LUMINAL NH_3 ON ALKALINE SECRETION BY GUINEA-PIG STOMACH *IN VITRO*

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Helicobacter (formerly Campylobacter) pyloris infection of the stomach is associated with increased luminal NH_3 concentration and NH_3 has been reported to damage the mucosa of rat stomach in vivo (Murukami et al, 1988). We have examined the effect of luminal NH_3 on alkaline secretion (AS) by guinea pig stomach under in vitro conditions.

The methods have been described to the Society previously (Canfield & Spencer, 1989). Acid secretion was supressed by SCH 28080 (50 uM) and the serosal side buffered by HCO_3^-/CO_2. Measurement of AS is complicated by the alkalinising effect of NH_3 and no attempt was made to measure AS during the 15 min exposure to NH_3. Subsequent measurements were contaminated by NH_3 washout from the tissues so control experiments were performed where the serosal side was buffered with Hepes/O_2 which abolishes AS in this tissue. NH_3 is expresed as % (v/v) dilution of a solution containing 28-30% NH_3 (Aldrich Chemical, Poole). AS is expressed as the change from preceeding basal values (ΔAS) in μmole $cm^{-2}h^{-1}$ and are mean and s.e. mean (n = 5 or 6 for all). A typical basal value for AS was 1.1 ± 0.21 μmole $cm^{-2}h^{-1}$.

Below 0.35% NH_3 there were no significant differences between HCO_3^- and Hepes buffered tissues except for the first 15 min period after washout when HCO_3^- was greater. Increasing NH_3 up to 2% lead to a sustained increase in AS of 2-4 μmole $cm^{-2}h^{-1}$ in HCO_3^- tissue whereas in Hepes tissue AS fell to basal levels within 75 min. Hepes tissues treated with 0.75% NH_3 and allowed to recover to basal value were then exposed to HCO_3/CO_2 buffer. There was a rapid increase in AS to values not significantly different from those seen in tissues in HCO_3^-/CO_2 throughout. Prior exposure to 0.1% NH_3 had no effect on subsequent exposure to 0.75% compared with control tissues exposed only to 0.75% (AS test 2.49 ± 0.32, control 2.16 ± 0.43). Luminal exposure to either NaOH or NH_4Cl (1 mM) had no effect on AS.

Luminal exposure for 15 mins to NH_3 above 0.35% in HCO_3^-/CO_2 buffered tissues lead to a sustained increase in AS. In view of the ability of NH_3 to damage gastric mucosa in vivo (Murukami et al, 1988) and gastric cells in vitro (Canfield & Gillen, this meeting), this probably represents an increase in passive permeability to HCO_3^- through damaged tissue and is qualitatively similar to results obtained with luminal ethanol (Canfield & Spencer, 1989).

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224P OESTRADIOL INCREASES HISTAMINE LEVELS AND ACID CONTENT OF MOUSE STOMACH

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Nausea and vomiting are the most frequent gastrointestinal disturbances associated with the use of oestrogens in clinical practice. The mechanism of this is not clear. Recent evidence has suggested that histamine may be involved in the reflex pathway that controls vomiting. H_1 receptors have been located in areas which form an integral part of this pathway including the nucleus of tractus solitarius, dorsal motor nucleus and vestibular nuclei (see Rang & Dale, 1987). In the present study we have investigated if oestrogen can affect histamine levels and acidity in the mouse stomach.

Ovariectomized mice of LACA breed were randomly divided into groups of six and oestradiol dipropionate (ED) in 0.5 ml ES (2% ethanol in 0.9% NaCl) was injected s.c. Control animals were given 0.5 ml ES. The animals were killed at various intervals following injection and their stomach removed. This was washed free of gastric contents using 10 ml distd.water. The effluent was centrifuged and supernatant estimated for acidity using a pH meter and also by the titration method. The histamine content of the stomach was measured as described earlier (Sharma & Jande, 1989). The results shown in table 1 indicate that oestradiol

Table 1 Effect of oestradiol on gastric histamine in the mouse

ED (ng/g body wt.)	Tissue histamine (µg/g) mean ± SEM			
	At 3 h		At 24 h	
	Control	Injected	Control	Injected
5	6.39 ± 0.81	9.48 ± 1.17*	7.11 ± 0.70	11.38 ± 0.97**
10	7.85 ± 0.71	11.86 ± 1.01**	6.47 ± 0.61	13.39 ± 1.08**
20	6.14 ± 0.70	14.03 ± 1.12**	7.06 ± 0.85	12.80 ± 1.17**

$P < 0.05$* or < 0.01**

causes a dose dependent increase in histamine content of stomach. A significant increase is seen within 3 h and is persistant even at 24 h after ED injection. This is unlike the rat uteruswhich is depleted of histamine following oestradiol injection (Spaziani & Szego, 1958). In the present study ED also caused an increase in gastric acidity.

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225P COLLOIDAL BISMUTH SUBCITRATE AND ARACHIDONIC ACID PROTECT GUINEA-PIG ISOLATED GASTRIC CELLS FROM DAMAGE BY AMMONIA

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Helicobacter (formerly Campylobacter) (HP) infection may be associated with gastritis and peptic ulceration (Dooley & Cohen, 1988). HP has high urease activity and patients show elevated gastric juice NH_3 concentration (Yoshida et al, 1989). We have examined the effect of NH_3 on the viability of cells isolated from guinea pig gastric mucosa.

Cells were isolated from female guinea pigs (250-350g) using the method described by Lewin et al (1974) for rats. Everted gastric sacs were filled with a Ca^{2+}-Mg^{2+} - free saline containing pronase 10 mg ml^{-1}, 2mM EDTA, 2% BSA buffered with Hepes to pH 7.4 and suspended in a similar solution but lacking EDTA and BSA for 90 min at 37°C. Sacs were then transferred to a solution containing Ca^{2+} and Mg^{2+} (1mM) and 1% BSA and mechanically stirred for 30 min to release cells which were collected by centrifugation (100g, 10 min), washed 3x and then resuspended in fresh solution at 37°C (approx 10^7 cell/ml). Average yield per stomach was 1.1 ± 0.11 x 10^8 and viability by trypan blue exclusion 92.8 ± 1.2% (mean, s.e.mean, n=16). Cell number and viability did not change significantly over 3h. NH_3 is expressed as % (v/v) dilution of a solution of NH_4OH containing 28-30% NH_3 (Aldrich Chemical, 16.5M).

Exposing cells to NH_3 for 5 mins (0.03-2%) progressively damaged them mainly by reducing cell number with only small effects on the viability of surviving cells. At 0.25% NH_3 cell survival was 50% of control and at 1% NH_3 it was 30%. Treating cells with KOH or NH_4CL at concentrations equivalent to 1% NH_3 gave survival rates of 90% and 68% respectively. 1%NH_3 has an osmolarity of 378 mOsm kg^{-1}, treating cells with 100 mM urea (362 mOsm kg^{-1}) gave 70% survival compared with 38% with 1% NH_3. Pretreatment of cells for 30 min, prior to exposure to 0.5% NH_3, with either arachidonic acid (30μM) or DeNol (10mg ml^{-1}) increased survival from 49 ± 1% to 75 ± 2% (n=4) and 45 ± 4% to 88 ± 7% (n=4) respectively. Similar results were obtained with acetaminophen pretreatment (10mM). In all three cases the effects were blocked by indomethacin (10μM).

The results suggest that NH_3 at concentrations similar to that found in gastric juice of HP-patients can damage gastric cells and that De Nol and arachidonic acid can antagonise this via products of cycloxygenase activity.

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226P AN *IN VITRO* INVESTIGATION OF THE PEPSIN INHIBITORY PROPERTIES OF SEVERAL BISMUTH COMPOUNDS

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The activity of pepsins is greatest at low pH, and this family of enzymes has been implicated in the pathogenesis of peptic ulceration (Samloff & Taggart, 1987). Bismuth compounds have been used successfully to treat peptic ulcers but it has long been recognised that the antacid properties of most bismuth compounds are minimal. A series of in vitro experiments was undertaken to investigate if eight bismuth compounds, including the recently introduced tripotassium dicitratobismuthate (TDB) have any pepsin inhibition properties independent of the pH effect. Using a simulated gastric juice containing porcine pepsin A at 37°C (United States Pharmacopeia, 1985) a range of concentrations of the compounds was incubated for 20 min and the pH of each mixture was recorded after incubation. Pepsin activity was assayed using a modified method of Anson & Mirsky (1933).
All the bismuth compounds examined were able to inhibit pepsin activity to some extent and this inhibition was largely independent of pH changes (Table 1). The significance of this finding is important in the treatment of gastric ulceration.

Table 1 lists the concentration of eight bismuth compounds which inhibit 50% of the porcine pepsin in the simulated gastric juice (IC_{50}) and the pH change in this juice at this value after 20mins incubation.

Compound	IC_{50} value (mM)	Δ pH
Bismuth Carbonate	10.4	-
Bismuth Nitrate Pentahydrate	5.8	- 0.14
Bismuth Oxide	28.3	< + 0.10
Bismuth Oxychloride	103.6	-
Bismuth Salicylate	2.7	+ 0.02
Bismuth Subgallate	118.8	-
Bismuth Subnitrate	36.9	+ 0.05
Tripotassium Dicitratobismuthate	2.2	+ 0.05

We wish to thank Gist-Brocades Pharmaceuticals for the gift of TDB.

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227P EVIDENCE THAT PHRENIC NERVE ENDINGS POSSESS INHIBITORY AND EXCITATORY ADENOSINE RECEPTORS

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The effect of the adenosine analogue 5'-N-ethylcarboxamide adenosine (NECA) on [^{3}H]-acetylcholine ([H]ACh) release from phrenic nerve endings of the rat was compared in the absence and in the presence of 1,3-dipropyl-8-cyclopenthylxanthine (DPCPX), a potent antagonist of the adenosine receptor mediating inhibition of synaptic transmission in the rat neuromuscular junction (Ribeiro & Sebastião, 1990), or DPCPX plus PD 115.199, an antagonist of the A_2 adenosine receptor.

The experiments were performed at 37°C on rat phrenic nerve-hemidiaphragm preparations gassed with 95% O_2 and 5% CO_2. The bathing solution contained (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_3PO_4 0.4, $NaHCO_3$ 11.9, glucose 11.2, unlabelled choline chloride 0.001). Labelling was carried out for 40 min by incubation with 2.5 µCi/ml (methyl-[^{3}H])choline chloride (see Wessler & Kilbinger, 1986). During this period the phrenic nerve was stimulated at 1 Hz. After the labelling, hemicholinium-3 (10 µM) was added to the bathing solution. [^{3}H]ACh release was evoked by nerve stimulation at 5 Hz during 3 min. Three stimulating periods, separated by resting periods of 24 min, were used. The evoked release of [^{3}H]ACh was calculated by subtracting the spontaneous tritium outflow from the total tritium outflow during each stimulation period. In the absence of test drugs the evoked tritium outflow during each stimulation period (S_1, S_2 and S_3) was of similar magnitude. NECA was usually applied between S_2 and S_3 and its effect was calculated by comparing the S_3/S_2 ratio with the S_2/S_1 ratio. DPCPX and PD 115.199 were applied 15 min before S_1.

NECA (30-300 nM) decreased in a concentration-dependent manner the evoked release of [^{3}H]ACh, the average effect of 300 nM NECA being 64±8% (n=3) inhibition. This inhibitory effect of NECA was reversed into an excitatory effect by DPCPX; in the presence of DPCPX (2.5 nM), NECA (300 nM) increased the evoked release of [^{3}H]ACh by 65±7% (n=3). The excitatory effect of NECA (300 nM) on [^{3}H]ACh release was antagonized in a concentration-dependent manner by PD 115.199 (5-25 nM), i.e. NECA (300 nM) increased [^{3}H]ACh release by 34±11% (n=2) in the presence of DPCPX (2.5 nM) + PD 115.199 (5 nM) and by 7±9% (n=2) in the presence of DPCPX (2.5 nM) + PD 115,199 (25 nM). Neither DPCPX (2.5 nM) nor PD 115.199 (5-25 nM) affected the release of [^{3}H]ACh when applied in the absence of NECA.

The results suggest that at the phrenic motor nerve endings are present both inhibitory and excitatory xanthine-sensitive adenosine receptors, which modulate the evoked release of acetylcholine.

We thank Dr. R.F. Bruns and Parke-Davis Pharmaceutical for gift of PD 115.199.

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228P EFFECTS OF LONG-TERM ADMINISTRATION OF DILEVALOL AND PINDOLOL ON BLOOD PRESSURE AND MYOCARDIAL HYPERTROPHY IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS

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Dilevalol (D) and pindolol (P) are nonselective ß-blocking agents with partial agonist activity occurring through the stimulation of ß-2 adrenoceptors (D) or of both ß-1 and ß-2 receptor subtypes (P). To determine whether the different activity of these drugs may prevent the development of hypertension and left ventricular hypertrophy (LVH), we conducted experiments in spontaneously hypertensive rats (SHRs).

Groups of 7-week-old male SHRs were treated with D (30 mg/kg/day) or P (3 mg/kg/day) in drinking water for 3 months. Age-matched, untreated SHRs and Wistar Kyoto rats (WKY) were used as controls. Systolic blood pressure (SBP) and heart rate (HR) were measured weakly during the first month and monthly thereafter by the tail cuff method. The animals were then sacrificed, the hearts were fixed by perfusion, dissected into right and left ventricle including the septum and weighed. Ten blocks of each LV were processed for light microscopy and the fraction of viable and scarred tissue evaluated.

Both treatments attenuated the rise in SBP ($p<0.01$) maintaining the pressure within the level found in WKY. HR was significantly lowered in D-group ($p < 0.01$) in comparison with other groups. In contrast, LV weight (LVW) to body weight (BW) ratios were not statistically different from those of control SHRs.

	SBP (mm Hg)		HR (beat/min)		LVW/BW
	7-Week	19-Week	7-Week	19-Week	(mg/g)
Control WKY	100 ± 3.6	143 ± 2.5	410 ± 10.5	377 ± 7.0	2.44 ± 0.07*
Control SHR	121 ± 2.7	207 ± 3.0**	433 ± 7.3	409 ± 7.1	2.92 ± 0.12
D 30 mg/kg	118 ± 1.8	160 ± 4.0	442 ± 6.5	344 ± 7.3**	2.89 ± 0.08
P 3 mg/kg	122 ± 3.0	167 ± 2.9	440 ± 6.3	389 ± 3.8	3.05 ± 0.07

* $p<.05$; ** $p<.01$ compared with other groups (n = 10)

The morphological analysis of the myocardium demonstrated a significant increase of scarred tissue in SHRs with respect to WKY. The D-treatment decreased the damage, whereas P-treatment increased the extension of fibrotic tissue.

These data indicate that both D and P are able to prevent the rise in pressure in young SHRs, but do not influence the degree of LVH. LV fibrosis, was partially reduced in D-group. Thus, the different partial agonist properties of the two drugs may be of importance in the maintenance of myocardial integrity in SHRs.

229P EFFECTS OF VARIOUS CALCIUM ANTAGONISTS AND OF CALCIUM IONS IN ISOLATED HEARTS OF DIABETIC AND NORMAL RATS

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Previous studies have demonstrated that changes in calcium metabolism may play an important role in the pathogenesis of cardiovascular damage in diabetes mellitus (Gøtzche, 1986). The present study was undertaken to investigate the effects of the calcium antagonists nifedipine, diltiazem, verapamil, two stereoisomers of devapamil and Ca^{++} ions, respectively, upon the left ventricular function in hearts of diabetic rats (DR), in comparison with hearts of age-matched control rats (CR).
Diabetes was induced by i.v. injection of streptozotocin (50 mg/kg), dissolved in citrate buffer (pH 4.5). CR were injected with citrate buffer alone. At 45 days after STZ injection, hearts of both groups were perfused with Tyrode solution (Ca^{++}, 1.8 mM, 37°C) according to the Langendorff technique and stimulated at 5 Hz. The left ventricular pressure (via an intraventricular balloon), the first derivate of the LVP (dP_{max}/dT) and the coronary flow (ml/min/g wet heart weight) were determined.
Drugs were added to the perfusate via a PE 100 catheter placed just above the heart. Body weight (396±10 vs 260±8 g), heart weight (1.53±0.18 vs 1.22±0.26 g, $p<0.05$) and glucose levels (5.8±1.2 vs 22.4±3.0 mM) were determined, for CR and DR, respectively.
The initial values for contractile force (LVP, $dP_{max}dT$) were not significantly different in hearts of DR and CR.
Calcium ions (0.9-9.9 mM) induced a similar positive inotropic effect (LVP, $dP_{max}dT$) in hearts of DR and CR, respectively.
A strong leftward shift of the concentration-response curve for diltiazem was observed in hearts of DR in comparison with those of CR (EC_{50} values, 1.8×10^{-6} vs 10^{-5} M). For the diphenylalkylamines verapamil and the l- and d-isomers of devapamil a moderate leftward shift was observed. Nifedipine did not induce a differential effect with respect to the inotropy. In addition, coronary flow was not significantly different in DR and CR (6.9±1.7 vs 6.5±1.4 ml/min/g). All calcium antagonists induced an increase in coronary flow (range 64-101% of the initial value). However, in hearts of DR a stronger vasodilator activity was observed for all calcium antagonists with the exception of verapamil (range 95-128% of the initial coronary flow). We conclude that the effects of calcium antagonists from various classes are differential in hearts of DR in comparison with CR.

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230P ESTIMATION OF SYNCHRONY OF CARDIAC REPOLARISATION BY SIMULTANEOUS ON-LINE ACQUISITION OF 31 LOCAL ELECTROCARDIOGRAMS FROM THE DOG HEART *IN SITU* USING A MICROCOMPUTER

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Many studies concerned with cardiac electrophysiology and, in particular, with the mode of action of antiarrhythmic drugs, have depended on recordings of action potentials *in vitro* or on measurements of repolarisation or refractoriness at single sites on the heart *in vivo*. Such procedures provide information on cellular mechanisms of drugs, but not on the ways in which these mechanisms may be expressed in the whole heart. Normally, repolarisation occurs synchronously within the myocardium. However, local disturbances in conduction or repolarisation can induce electrical inhomogeneities observed as dispersion of repolarisation across the heart. This may indicate increased vulnerability to malignant cardiac arrhythmias, such as ventricular tachycardia and fibrillation (Han & Moe, 1964). We have developed a microprocessor system to measure dispersion of repolarisation, based on the Motorola 68000 with 128kB of memory, fast (1kHz) analogue to digital conversion and high resolution graphics display which can simultaneously acquire 31 separate local electrocardiograms from discrete areas of the heart and store them to disk for subsequent analysis.

Individual unipolar electrograms are first fed into a 31 channel amplifier (designed and built by Pfizer electronics section). This has an input impedance of 3.3MΩ and amplifies each signal to a peak amplitude of 1.25V by means of an autogain circuit (acceptable input range is 20μV - 50mV). Analogue filtering is then applied to give 3dB points of 0.1Hz and 500Hz. Data acquisition during cardiac pacing is initiated by a pulse (2ms, >5V, synchronised with the pacing stimulus) *via* one channel of the A/D converter. 500ms of data on each channel is then acquired. All signals are then written to disk, and then immediately read back and compared with the original data to ensure that there are no disk errors; if errors do exist it is possible to replace the faulty disk with no loss of data. Subsequent to this, the records are displayed on the VDU for inspection. Analysis of data takes place off-line; the records for any acquisition run are read from disk into memory. Each individual record is displayed on the VDU together with its differential, used to define the period of both activation and repolarisation times by the method of Millar *et al* (1985), and cursors are drawn to indicate the points of activation and repolaisation. Activation time, repolarisation time and activation repolarisation interval are displayed in milliseconds; median values and dispersions (interquartile ranges) of parameters from each run are then calculated. Displayed electrograms can be transferred to an on-line plotter (Epson HI-80) where they are scaled down to allow all electrograms for any acquisition run to be presented on a single sheet.

Thus, we have assembled a powerful system for the simultaneous acquisition of a number of channels of data at high resolution from readily available and relatively inexpensive components. Such systems have an important role in the investigation of the actions of new antiarrhythmic drugs (Gwilt *et al*, 1990).

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231P TEDISAMIL POSSESSES DIRECT DEFIBRILLATORY ACTIVITY DURING MYOCARDIAL ISCHAEMIA AND DURING REPERFUSION

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In ventricular tissue, at 1μM, tedisamil (Kali-Chemie AG, Hannover) prolongs functional refractory period, prolongs action potential duration and slows sinus rate without reducing resting potential, V_{max}, action potential amplitude or contractility (Kühl and Buschmann, 1987; Oexle et al., 1987). The Class III aspect of these actions can be expected to disfavour maintenance of ventricular fibrillation (VF). This hypothesis was tested in the isolated perfused rat heart.

Left regional ischaemia was induced in hearts (n=12/group) perfused with Krebs-Henseleit solution modified to contain 1.4 meq/l Ca^{++} and 3 meq/l K^{+}. Reperfusion was begun either 10 or 30 min after occlusion. Occlusion and reperfusion were verified by the dye exclusion method (Curtis and Hearse 1989). Arrhythmias were examined according to the Lambeth Conventions (Walker et al., 1988). Continuous VF lasting >120 sec was defined as sustained (SVF; Curtis and Hearse 1989). Tedisamil-containing solution (3μM) was introduced 5 min before occlusion and delivered continuously thereafter. Sinus bradycardia was expected (Oexle et al., 1987) so the study was repeated using hearts with right atrial excision plus left atrial pacing at 300 cycles/min (similar to drug-free heart rate); this rate achieved 1:1 atrial:ventricular coupling during arrhythmia-free periods. The threshold for statistical significance was set at $p<0.05$.

In unpaced hearts the incidence of VF during 30 min of ischaemia was not affected by tedisamil (83% vs 92% in controls). However, the incidence of SVF was reduced from 67% to 0% ($p<0.05$). This was neither a consequence of any variability in occluded zone size (37±3 vs 40±2 % of ventricular weight in control and tedisamil hearts, respectively) nor was it related to coronary flow (12.9±0.5 vs 12.9±0.3 ml/min/g wet wt., respectively, 1 min before occlusion), athough the possibility arose that bradycardia may have contributed, since tedisamil reduced sinus rate (measured 29 min after occlusion) from 288±8 beats/min in controls to 153±5 beats/min ($p<0.05$). However, in pacing experiments the total incidence of VF was again unchanged (83% with and without drug) whilst SVF was abolished by tedisamil (from a 75% incidence in paced controls) showing that the defibrillatory effect was unrelated to sinus bradycardia. Reperfusion after 10 min ischaemia elicited VF in 100% of unpaced controls, with an SVF incidence of 75%. Tedisamil had no significant effect on VF incidence (83%), but abolished SVF ($p<0.05$), as it had done during ischaemia. A similar profile of activity was seen in paced hearts with no effect of tedisamil on reperfusion-induced VF incidence (100% vs 100% in paced controls), but an abolition of SVF (from a 42% incidence in controls). Tedisamil's effects on reperfusion-induced VF were not dependent on the duration of preceding ischaemia since in hearts reperfused after 30 min ischaemia tedisamil again abolished SVF (from control incidences of 25% and 67% in unpaced and paced hearts, respectively) without affecting the total incidence of VF (which was 67% and 83% in unpaced and paced controls, respectively, and 75% and 50% with tedisamil).

In conclusion, tedisamil did not prevent VF during ischaemia or reperfusion. However, it abolished sustained VF during ischaemia and during reperfusion, as the hypothesis predicted, consistent with a direct (Class III) electrophysiological mechanism of action (independent of haemodynamic variations).

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232P CARDIOVASCULAR EFFECTS OF ELGODIPINE, A NEW DIHYDROPYRIDINE DERIVATIVE

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Elgodipine (IQB-875) is a new dihydropyridine derivative chemically related to oxodipine. In isolated rat aortae elgodipine potently inhibited high K-induced contractions and Ca influx which indicated that it inhibited Ca entry through potential-operated channels (Tejerina et al., 1989). In this communication we have studied the electromechanical and haemodynamic effects of elgodipine.

Experiments were performed in isolated atria and papillary muscles and in the isolated-perfused guinea-pig heart as well as in anaesthetized dogs. Transmembrane action potentials were recorded through standard microelectrode techniques (Valenzuela et al., 1987).

In isolated guinea-pig right atria, elgodipine (10^{-10}M - 10^{-6}M) produced a concentration-dependent negative inotropic (IC_{50} : 4.5 ± 1.6 x 10^{-9}M) and chronotropic effect (1.5 ± 1.7 x 10^{-8}M). In left atria and papillary muscles driven at 1 Hz, elgodipine at concentrations at which it had no effect on resting membrane potential or the maximum upstroke velocity (Vmax) produced a parallel shortening of the action potential duration and effective refractory period. Elgodipine also inhibited slow contractions (IC_{50} : 5.8 ± 2.3 x 10^{-9}M) as well as the amplitude and Vmax and shortened the duration of the slow action potentials elicited by isoproterenol in 27 mM K Tyrode solution. In isolated perfused guinea-pig hearts elgodipine produced a concentration-dependent negative inotropic effect (IC_{50} : 1.0 ± 0.4 x 10^{-9}M), slowed the conduction time through the atrio-ventricular node (lengthned the A-H interval) and increased coronary blood flow (ED_{20}: 5.5 ± 3.3 x 10^{-10} M), while it had no effect on intraatrial or intraventricular conduction times.

In anaesthetized dogs elgodipine (0.1, 1, 10, 50 and 100 ug/kg) produced a concentration-dependent decrease in heart rate, mean, systolic and diastolic blood pressure and peripheral vascular resistances, increased cardiac output and stroke volume but had no effect on left ventricular end-diastolic pressure.

All these effects are qualitatively similar to the actions of other dihydropyridines and indicated that elgodipine exhibits a potent and selective inhibition of Ca entry via the slow inward Ca current.

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233P EFFECTS OF THE COMBINATION OF MEXILETINE AND FLECAINIDE IN GUINEA-PIG PAPILLARY MUSCLES

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Mexiletine (M) and flecainide (F) are two antiarrhythmic drugs included in groups Ib and Ic, respectively. In this study the electrophysiological interactions between M and F at the sodium channel level were analyzed.

Experiments were performed in guinea-pig papillary muscles (less than 1 mm in diameter) perfused with Tyrode solution (34ºC). Maximum upstroke velocity (Vmax) of the action potentials was obtained by electronic differentiation (Delpón et al., 1989).

M (10^{-5}M) or F (10^{-6}M) alone or in combination had no effect on the Vmax of the ventricular action potentials. However, M but not F prolonged the ratio effective refractory period/action potential duration, while the combination increased this ratio more than M alone. M alone produced a 1.3 ± 0.5% of tonic Vmax block, F 1.7 ± 3.5% and the combination 3.4 ± 1.2% ($P < 0.05$). At 2 Hz the frequency(use)-dependent Vmax block produced by M was 19.8 ± 2.4% whereas that produced by F was 18.0 ± 2.2% and in the presence of the combination increased to 27.3 ± 1.4% ($P < 0.01$). The rate constants for the onset kinetics of use-dependent Vmax block induced by M and F were 0.162 ± 0.03 and 0.054 ± 0.004 AP^{-1}, respectively, while in the presence of the combination it was 0.099 ± 0.02 AP^{-1} ($P < 0.05$). In the presence of M or F alone the time constants of recovery of Vmax (τoff) were 314.8 ± 51.6 ms and 16.3 ± 2.3 s, respectively. However, when the two drugs were present two components in the reactivation process can be observed, a fast initial component with a τoff of 2.8 ± 0.3 s followed by a second slope component of similar kinetics to that observed in the presence of F alone (16.7 ± 2.7 s). M did not modify the fraction of sodium channels blocked by F alone (9.4 ± 0.6% vs 7.9 ± 0.7%. $P < 0.05$). The apparent Hill coefficient, nH, was 0.37 in the presence of F alone and 0.32 in the presence of both drugs.

These results demonstrated that the combination M plus F is a synergistic one, since it increased the onset rate and the steady-state value of the use-dependent Vmax block produced by each drug alone.

This work was supported by a FISS Grant

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234P EFFECT OF α_1-ADRENOCEPTOR MODULATION AND UK-52,046 ON ATRIO-VENTRICULAR CONDUCTION IN THE ANAESTHETISED DOG

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UK-52,046 is an α_1-adrenoceptor antagonist which abolishes arrhythmias in experimental models with little effect on blood pressure (BP) and heart rate (HR); (Uprichard et al, 1988). As drug induced alterations in intracardiac conduction may be hazardous, the present study investigated the effects of UK-52,046 on the specialized conduction system and compared the results to those of another α_1-adrenoceptor antagonist (prazosin), an α-adrenoceptor agonist (phenylephrine), a Class Ic antiarrhythmic drug (flecainide), and placebo.

Five groups of adult greyhounds were anaesthetized with sodium pentobarbitone (30 mg/kg iv) and ventilated. An atrial pacing catheter was placed in the right atrium and a 6F electrode catheter placed across the tricuspid valve (Gallagher & Damato, 1988) to record the His Bundle potential from which AH and HV intervals were measured. All catheters were positioned fluoroscopically. Arterial BP and HR (lead II) were simultaneously recorded. Increasing doses of UK-52,046 (1, 2, 4, 8, 16 and 32 μg/kg), flecainide (0.5, 1, and 2 mg/kg), prazosin (5, 10, 20 and 40 μg/kg), phenylephrine (continuous infusion, 50 μg/ml at 50 ml/hr) and placebo were administered in random order. Comparisons were made between placebo and active drugs using ANOVA and an unpaired Student's t-test. Following placebo there was no change in BP and HR, or in the AH or HV interval during sinus rhythm (SR) or atrial pacing (PA; 200 per min). UK-52,046 (1-8 μg/kg) had no effect on BP or HR , but reduced ($P<0.05$) BP at doses of 16 and 32 μg/kg, with no change in HR; during SR there was no effect on AH or HV intervals compared with placebo (Table). AH intervals decreased on pacing after UK-52,046 (4-16 μg/kg). Prazosin (5-40 μg/kg) increased HR, but BP fell only after 20-40 μg/kg. The HV interval was unaltered, but the AH interval fell after 20μg/kg during SR and after 40μg/kg during both SR and PA. Infusion of the α-adrenoceptor agonist phenylephrine reduced HR at 23 and 33 min, and after 33 min increased BP and the AH interval during PA (98.3 ± 0.8 ms; $P<0.05$), but had no effect on HV intervals. The Class Ic antiarrhythmic drug flecainide (0.5-2.0 mg/kg) had no effect on BP or HR but, as expected, significantly increased both AH and HV intervals during SR and PA ($P<0.05$).

TABLE

	Placebo control	final dose	UK-52,046 8μg /kg	16μg /kg	Prazosin 10μg /kg	20μg /kg	Phenylephrine 23† mins	33† mins
SBP(mmHg)	172	178	158	151*	163	148*	189	201*
HR(bpm)	149	153	159	162	187*	184*	132*	122*
AH(ms)	57.7	60.1	55.2	54.6	49.0	49.7*	75.4	80.8
HV(ms)	36.8	35.5	35.7	36.8	34.2	34.8	37.5	38.0

* $P<0.05$ † time following 50μg/ml/min n=6

In conclusion the α_1-agonist phenylephrine and the α-adrenoceptor antagonist prazosin altered AH (but not HV) intervals, in association with changes in HR and BP. However the new α_1-antagonist UK-52,046 at antiarrhythmic doses (2-8 μg/kg; Uprichard et al, 1988), did not significantly affect AH and HV intervals (during SR), BP or HR.

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235P COMPARATIVE EFFECTS OF ATRACURIUM AND LIGNOCAINE AT THE RAT NEUROMUSCULAR JUNCTION

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Like tubocurarine, atracurium acts mainly postjunctionally, to block acetylcholine nicotinic receptors. However, atracurium, may also affect presynaptic mechanism of transmitter release (Wali & Payne, 1983; Gibb & Marshall, 1986).

The local anaesthetic lignocaine, is known to inhibit impulse conduction by blocking sodium (Na^+) channels responsible for the generation of nerve compound action potential (NCAP).

The present investigation was carried out to test the possibility that atracurium, in very low, or high concentrations, may posses other actions, such as an anticholinesterase effect and/or a local anaesthetic-like action at the neuromuscular junction.

The phrenic nerve-diaphragm preparation of the rat was dissected and set up in an organ bath containing 80 ml of Krebs-Henseleit solution, maintained at 38±2°C and bubbled with 5% CO_2 in O_2 . The mechanical responses of the diaphragm, produced by indirectly- elicited twitch (0.2 Hz), tetanus (50 Hz), were recorded isometrically, in the absence or presence of atracurium or lignocaine. The NCAP was also recorded before and after addition of drugs.

At very low concentrations, atracurium (0.01 µM), and lignocaine (0.01 µM), both, slightly increased the twitch tension (by 13±2.1% and 15±1.1% of their respective control values). The control value was 2.4±0.1 g tension (mean±s.e.,n=6 rats).

In intermediate and high concentrations (1-100 µM), atracurium and lignocaine ,both, reduced and then blocked the twitch and tetanic tensions significantly ($p < 0.001$). Atracurium also produced a marked tetanic fade . In contrast, lignocaine produced no tetanic fade but instead a marked contracture was produced in the muscle.
Both atracurium and lignocaine reduced the amplitude and duration of the NCAP. The control amplitude and duration of the NCAP was 3.2±0.1 mV and 5.0±1.0 ms, respectively. Atracurium reduced the amplitude by 26± 1.5%, whereas lignocaine reduced it by 58±2.7%. Atracurium reduced the duration by 20±1.3%, whereas lignocaine reduced it by 40±5.1%. Thus, the effect of atracurium on the duration of the NCAP was not significant. All other effects were significant at 5% and 0.1% level.

The results showed that atracurium had a powerful neuromuscular blocking effect, at the neuromuscular junction or at the muscle. At no concentration, did atracurium produce a local anaesthetic like action (i.e., blocking the ion channel) at the rat neuromuscular junction.

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236P 8-OH-DPAT LABELS TWO SITES IN THE RAPHÉ NUCLEUS AREA OF RAT BRAINS

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It is generally thought that the main 5-HT receptors in the hippocampus and raphe nucleus areas belong to the 5-HT_{1A} sub class. Although numerous binding studies have been performed for a large number of compounds in hippocampal tissue (Hoyer, 1988), very little has been reported for the raphe area. We have carried out displacement binding studies using [^{3}H]-8-OH-DPAT in homogenates from the raphe nucleus area of rat brains and have found 2 sites labelled with this ligand; the higher affinity sites correspond to the 5-HT_{1A} binding site and the lower to 5-HT uptake sites as shown previously in rat striatum (Alexander & Wood, 1988).

Table 1 pKi values for displacement of [^{3}H]-8-OH-DPAT from rat raphe nucleus

	5-HT_{1A} pKi	5-HT uptake pKi
5-CT	9.28 ± 0.30	4.96 ± 0.04
NNdiPr-5-CT	9.40 ± 0.20	6.10 ± 0.21
8-OH-DPAT	8.58 ± 0.21	7.57 ± 0.05
5-HT	8.75 ± 0.18	5.71 ± 0.22
Ipsapirone	7.98 ± 0.18	4.56 ± 0.13
Buspirone	7.48 ± 0.11	4.28 ± 0.19
5-MeT	7.66 ± 0.12	5.03 ± 0.32
α-Me-5-HT	7.34 ± 0.11	6.13 ± 0.34
NNdiMeT	6.53 ± 0.17	5.32 ± 0.09
Ketanserin	5.64 ± 0.17	< 4
Fluoxetine	4.91 ± 0.08	6.94 ± 0.08
Citalopram	4.36 ± 0.16	6.92 ± 0.29

A 2mm thick coronal section of rat brain (Bregma -7 to -9mm, Paxinos & Watson, 1986) was dissected and an area 3mm by 2mm below the aqueduct was punched out with an oval punch and frozen at -80°C until use. Binding studies were carried out as normal but incubating for 2h at 27°C in a medium containing 1nM [^{3}H]-8-OH-DPAT, 50mM Tris buffer pH 7.4, 0.1% ascorbate and 10µM pargyline. 12 concentrations in duplicate were used for each compound with 6 compounds in each assay. Non-linear regression analysis was used to estimate common lower, middle and upper asymptotes for each data set and 2 pIC_{50} values for each compound. pKi values are reported as means ± s.e. mean from 3 or more determinations.

In most cases the displacement curves were clearly biphasic with ca. 50% each of the 2 components. Generally the higher affinities correspond to affinities determined in 5-HT_{1A} assays with hippocampal homogenates, but for the 5-HT uptake inhibitors, citalopram and fluoxetine, the order was reversed. This was evident by inclusion of 1µM 5-CT in the assay medium which blocked the low affinity site for these compounds but the high affinity site for the others. Saturation curves were also measured giving pK_D values of 8.82 ± 0.09 and 7.38 ± 0.06 for the 5-HT_{1A} and 5-HT uptake sites respectively.

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237P SYMMETRICAL HEMISPHERIC DISTRIBUTION OF BRAIN [^{3}H]-PAROXETINE BINDING SITES IN POST-MORTEM SAMPLES FROM DEPRESSED SUICIDES AND CONTROLS

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Two recent reports indicate marked differences in the Bmax of [3H]imipramine (which labels 5-HT uptake sites) binding sites between left and right hemispheres of post-mortem human frontal cortex. In controls Bmax values were more than two-fold higher in right than left hemispheres, whereas the converse was true in suicides (Arato et al., 1987) and in subjects with schizophrenia, depression and alcoholism (Demeter et al., 1989). This asymmetry may in part explain discrepancies between previous studies of [^{3}H]imipramine binding in depressed subjects and controls. We now report 5-HT uptake sites in frontal cortex (Brodmann area 11), putamen and substantia nigra from suicides and controls, using the more selective ligand [^{3}H]paroxetine.

Eight controls (4M, 4F; mean age ± sem, 51±5 years; post-mortem delay, 40±6 h) who died suddenly from natural causes and 8 suicides (5M, 3F; 44±6 years; 35±4 h), with a firm retrospective diagnosis of depression, were studied. Saturation binding of [^{3}H]paroxetine (Lawrence et al., 1989) was performed on coded samples arranged such that both hemispheres from the same subject were assayed concurrently.

No significant differences in K_d or Bmax (Table 1) were found between left and right hemispheres (paired Student's t-test, two tailed) in any of the brain areas from either controls or depressed suicides.

Table 1. Bmax of [^{3}H]paroxetine binding (fmol/mg protein, means ± sem)

	Controls		Suicides	
Hemisphere	Right	Left	Right	Left
Frontal cortex	46 ± 5	40 ± 5	46 ± 6	47 ± 8
Putamen	209 ± 19	181 ± 18	190 ± 21	184 ± 19
Substantia nigra	526 ± 40	512 ± 54	408 ± 37	445 ± 49

The present results are in contrast to the marked asymmetry previously reported using [^{3}H]imipramine. The asymmetry may be related to sites other than 5-HT uptake sites labelled by [^{3}H]imipramine.

We thank the Wellcome Trust and the Sir Jules Thorn Charitable Trust for financial support.

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238P MICROSTRUCTURAL ANALYSIS OF THE EFFECTS OF THE SELECTIVE 5-HT$_3$ ANTAGONIST, ONDANSETRON, ON FEEDING AND OTHER BEHAVIOURAL RESPONSES

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Detailed investigations of the changes in feeding behaviour (microstructural analysis) which underlie anorectic effects of drugs are indispensable to a clear understanding of the different mechanisms that underlie such effects (Blundell & Latham, 1980). Central 5-hydroxytryptamine (5-HT) and dopamine systems have been strongly implicated in the control of feeding responses (Blundell & Latham, 1980), and there is growing evidence that selective 5-HT$_3$ receptor agonists and antagonists interact with central dopamine systems in the rat (Blandina et al., 1988; Costall et al., 1987; Hagan et al., 1987). The aim of the present study was to determine if a selective 5-HT$_3$ receptor antagonist modulates appetite and feeding responses.

Thirty-two adult, male hooded rats (250-400g) were familiarised with eating a palatable, sweetened mash in a clear plastic tank. They were not food-deprived. The animals were assigned to four equal groups, and were injected with ondansetron (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, HCl $2H_2O$) in doses of 0, 3, 10 and 30μg.kg^{-1} respectively, by i.p. route, 30 min prior to a 60 min observation period. The injection vehicle was distilled water. An observer recorded every instance of behaviour, according to pre-designated categories (feeding, locomotion, rearing, grooming, sniffing, oral behaviour, immobility), and the data were stored using a BBC Master microcomputer. The microstructural analysis provided information about the frequency and duration of behaviour within each response category. The data were analysed by ANOVA and Dunnett's t-test.

The results indicated that ondansetron had a highly significant effect on palatable food intake, $F_{3,28}$= 7.49, $p<0.01$. Significant reductions in intake were found at each dose level ($p<0.05$), with 10μg.kg^{-1} being most effective, producing a 42% reduction in intake ($p<0.01$). The reduction in intake was due to reductions in the duration of eating bouts and in the eating rate, but not in the frequency of eating bouts. Ondansetron did not produce any general stimulant or depressant effects, since measures of general activity (locomotion, rearing) were not affected at any dose tested. We conclude that ondansetron produces behaviourally-specific anorexia in the rat.

GAvdH is supported by a Glaxo Research studentship.
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239P EFFECTS OF RUBIDIUM ON 5-HT-MEDIATED BEHAVIOUR IN RAT AND MOUSE

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Judd et al. (1975) reported that the administration of tranylcypromine to rats after 14 days of rubidium chloride (RbCl) in the diet produced the 5HT behavioural syndrome. The similarities with the effects of lithium known at that time (Grahame-Smith and Green, 1974) were noted. We have re-examined the effects of rubidium on 5HT-mediated behaviour in rat and mouse in the light of recent advances in 5HT neuropharmacology. Sprague-Dawley derived rats (160-250g) were given either RbCl or KCl (30 mmol/Kg diet) for 14 days. Tranylcypromine [TCP] (15 mg/kg i.p.) was then given. The rats were observed and rated for the components of the behavioural syndrome, (Deakin and Green, 1978) and activity was monitored on Opto-Varimex meters. In RbCl diet fed rats, the complete 5HT behavioural syndrome occurred with onset at about 40 min after TCP, peaking at about 200 min. The syndrome was not seen in the controls (KCl). Similar effects were seen after TCP in rats pre-treated with RbCl i.p., 3 mmol/kg twice daily for 5 days, but not after one day. In rats given dietary RbCl for 14 days, the administration of p-chlorophenylalanine (300 mg/kg i.p.) 24 and 3 h before TCP almost completely prevented the occurrence of the 5HT syndrome. The 5HT syndrome produced by dietary RbCl and TCP was also inhibited by treatment with (-)-propranolol (20 mg/kg i.p.), pindolol (4 mg/kg i.p.) and ritanserin (0.4 mg/kg s.c.). RbCl pretreatment had no effect upon the 5HT syndrome produced by 8OHDPAT (0.5 mg/kg s.c.), or 5MeODMT (2 mg/kg i.p.). RbCl pretreatment enhanced hyperactivity, head weaving, forepaw treading, flat-body posture and total behavioural scores produced by quipazine (20 mg/kg i.p.), DOI (8 mg/kg s.c.), and p-chloroamphetamine (4 mg/kg i.p.). Dietary RbCl administration for 14 days resulted in the enhancement of the mouse head-twitch response to 5MeODMT (5 mg/kg i.p.), carbidopa (25 mg/kg i.p.) plus 5HTP (100 mg/kg i.p.), quipazine (10 mg/kg i.p.) and DOI (2 mg/kg s.c.). RbCl administration did not affect the hypothermic response of mice to 8OHDPAT (0.5 mg/kg s.c.). The accumulation of 5HT in whole brain (minus cerebellum) over 3 h following TCP (15 mg/kg) was increased by 33% ($p<0.005$) in rats fed RbCl for 14 days. Chronic but not acute RbCl administration clearly affects 5HT mediated behaviours. These effects of rubidium are quite different to those produced by lithium (Grahame-Smith, 1988) and are most probably mediated through a different mechanism.

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240P EFFECT OF 5-HT$_1$ AGONISTS ON CARBOHYDRATE/PROTEIN DIETARY SELECTION OF RATS

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Previous evidence of the involvement of 5-HT in carbohydrate/protein dietary selection has largely been obtained by altering 5-HT availability to receptors. We now report the effects of choice of 8-OH-DPAT which causes hyperphagia by activating cell body 1A receptors. (Dourish et al., 1985) and of RU24969 and mCPP which cause hyperphagia by activating postsynaptic 1B and 1C sites respectively (Kennett & Curzon, 1988).

Male Sprague-Dawley rats (140-150g) singly housed with free access to water were adapted over two weeks to a choice of two isocaloric diets of different protein-carbohydrate composition (0% and 55% protein). 0.9% NaCl (1 mg/kg) or 8-OH-DPAT were given and 4h intakes measured. Using a separate group of rats, food but not water was removed and 24hr later, vehicle, RU24969 or mCPP injected and intakes measured 2h after replacement of diets as described by Kennett and Curzon (1988). 8-OH-DPAT significantly and dose dependently increased intakes of both diets. % increases were significantly greater for the % protein diet (Table 1). RU24969 and mCPP significantly and dose dependently decreased intakes but % decreases were similar for both diets.

Table 1. Effects of 5-HT$_1$ agonists on food choice

	8-OH-DPAT(s.c)		RU24969 (i.p.)		mCPP (i.p.)	
Diet	60 ug/kg	500ug/kg	2.5 mg/kg	10 mg/kg	2.5 mg/kg	10 mg/kg
	% increase of intake(4h)		% decrease of intake (2h)			
0% protein	233 ± 39	654 ± 155	63 ± 7	90 ± 3	55 ± 8	77 ± 8
	$P < 0.01$	$P < 0.05$	ns	ns	ns	
55% protein	82 ± 17	292 ± 100	58 ± 11	98 ± 1	52 ± 7	76 ± 11

Means ± s.e.m. (n = 8-10). Mann-Whitney U test after non-parametric ANOVA.

The selective increase of carbohydrate intake on giving 8-OH-DPAT which reduces 5-HT release agrees with the selective decrease of carbohydrate intake when 5-HT release is elevated (Wurtman & Wurtman, 1979). However, the lack of selectivity of the previously food-deprived rats given RU24969 and mCPP appears inconsistent with the above relationships.

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241P ID_{50} VALUES OF ANTAGONISTS *versus* MCPP-INDUCED HYPOPHAGIA AND 5-HT_2-MEDIATED HEAD SHAKES INDICATE 5-HT_{1C} SITES MEDIATE THE HYPOPHAGIA IN RATS

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Evidence suggests that 1-(3-chlorophenyl) piperazine (mCPP) causes hypophagia by stimulating 5-HT1C receptors (Kennett and Curzon, 1988). However 5-HT1C and 5-HT2 sites have major similarities and activation of the latter has been suggested to cause hypophagia (e.g. Schechter and Simansky, 1988). We have investigated this problem by determining the potencies of nine 5-HT antagonists against mCPP induced hypophagia and 5-HT2 mediated head shakes.

Male S.D. rats 250-300g were singly housed. To determine ID50 (hypophagia), food was withdrawn between 13.00 and 14.00h. 24h later drug or vehicle was given s.c. 20 min prior to 0.9% NaCl or mCPP (5mg/kg i.p.) in 0.9% NaCl. Food was restored after 20 min and 2 h intake measured. To determine 1D50 (head shake), normally fed rats were placed in observation cages and given carbidopa (25mg/kg i.p) and antagonists (s.c.). 5-HTP (100mg/kg) was given i.p. 30 min later. Head shakes during 2 min. periods 30, 60, 90 and 120 min later were summated. The drugs LY53857, metergoline, 1-naphthyl piperazine and (-) propranolol were given in 0.9% NaCl: altanserin, ketanserin, methysergide, mianserin and ritanserin were dissolved in acetic acid in 0.9% NaCl and made to pH 6.5.

ID50s VS hypophagia and head shakes respectively were (mg/kg): metergoline 0.2, 0.034; 1-naphthyl piperazine 1.03, 1.75; mianserin 2.1, 0.12; ritanserin 4.6, 0.19; methysergide 5.1, 1.08; LY53857 6.4, 1.18; ketanserin 12.9, 0.036; (-) propranolol 41.9, 1.98. Log ID50 (hypophagia) correlated significantly with log affinity (Hoyer, 1988) for 5-HT1C sites ($r = 0.84$, $P < 0.01$) but not for 5-HT2 or 5-HT1A, 5-HT1B or 5-HT1D sites. Log ID50 (head shakes) correlated significantly with log affinity for 5-HT2 sites ($r = 0.75$, $P < 0.05$) but not for the above 5-HT1 sites. The ratios of ID50 values correlated highly significantly with the ratios of 5-HT1C/5-HT2 site affinities ($r = 0.95$ $P < 0.001$). The data thus strengthens evidence that mCPP causes hypophagia by activating 5-HT1C sites.

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242P INHIBITION OF 5-HT_{1B} AUTORECEPTORS BY METHIOTHEPIN DOES NOT REVEAL A CIRCADIAN VARIATION IN AUTORECEPTOR SENSITIVITY IN THE CEREBRAL CORTEX OF THE RAT

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In the rat, terminal 5-HT_{1B} autoreceptors are reported to play a crucial role in the regulation of release and possibly synthesis of 5-HT in central serotoninergic neurones (Sanders-Bush 1982). It has been reported that there exists a circadian variation in behavioural responses to stimulation of 5-HT_2, but not 5-HT_1 receptors, (Moser and Redfern, 1986).Earlier work using 5-HT to inhibit release from brain slices in-vitro failed to reveal a circadian variation in autoreceptor sensitivity (Redfern et al, 1988). The present study was undertaken to investigate the sensitivity of these autoreceptors using the 5-HT antagonist methiothepin.

Male Wistar rats were housed under a constant 12:12 Light:Dark cycle. Slices of the cerebral cortex were incubated for 15 min in Krebs solution containing 0.1 μM tritiated 5-HT and 10 μM pargyline. After 3x5 ml washes, 50 μl aliquots of the tissue suspension were superfused for 30 min with Krebs containing 3.2 μM paroxetine. The superfusion medium was then changed to iso-osmotic modified Krebs solution containing 25 mM potassium and 3.2 μM paroxetine. The potassium-evoked release of tritium was measured in samples collected every 4 min. The cumulative addition of 5-HT (30-1000 nM) caused a dose-related inhibition of tritium release. These inhibitory effects of 5-HT were attenuated by the 5-HT antagonist methiothepin (1 μM). Apparent pA_2 values were calculated as described by Schlicker and Gothert (1981). Results are expressed as means ± s.e.m. The number of experiments is shown in parenthesis.

Hours after lights on	0	6	12	18
Apparent pA_2 Methiothepin	6.780±0.06(5)	6.648±0.11(5)	6.724±0.08(5)	6.763±0.05(4)

Basal ^3H release was unchanged by 5-HT (1μM) or methiothepin (1 μM). Methiothepin, however, caused an enhancement of the potassium-evoked release, the magnitude of which was similar at the four time points. This enhancement is generally interpreted as the presence of an endogenous inhibitory tone. These results are generally indicative of a lack of circadian variation seen on stimulation of receptors of the 5-HT_1 type and suggest that factors other than terminal autoreceptor sensitivity are responsible for the reported circadian variation in release.

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243P EFFECTS OF THE 5-HT_3 ANTAGONIST GR38032F ON BENZODIAZEPINE WITHDRAWAL IN RATS

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To investigate the ability of the 5-HT_3 antagonist GR38032F to block benzodiazepine (BZ) withdrawal (Costall et al. 1988; Oakley et al. 1988), groups of female rats (n = 10) received i.p. injections b.i.d. (1000, 1600 hours) of chlordiazepoxide (C or CDP). Doses administered (b.i.d.) increased daily by 2 mg/kg from 10 (day 1) to 40 mg/kg (day 16). Subsequently, animals received 40 mg/kg CDP b.i.d. for 5 further days - i.e. 21 days treatment in toto. Over the next 8 days one group (coded C-C) continued to receive the same CDP treatment they had received for the previous 21 days. One group (coded C-S) received b.i.d. saline injections. A further three groups (coded C-G 1.0, C-G 0.1 and C-G 0.01) received b.i.d. injections of GR38032F at doses of 1.0, 0.1 and 0.01 mg/kg respectively.

Withdrawal was assessed 18 hours after the final CDP injection on day 21, and at subsequent 24 hour intervals, up to day 30. Indices of withdrawal recorded were:- bodyweight and food intake. Effects of BZ withdrawal were seen in groups C-S, C-G 1.0, C-G 0.1 and C-G 0.01, as determined by loss of bodyweight and reduced food intake. Both indices fell progressively and then recovered over 9 days. During withdrawal the body weight of group C-S fell by a maximum of 5.6%. Maximal falls in bodyweight for other groups were: C-G 1.0 (4.1%), C-G 0.1 (2.5%), C-G 0.01 (4.3%). A similar pattern of data was seen for the food intake index. Withdrawal peaked 3 or 4 days after cessation of chronic BZ treatment. No evidence of "withdrawal" was seen in group C-C, which gained weight and showed stable food intake. Analysis of the body weight data indicated that group C-G 0.1 lost less weight than group C-S (α = 0.05, Tukey HSD test after ANOVA). Analysis of the food intake data showed a similar effect, animals in Group C-G 0.1 ate more during withdrawal than group C-S (α = 0.01 two tailed, Mann Whitney U test after Kruskal Wallis ANOVA). Thus GRF38032F attenuated, but did not fully block, BZ withdrawal, and this effect was only seen at the intermediate dose studied (0.1 mg/kg). These data suggest that, over a narrow dose range, GR38032F ameliorates signs of BZ withdrawal, at least when these are measured in terms of loss of bodyweight and reduced food intake. One potential explanation for these findings, which requires further investigation, is that GR38032F at 0.1 mg/kg may stimulate food intake rather than specifically attenuating BZ withdrawal.

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Acknowledgement: Supported by a grant from the University of Liverpool Research Development Fund. We are indebted to Glaxo Group Research Ltd. for generous supplies of GR38032F.

244P DIFFERENCES IN OPEN-FIELD BEHAVIOUR OF RATS AFTER INJECTION OF 5-HT_{1A} SELECTIVE COMPOUNDS INTO DORSAL AND MEDIAN RAPHÉ NUCLEI

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Previous studies have reported both hyper- and hypoactivity after systemic injection of the selective 5HT_{1A} agonist, 8-hydroxy-di-N-propylamino tetralin (8-OH DPAT) in the rat. Furthermore, the administration of 8-OH DPAT into the dorsal (DRN) and median (MRN) raphé nuclei has been reported to respectively decrease, and increase locomotor activity (LMA) (Hillegaart and Hjorth, 1989). An attempt to confirm and extend the latter findings using a more extensive analysis of open field behaviour is now reported. In addition to 8-OH DPAT, the selective 5HT_{1A} compounds, gepirone and BMY7378 were also examined.

Male hooded Lister rats (200-250g; Glaxo) were cannulated according to a previous study (Higgins et al 1988) and allowed 7 days for recovery prior to testing. Microinjections of 5HT_{1A} receptor-selective drugs (0.5µl/1min) into the DRN or MRN were made 5min before placing the animal in a novel open-field (62 x 62 x 33cm) arena for 8min. Behaviours (including LMA, flat body posture (FBP) and rearing) were recorded on video-tape and scored later by an observer blind to the drug treatments.

Drug	Dose	DRN				MRN			
	µg	n	LMA	REARS	FBP	n	LMA	REARS	FBP
8-OH DPAT	0.1	(6)	75±12	90±16	1/6	(6)	201±13**	66±14*	0/6
	0.5	(5)	71±43	17±10*	3/5	(9)	188±19**	63±6**	0/9
	2.5	(5)	9±6**	2±2**	5/5	(8)	105±22	5±4**	2/8
GEPIRONE	5.0	(4)	127±14	69±18	2/4	(9)	145±17	53±10**	0/9
	25.0	(5)	42±12*	10±6**	5/5	(6)	134±43	32±12**	1/6
BMY7378	0.5	(6)	78±14	102±16	0/6		NOT TESTED		
	2.5	(6)	70±7*	73±9	1/6		NOT TESTED		
	12.5	(6)	26±11**	17±10**	6/6		NOT TESTED		

LMA and rears are presented as percentage of controls (= 100%); FBP given as number/ group; *p<0.05, **p<0.01 compared to controls (unpaired t-test).

The present results indicate that injection of 8-OH DPAT, gepirone and BMY7378 into the DRN produce FBP and a profound hypoactivity whereas equivalent doses of 8-OH DPAT and gepirone given into the MRN elicit hyperactivity without FBP. The latter increase in motor activity appeared to be unrelated to exploration since rearing was reduced in the same animals. These results also suggest that BMY7378 has agonist activity at 5HT_{1A} receptors localised within the DRN. The contrasting effects on motor activity seen after discrete injections into the DRN and MRN are probably related to their differential innervation of forebrain structures; the preferential activation of either nuclei may therefore account for the conflicting observations reported with systemically administered 8-OH DPAT.

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245P AVERSIVE ENVIRONMENTAL STIMULI DO NOT EVOKE BEHAVIOURAL RESPONSES TO GR38032F FOLLOWING CHRONIC TREATMENT AND WITHDRAWAL

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Behavioural studies using rodent models have shown that the anxiolytic properties of the $5HT_3$ antagonist GR38032F are maintained during chronic drug administration and that there is no withdrawal anxiogenesis (Oakley et al 1988). This study tested the hypothesis that aversive environmental stimuli might be necessary to reveal the effects of chronic GR38032F on locomotor activity.

Male Sprague Dawley rats (n=8/group) received i.p. injections of either GR38032F (1mg/kg) or saline twice daily for 13 days, followed by a 48 hr withdrawal period on days 14 and 15. On days 16 and 17, drug was again given twice daily. The spontaneous locomotor activity of rats repeatedly exposed to either an elevated open platform or a less aversive enclosed platform (Vale and Balfour 1989) was monitored for 15 min using infrared photobeams every second day. Further groups of chronically treated rats were tested on the platforms on days 13, 15 and 17 only. Exposure to the open platform resulted in a significant reduction in total activity compared to that on the enclosed platform ($F(1,28)=28.7;P<0.001$). Repeated testing of the animals on both the open and enclosed platforms resulted in a reduction in locomotor activity ($F(8,224)=6.8;P<0.001$). However, concurrent treatment with GR38032F did not alter the habituation of the rats to either platform. Following drug withdrawal (wdal) and a challenge dose of GR38032F (1mg/kg) 48h post withdrawal there was no change in locomotor activity compared to saline treated controls on either platform. Similarly, spontaneous locomotor responses remained unchanged in both test environments in rats that were naive to the test apparatus.(Table 1).

Table 1 Effect of chronic GR38032F administration on locomotor responses in an open and enclosed platform.

	Repeated Exposure				Acute Exposure			
DAY	open		enclosed		open		enclosed	
	sal	drug	sal	drug	sal	drug	sal	drug
1	178±20	153±18	283±25	265±14	-	-	-	-
13	141±23	137±24	210±28	182±21	113±18	144±27	324±18	326±26
15(wdal)	107±21	113±24	205±21	166±15	124±33	154±35	234±16	254±18

Data are expressed as mean ± SEM and were analysed using ANOVA for repeated measures.

The study therefore has failed to provide any evidence that GR38032F influences behavioural habituation to an aversive environment, or, that exposure to aversive stimuli reveals behavioural responses to GR38032F withdrawal.

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246P MANIPULATION OF CENTRAL 5-HT FUNCTION DISRUPTS PERFORMANCE OF A DELAYED-NON-MATCH-TO-SAMPLE TASK IN RATS

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The role of 5-hydroxytryptamine (5-HT) in learning and memory has mainly been studied in tasks involving aversive stimulation (see Hunter, 1989). Few studies have used appetitive tasks, e.g. Winter & Peti (1987). The aim of the present experiments was to characterise the effects of 5-HT depletion and 5-HT antagonists on delayed-non-match-to-sample (DNMS) performance in rats.

Male Lister Hooded rats (250-350g), maintained at 90% of their free-feeding body weight were trained on DNMS (Saghal, 1987). Rats received either sham lesions or bilateral injections of 5,7,dihydroxytryptamine (5,7DHT, 5µg in 1µl) into the fimbria-fornix following $25mgkg^{-1}$ i.p. desmethylimipramine 30 min earlier. A second group received $5mgkg^{-1}$ p-chloroamphetamine (PCA) or saline i.p. daily for 2 days and were tested on DNMS on day 3. 5-HT antagonists were administered i.p. to another group 20 min before testing.

Table Delay-dependent responding following either 5,7DHT lesions or PCA treatment

	Delay(s)						5-HT depletion (% of controls)
Treatment	0	2	4	8	10	16	
Sham	87.5±4.5	83.5±5.7	77.2±6.9	63.7±3.4	64.3±3.3	62.5±11.7	
5,7DHT	64.1±11.1	59.3±10.3	55 ±10.9	46.8±6.3	42.4±8.2	46.1± 7.7	80% (a)
Saline	96.8±1.1	92.4±3.6	88.6±3.2	79.9±3.5	66.6±3.7	66.9±4.3	
PCA	88.3±3.6	80.7±4.3	75.4±5.4	70.3±3.9	70.9±3.7	56.7±2.1	82% (b)

Values are% correct responses mean ± SEM. (a) hippocampus; (b) frontal cortex.

PCA treatment impaired performance, but this effect was not delay dependent ($p<0·01$). Similarly 5,7DHT lesioned rats were impaired on DNMS compared to sham lesioned ($p<0.05$). Both groups had large depletions of cortical or hippocampal 5-HT respectively. Ritanserin, a $5\text{-}HT_2/5\text{-}HT_{1C}$ antagonist produced no effect at a low dose ($0.3mgkg^{-1}$) but $0.6mgkg^{-1}$ increased the number of missed trials while not impairing correct responding. The $5\text{-}HT_{1A}/5\text{-}HT_{1B}$ receptor antagonists, (±)pindolol and (-)propranolol, and methysergide, were without effect. Therefore severe depletion of cerebral 5-HT impairs performance but this is not mimicked by administration of 5-HT antagonists.

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247P PRELIMINARY ASSESMENT OF FEVERFEW (*Tanacetum Parthenium*) TO ANTAGONISE CISPLATIN-INDUCED EMESIS IN THE FERRET

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Agents capable of antagonising 5-HT function by synthesis inhibition or 5-HT_3 receptor blockade can prevent emesis induced by chemotherapeutic agents (Barnes et al. 1988). The herbal product feverfew (Tanacetum parthenium) is known to reduce 5-HT release and other secretory activities of platelets (Heptinstall et al. 1985), and is reported to reduce migraine and the vomiting associated with such attacks (Murphy et al. 1988). Here we investigate the actions of feverfew to modify cisplatin induced emesis in the ferret.

Albino or fitch ferrets of either sex (0.66-0.9kg) were administered cisplatin (10mg/kg i.v.) under anaesthesia as previously described by Barnes et al. (1988). Feverfew suspensions (125 and 62.5mg/ml) were prepared by sonication of feverfew herb powder (Lifeplan Products Ltd.) in saline and were administered intraperitoneally (2ml/kg) immediately following the injection of cisplatin.

Table 1. Effect of feverfew on cisplatin induced emesis

Treatment mg/kg	Group Size	Onset of Emesis (min)	No. of Emetic Episodes	No. of Retches	No. of Vomits
Cisplatin 10	5	68.4 ± 3.9	18.0 ± 2.6	101.2 ± 26.8	13.0 ± 2.7
Cisplatin 10 + Feverfew 125	5	102.2 ± 26.8	8.8 ± 2.6	42.6 ± 17.7	3.6 ± 1.1*
Cisplatin 10 + Feverfew 250	4	93.2 ± 7.0*	7.8 ± 2.0*	45.3 ± 11.9	4.5 ± 1.9

Significant differences between cisplatin and feverfew treated animals are indicated as *<0.05 (Mann-Whitney U test).

The injection of feverfew caused a transient (less than 5 min) and inconsistent ($^3/_8$ animals) emetic response within 5 min of injection. Subsequently, both doses of feverfew caused a clear trend for a delay in the onset of cisplatin-induced emesis and a reduction in the number of episodes, retches and vomits; in some cases this reached statistical significance. The antagonism by feverfew of cisplatin-induced emesis requires further investigation of the active constituent(s) for detailed dose-ranging studies.

J.A. Rudd is supported by a Glaxo studentship.

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248P THE EFFECT OF NOVEL ANXIOLYTICS ON THE HEAD-TWITCH RESPONSE IN MICE

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Benzodiazepine anxiolytics and the putative anxiolytic ipsapirone have been shown to potentiate head-twitches induced by the direct 5-hydroxytryptamine (5-HT) receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) but not those induced by the 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP) (Moser & Redfern, 1988; Goodwin *et al.*, 1986). The present study was undertaken to examine the effects of the novel anxiolytics buspirone and MDL 73005EF (Moser *et al.*, 1989) and to explore the mechanisms involved.

Head-twitches were induced in male Swiss albino mice (Charles River, France) using either 5-MeODMT or 5-HTP (in the presence of carbidopa, 25 mg kg^{-1} i.p.), as previously described in detail (Moser & Redfern, 1988). Mice were observed between 1 and 4 min post-5-MeODMT and between 20 and 23 min post-5-HTP. The number of head-twitches observed in these periods were recorded. All compounds were administered i.p. (unless otherwise stated) and a 30 min pretreatment time was used.

Buspirone (0.125 - 1 mg kg^{-1}) and MDL 73005EF (0.25 - 2mg kg^{-1} s.c.) dose-dependently and significantly potentiated head-twitches induced by 5-MeODMT (2.5 mg kg^{-1}), as did diazepam (1 - 4 mg kg^{-1}) and ipsapirone (0.5 - 8 mg kg^{-1}), in confirmation of previous results. Table 1 shows the maximum effect of each compound. None of these compounds were able to potentiate head-twitches induced by 5-HTP (50 mg kg^{-1}) and in the case of diazepam (16 mg kg^{-1}) and MDL 73005EF (8 mg kg^{-1} s.c.) a significant inhibition was obtained. 8-OH-DPAT (25 - 800 µg kg^{-1} s.c.) had no significant effect on 5-MeODMT-induced head-twitches. 1-pyrimidinyl piperazine (1-PP; 0.125 - 2 mg kg^{-1}), a metabolite of buspirone and ipsapirone, potentiated 5-MeODMT-induced head-twitches but not those induced by 5-HTP.

The role that the α_2-adrenoceptor antagonist properties of 1-PP might play in the effects of buspirone and ipsapirone was studied in more detail as α_2-adrenoceptor antagonists have been shown to potentiate head-twitches (Handley & Brown, 1982). Idazoxan (0.13 - 2 mg kg^{-1}) dose dependently potentiated 5-MeODMT-induced head-twitches and, unlike the other compounds tested, also those induced by 5-HTP. Clonidine inhibited twitches induced by 5-MeODMT (10 mg kg^{-1}) with an ED_{50} of 32 µg kg^{-1}. In the presence of idazoxan (1 mg kg^{-1}) the dose-response curve for clonidine was shifted rightwards (ED_{50} 178 µg kg^{-1}) whereas no such shift occurred in the presence of buspirone (1 mg kg^{-1}; ED_{50} 25 µg kg^{-1}), diazepam (4 mg kg^{-1}; ED_{50} 38 µg kg^{-1}) or 1-PP (1 mg kg^{-1}; ED_{50} 47 µg kg^{-1}).

Table 1. Potentiation of 5-MeODMT-induced head-twitches. Values are the mean ± s.e.mean (n = 6). The differences between saline (first column) and drug pretreatment were significant ($P < 0.01$, Student's t test).

Compound	Dose (mg kg^{-1})	Number of head-twitches	
Diazepam	4	4.7 ± 0.4	14.0 ± 1.4
Buspirone	1	6.8 ± 1.1	14.7 ± 1.8
Ipsapirone	8	5.5 ± 1.0	14.3 ± 2.0
MDL 73005EF	2	6.0 ± 0.5	20.8 ± 1.5
1-PP	2	4.8 ± 0.9	19.3 ± 0.7

These results suggest that potentiation of the 5-MeODMT-induced head-twitches by buspirone, an effect it shares with other anxiolytics, does not involve interactions with α_2-adrenoceptors or 5-HT_{1A} receptors. The different effects of anxiolytic compounds on 5-MeODMT and 5-HTP-induced head-twitches might prove useful in examining their pharmacological properties.

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249P AN AUTOMATED DARK/LIGHT BOX TO ASSESS ANXIOLYTIC POTENTIAL

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A black/white test box has been valuable for assessing the ability of anxiolytic agents to release a suppressed behaviour (Costall *et al.*, 1989; Kilfoil *et al.*, 1989). The disadvantage of the test described is the time taken to analyse the data from the videotapes. An automated system has been developed which consists of an open topped box (43 x 25 x 25cm) divided into two sections in the ratio 2:3 by a black partition. An opening (7.5 x 7.5cm) located at floor level allowed free access between the two compartments. The smaller dark chamber was constructed of black perspex, through which infra-red beams could pass, and was illuminated by a dim red light. The light chamber had clear perspex sides with a white floor and was illuminated by a white light. The box was placed within the arena of an Optovarimex which had two banks of infra-red beams, one set to measure locomotor activity, the other to record rearing behaviour. The apparatus was linked to an IBM computer and software which enabled a plot of the animal's path within the test box to be obtained, in addition to the standard measures of time spent, number of rears and distance travelled in each section. In this study the effect of chlordiazepoxide on behaviour was assessed both automatically and visually by remote video recording so that a comparison between the two protocols could be made.

A comparison of the results obtained by visual assessment and those from the automated apparatus showed good correlation for the time spent ($r = 0.95$), number of rears ($r = 0.95$) and distance travelled ($r = 0.93$) in the light section. The values obtained from the automated system are shown in Table 1.

Table 1 The effect of chlordiazepoxide (CDP) on mouse behaviour in an automated dark/light box

CDP	Time in white	White section		Black section	
mg/kg i.p.	(s)	Rears	Distance (cm)	Rears	Distance (cm)
V	143.9±12.1	24.5±4.1	927.5±58.5	29.4±4.3	921.0±98.3
2.5	173.6±16.4	24.3±4.3	1015.0±115.4	24.8±3.7	804.3±78.9
5.0	215.8±19.2*	28.5±4.0	1108.1±122.4	16.6±2.8*	569.8±125.6*
7.5	181.4±17.7	18.5±4.4	843.5±172.2	15.6±3.1*	568.1±73.6*

*P<0.05, ANOVA,Dunnett's t-test. n = 10.

Thus the measures obtained from the automated model employed in this study show good correlation with those obtained by visual assessment and are sensitive to modification by the anxiolytic, chlordiazepoxide.

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250P LACK OF EFFECT OF AVERMECTIN ON CALCIUM CHANNELS IN MUSCLE FIBRES FROM MOTH LARVAE

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The potent anthelmintic drug, avermectin, has GABA-like effects in many systems, but also acts on preparations which are insensitive to GABA (Duce & Scott, 1985). Besides acting on chloride channels, a possible action on calcium channels has also been suggested (Lacey, 1987). We have therefore investigated the effect of avermectin on calcium channels, using skeletal muscle fibres from larvae of the moth Plutella xylostella.

Recordings were made continuously from muscle fibres using KCl-filled microelectrodes under perfusion firstly with control saline and then with avermectin B_1 (10 nM). Hyperpolarising current pulses were applied via the same microelectrode at 0.2 Hz (10 nA, 500 ms duration) for measurements of cell input resistance. In other experiments, cells were kept near the resting potential by passage of steady DC current and depolarising current pulses (0.1 Hz, 10-20 nA, 40 ms duration) were applied to trigger action potentials. Tetraethylammonium was present to block potassium channels, and hypertonic sucrose was used to prevent muscle contraction. Under these conditions, calcium channel currents underly action potentials in this preparation (Lees et al, 1988).

Avermectin (10 nM) produced a depolarisation (10.4 ± 0.5 mV) and a fall in input resistance (from 0.61 ±0.03 MΩ to 0.27 ± 0.03 MΩ, $P < 0.02$, n=3 cells). At the same concentration, avermectin had no significant effect on the measured action potential parameters (control values: overshoot 18.5 ± 1.4 mV, maximum rate of rise 11.9 ± 2.1 Vs^{-1}, duration 35.0 ± 7.8 ms, n=5 cells).

These results show that, although avermectin caused a decrease in input resistance, possibly due to the opening of chloride channels in the membrane, it had no effect on voltage-dependent calcium channels in this insect preparation.

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251P PHACLOFEN INCREASES GABA RELEASE FROM VALPROATE TREATED RATS

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The GABA-transaminase inhibitor, gamma-vinyl-GABA, increases the K-evoked release of GABA from the rat cerebral cortex and this increase is greatly potentiated by phaclofen (Neal & Shah, 1989). Valproate, like gamma-vinyl-GABA, is also able to increase brain GABA levels and the present experiments were undertaken to see whether the effects of valproate on GABA release were also affected by phaclofen.

Rats were injected (I.P.) with either sodium valproate (400mg kg^{-1} or 600mg kg^{-1}) or saline (0.9%) 30min before they were killed by cervical dislocation. Cortical GABA levels and the release of GABA from cortical slices were measured as described previously (Neal & Shah, 1989).

The GABA content of the cerebral cortex in control rats was 2.9±0.37μmol g^{-1} wet wt (mean ± s.e. mean n=6). In valproate injected animals, 400 and 600 mg kg^{-1}, the cortical GABA levels were 3.8±0.56 and 4.2±0.44μmol g^{-1} ($P<0.02$) respectively.

The resting release of GABA from cortical slices prepared from saline injected (control) rats was approximately 6pmolmg^{-1} $10min^{-1}$, this was increased by KCl (50mM) to 26pmolmg^{-1} $10min^{-1}$ ($P<0.001$). The administration of valproate to the rats did not affect the subsequent resting release of GABA from cortical slices, but in tissue from rats treated with the higher dose of valproate (600mg kg^{-1}), the K-evoked release of GABA was significantly increased ($P<0.02$). In cortical slices taken from valproate (600mg kg^{-1}) treated rats the K-evoked release of GABA was progressively increased by phaclofen, being almost doubled by phaclofen (1mM) ($P<0.001$ n=9).

The results show that high doses of valproate increase both cortical GABA levels and the K-evoked release of GABA, phaclofen producing a further increase in the latter.

Neal, M.J. & Shah, M.A. (1989). Br. J. Pharmac. 98, 105-112

M.O. Ekwuru was an MRC supported intercalating BSc student. We are also grateful to the Physiological Society for a grant to M.O.E.

252P STUDIES ON THE EFFICACY OF LOW AFFINITY BENZODIAZEPINE RECEPTOR LIGAND ON RAT CEREBELLAR SLICE ACTIVITY

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This investigation arose from the observation that while the imidazopyrimidine derivative RU 34000 is a potent inverse agonist in vivo it has a low affinity, 0.98 µM, on benzodiazepine receptor binding (Gardner 1989). It is therefore of interest to determine the efficacy of this family of compounds on benzodiazepine receptors using RU 34347, a soluble methane sulphonate salt of RU 34000, on an in vitro test system. The system chosen was the modulation of γ-aminobutyric acid mediated inhibition in isolated cerebellar slices of rat where we have previously observed modulation with benzodiazepine receptor ligands (Bagust et al 1988).

In the present study electrical stimulation of the white matter (0.02-0.7mA- 1 ms) in the cerebellar slice preparation produced inhibition of spontaneous firing recorded from the Purkinje cell layer. The duration of inhibition was stimulus strength-dependent and could last several seconds at high current stimulation. Ro 194603 was used as a reference benzodiazepine inverse agonist and it showed a clear reduction of supra-threshold stimulus evoked inhibition at 1 µM and 10 nM in 7 of 7 cells tested using separate preparations for each observation, giving a reduction in inhibition of 20-100%. RU 34347, 10 µM, consistently induced a similar effect to Ro 194603 in 5 of 5 cells tested. However, with 1 µM RU 34347, a biphasic response was seen on 5 of 9 cells tested, when inhibition was initially increased over a 1-4 minute period by 120-320% but with continual perfusion this effect reduced and reversed to produce a decrease in inhibition of 20-100%. On the other 4 cells only reduction in inhibition occurred. At 10 nM, the biphasic response occurred consistently, 4 of 4 cells tested, and the increase in inhibition was more apparent, 120-470%. The effect of Ro 194603, 1-10 µM, was prevented or reversed by the benzodiazepine antagonist Ro 15-1788, 10 µM, in 4 of 7 cells tested. The enhanced inhibition due to RU 34347, 1-10 µM, was blocked by pre-incubation with RO 15-1788, 10 µM, in 6 of 6 cells tested but the following reduction in inhibition was more resistant to this antagonism.

These results provide evidence that Ro 194603 is acting on benzodiazepine receptors in this model and the increase in inhibition, i.e., agonist effect, seen with RU 34347 is also likely to be modulated via benzodiazepine receptors. This study also confirms that RU 34000 salts have potent inverse agonist-like effects. The mechanism underlying the novel biphasic effect of RU 34347 requires further study.

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253P THE PHARMACOLOGY OF CHOLINOCEPTORS ON THE SOMATIC MUSCLE CELLS OF THE PARASITIC NEMATODE *Ascaris Suum*

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Cholinoceptors on the somatic muscle cells of Ascaris are believed to be the site of action for several anthelmintics (Harrow & Gration, 1985). Using conventional 2-electrode electrophysiological recording techniques, the pharmacology of the cholinoceptor was studied. Acetylcholine (10 µM) elicited a depolarisation of 8.4 ± 0.7 mV (n=22, ± s.e. mean) accompanied by an increase in input conductance of 0.49 ± 0.09 µS, (n=22, ± s.e. mean). The relative potencies of muscarinic and nicotinic agonists (Table 1a) and antagonists (Table 1b) were investigated.

Table 1. a) The relative potency (R.P.) of agonists compared to acetylcholine was determined from the ratio of the concentration of acetylcholine to the concentration of agonist that produced equivalent responses for conductance (the results from the depolarization data were similar). b) Antagonists at the Ascaris ACh receptor: The IC_{50} was the concentration of antagonist that reduced the response to 10 µM ACh by 50%. The pA_2 value was determined from a single dose-ratio for which there was a parallel displacement of the dose-response curve (values are mean ± s.e. mean).

a)			b)					
AGONIST	R.P.	n	ANTAGONIST	IC_{50} µM		n	pA_2	n
Dimethylphenylpiperazinium	2	(6)	N-methyllycaconitine	0.23 ±	0.03	(4)	6.98	(1)
Acetylcholine	1		α-Bungarotoxin	-			5.85 ± 0.19	(3)
Carbachol	0.5	(4)	Mecamylamine	0.33 ±	0.04	(3)	-	
Nicotine	0.3	(3)	Strychnine	1.29 ±	0.29	(4)	-	
Trimethylammonium	0.05	(5)	d-Tubocurarine	3.1 ±	0.4	(3)	5.76	(2)
Muscarone	0.006	(3)	Pancuronium	3.2 ±	0.3	(3)	5.76	(1)
Furtrethonium	0.007	(3)	Atropine	6.7 ±	2.1	(3)	-	
Arecoline	0.002	(4)	Decamethonium	14.2 ±	3	(3)	-	
			Hexamethonium	43 ±	6	(3)	-	

The muscarinic agonists bethanechol and methacholine were without effect up to 1 mM (n=3). Pilocarpine (n=5) elicited a small, but distinct hyperpolarisation up to 3 mV (500 µM-3 mM). The results indicate that the receptor is nicotinic-like and the pharmacology may most closely resemble the vertebrate ganglionic nicotinic receptor. Further experiments are being conducted to examine this possibility.

We are grateful to the SERC and the Jersey Government for financial support.

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254P CHARACTERISATION OF SPATIAL LEARNING IN A HELICAL MAZE IN THE ABSENCE OF INTRA- OR EXTRA-MAZE CLUES

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One of the main deficits in Alzheimer patients is their inability to form and use 'internal maps' of their environment. The helical maze (Curry & Caan, 1988) was devised to provide a spatial learning task in which it is necessary to form an 'internal map' in order to find the reward, because there are no intra- or extra- maze cues. The maze is unlit and consists of ten storeys that are shuffled between trials to remove any cues to the rewarded floor. The interruption of infrared photobeams on each floor provide measures of the activity on each floor (horizontal movements), the time spent on each floor, and the latencies to move between floors.

Experiment 1 investigated the effects of the point of entry into the maze and of diazepam on the exploration of the maze by naive rats. Control rats descended more floors than they ascended and moved more rapidly down than up two floors. Diazepam (1 & 4 mg/kg) reduced the time spent on the bottom floor and the number of horizontal movements, but increased the speed of ascending the tower.

Experiment 2 investigated the effects on acquisition when the rats were required to ascend 2,3 or 4 floors to obtain a sugar puff reward, and of having to ascend versus descend 4 floors for reward. The task of ascending 4 versus 2 floors tended to be harder, but was only significantly so for the number of trials to obtain the reward without making a backwards turn. On the final criterion trial the rats ascending 4 floors took longer to reach the reward than those ascending 2 floors. The task of descending 4 floors tended to be easier than the ascending task, but the differences did not reach significance. Nor did the strength of the rat's turn preference (assessed independently) significantly change the speeed of acquisition.

Experiment 3 investigated the effects of scopolamine (2 & 4 mg/kg) and diazepam (1 & 4 mg/kg) on the acquisition of the task of ascending 4 floors for a sugar puff reward. Both doses of scopolamine significantly impaired acquisition, but diazepam was without significant effect.

Curry, P.E. & Caan, A.W. (1988) Neurosci. Lett. S32, S40

We are grateful to Peter Curry for technical assistance.

255P COMPARISON OF THE PHARMACOLOGICAL PROFILES OF FOUR HYPNOTICS WITH DIFFERENT CHEMICAL STRUCTURES: QUAZEPAM, BROTIZOLAM, ZOPICLONE AND ZOLPIDEM

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Because of their common ability to bind to the benzodiazepine recognition site within the GABA receptor complex (ω receptor), we compared the pharmacological properties in mice of four clinically active hypnotics, quazepam (a benzodiazepine), brotizolam (a thienotriazolodiazepine), zopiclone (a cyclopyrrolone) and zolpidem (an imidazopyridine). Zolpidem and quazepam show a high selectivity for the ω_1 (BZ_1) receptor subtype (Arbilla et al, 1985 ; Seighart, 1983). Anticonvulsant effects were evaluated by observing the presence of tonic convulsions induced by pentylenetetrazole (125 mg/kg, sc) or maximal electroshock (60 mA, 50 Hz, 0.4 s). Central depressant effects, ataxia and myorelaxation were assessed by measuring the decrease in exploratory activity and the impairment of performance in the rotarod and loaded grid tests. Disinhibitory activity was evaluated by measuring food intake in a novel environment. Intrinsic activity at the $GABA_A$ receptor was evaluated by observing the latency to convulsions induced by isoniazid (800 mg/kg, sc). Drugs were injected, ip, 30 min before the tests.

Compounds	ED_{50} mg/kg, ip					ED_{100}	% increase
	Anti-pentylene-tetrazole	Anti-Electroshock	Exploratory Activity	Rota-rod	Loaded Grid	Food Intake	Maximum delay to isoniazid convulsions
Zolpidem	9	9.6	1.2	18	17	Inactive	280
Quazepam	0.46	3.5	1.4	4.9	3.7	2	105
Brotizolam	0.04	0.2	0.11	0.36	0.16	0.3	200
Zopiclone	6.3	9.0	14.4	26.4	12.8	4	140

As shown in the table, zolpidem has a pharmacological profile different from the other hypnotics, dominated by sedative properties in comparison with its anticonvulsant and myorelaxant effects. Moreover zolpidem does not increase food intake indicating a lack of disinhibitory action. The high selectivity of zolpidem for the ω_1 receptor subtype (BZ_1) could be responsible to its preferential sedative properties. However, quazepam which also displays a selectivity for the ω_1 site showed a pharmacological profile similar to non-selective hypnotics. The observation that the intrinsic activity of zolpidem is much higher than that of quazepam could explain this discrepancy.

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256P PRE- AND POST-SYNAPTIC EFFECTS OF MIDAZOLAM ON RESPIRATORY SKELETAL MUSCLE

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The introduction of the potent benzodiazepine midazolam, often given in relatively high concentrations before surgery, has prompted a further evaluation of benzodiazepine effects on respiratory skeletal muscle.
In a previous study we confirmed findings of other authors (Driessen *et al*, 1984) of a biphasic action of chlordiazepoxide and diazepam on twitch and tetanic contraction of the rat diaphragm preparation *in vitro*. We also described effects of the benzodiazepines on the time constants of (miniature) endplate potentials (mepps) (Van Wilgenburg & Leeuwin, 1986). In the present study the effects of midazolam on twitch and tetanic contraction are compared with the effects caused by chlordiazepoxide, diazepam and flurazepam either after indirect and after direct stimulation. Furthermore attention has been paid to the resting potential and to the spontaneous release of acetylcholine.

Isolated rat diaphragm preparations *in vitro*, paralysed with pancuronium, were stimulated directly between a surgical steel wire attached to the tendon and to pins onto which the costal margin was impaled. Phrenic nerves were stimulated with bipolar platinum electrodes using rectangular waves (0.5 msec) at supramaximal voltages. Intracellular recordings were made with glass microelectrodes filled with 3 mol/l KCl. Benzodiazepines were given in increasing concentrations ranging from 10 µmol/l to 310 µmol/l. Between two concentrations the preparation was washed. Data were collected from three to six preparations for every experimental situation. The results were compared with the respective controls, taken as 100 %.

Midazolam causes a dose related enhancing followed by blocking action on twitch and tetanic contracture. The initial enhancing of the latter effect upon indirect stimulation, reaching 415 % of the control value at 30 µmol/l, is not sustained. The twitch is increased to 155 % followed by blocking at higher concentrations. To a two to three time lesser extent these effects are also found upon direct stimulation. Recovery of the contracture over 100 % only occurs with indirect stimulation. Compared to the other benzodiazepines midazolam causes a 3x, 6x and 8x stronger maximal response than diazepam, flurazepam and chlordiazepoxide respectively. Presynaptically midazolam increases dose related the spontaneous release of quanta to 6x the control values, while postsynaptically the membrane potential is reversibly depolarized from an average of 71 mV to 42 mV. Subsequently the amplitude of the mepps is reduced.

The results indicate that both presynaptic and postsynaptic effects of benzodiazepines might be involved in the biphasic effects of benzodiazepines on the contracture of skeletal muscles. Midazolam has the highest potency of the benzodiazepines studied. The quantitative similarity between the ratios of potency on peripheral and central sites suggests a relationship between the central and peripheral mechanism.

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257P FLUMAZENIL SUPPRESSES, AND CGS8216 ENHANCES, PHYSICAL SIGNS OF DIAZEPAM WITHDRAWAL IN RATS'

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Introduced by S.E.File

Withdrawal from chronic benzodiazepine (BZ) treatment produces a spontaneous withdrawal syndrome in animals, which can be reversed by the BZ receptor antagonist flumazenil (Baldwin and File, 1988; Gallager and Heninger, 1986). In contrast, the BZ receptor antagonist CGS 8216 has been reported to enhance anxiety and precipitate physical signs of abstinence after chronic BZ treatment (File and Pellow, 1985; McNicholas and Martin, 1982); but in these studies the antagonist was given while BZ was still present in the brain. The present studies sought to determine whether the effects of the two antagonists would differ when both were given 48h after diazepam (DZ) withdrawal.

Male Wistar rats were administered i.p. DZ (10 mg/kg/day, 28 days). 48h after the last DZ injection animals were administered i.p. vehicle, CGS 8216, flumazenil or CGS 8216 + flumazenil. Immediately afterwards the incidence of wet dog shakes and forepaw jerks (as indices of abstinence) were scored during a 30 min observation period.

CGS 8216 significantly ($P<0.01$) enhanced both signs of DZ withdrawal (Table 1). In contrast, flumazenil inhibited both signs ($P<0.05$ and $P<0.01$ respectively). Flumazenil also prevented the enhancement of the signs of abstinence by CGS 8216 (Table 1).

Table 1. Effect of CGS 8216 and flumazenil and their combined treatment on the physical signs of diazepam withdrawal (DW) in rats. The data are means ± SEM. * - $P<0.05$ vs control; + - $P<0.05$; ++ - $P<0.01$ vs DW; n - number of animals per group.

Drug treatment, dose	n	Number of wet dog shakes	Number of jerks of forepaws
Control: chronic vehicle	10	1.1±0.5	0.8±0.5
DW + Vehicle	12	6.1±1.4*	2.4±0.4*
DW + Flumazenil (10 mg/kg)	8	2.3±0.8+	0.4±0.2+
DW + CGS 8216 (5 mg/kg)	12	15.6±2.8++	10.7±1.3++
DW + CGS 8216 + Flumazenil	8	2.9±0.6	1.6±0.5

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258P REGIONAL DIFFERENCES IN EVOKED STRIATAL DOPAMINE OVERFLOW MEASURED USING FAST CYCLIC VOLTAMMETRY IN BRAIN SLICES

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Dopamine (DA) overflow, detected using Fast Cyclic Voltammetry (FCV), has been evoked by sine wave electrical stimulation *in vivo* (Stamford *et al.*, 1988). Palij *et al.* (1988) have used square wave stimulation to evoke DA overflow from rat striatal brain slices. In the present investigation, sine wave stimulation has been applied to brain slice preparations to study evoked neuronal DA overflow and to investigate this in different areas of the striatum.

Brain slices, including corpus striatum, (350 µm thick) from male Wistar rats (100 to 150 g) were superfused with oxygenated artificial cerebrospinal fluid at 32 °C. Stimulated DA overflow was monitored using FCV with a carbon fibre microelectrode located 80 µm below the surface of the slice. Waveform capture and analysis was performed using an IBM compatible computer, CED 1401 A/D converter and CED SIGAVG software. Bipolar tungsten stimulating electrodes placed 200 µm from the recording electrode were used to apply the electrical stimulus. Up to 2 µM DA could be detected per train (100 ms) of sine waves (50 Hz, amplitude 24 V); trains gave reproducible overflow over a period of at least 6 hours. The electrochemical signal recorded upon stimulation was indistinguishable from that produced by DA perfusion.

Overflow was directly proportional to stimulus strength over the range tested (6 to 42 V; 0.19 ± 0.02 to 1.36 ± 0.24 µM DA; n-4) and was also dependent on stimulus duration (50 to 500 ms; 0.60 ± 0.17 to 2.10 ± 0.43 µM DA; n-4 to 6). Stimulation in 'dark striatal bands' consistently evoked 15 to 32% less DA overflow: stimulating electrodes were always thereafter positioned to span a 'light band'. DA overflow was found to diminish with distance from stimulating electrodes (at 400 µm, overflow was 62 ± 22%; n-4). The central area of the striatum released more DA than any neighbouring medial or ventral area ($P<0.05$) while the medio-ventral area released less than all areas adjacent to it ($P<0.05$). This is interesting since Beal & Martin (1985) observed no difference in DA concentrations across a coronal section of striatum.

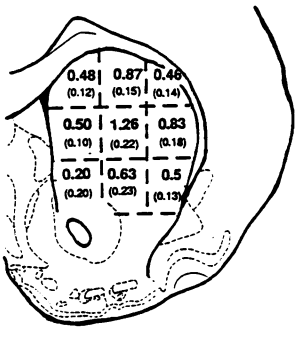

Figure 1 shows the average amounts of DA overflow (µM) in different areas of striatum (± s.e.mean; n-9 to 16).

Single square wave pulses (0.1 ms, 10 V) typically evoked 0.15 ± 0.05 µM DA (n-20); a train of square wave pulses (50 Hz, 10 V, 0.1 ms for 100 ms) evoked 0.61 ± 0.12 µM DA (n-4), which did not differ ($P>0.05$) from sine wave stimulation (10 V, 100 ms, 50 Hz; 0.41 ± 0.07 µM DA). These results have shown that sine wave stimulation can be utilised to evoke DA overflow in striatal slices, consistent with previous observations *in vivo*. Further, evoked overflow is not uniform from all areas of the striatum.

Supported by the Wellcome Trust (SJT) and Glaxo (JJO'C)

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259P THE EFFECTS OF NICOTINE AND AMPHETAMINE ON NUCLEUS ACCUMBENS DOPAMINE SECRETION: A MICRODIALYSIS STUDY

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Robinson et al (1988) have reported that the stimulation of locomotor activity and nucleus accumbens (NAC) dopamine (DA) secretion evoked by amphetamine can be enhanced by pretreatment with high doses of the drug. This study tested the hypothesis that the increased locomotor stimulant response to nicotine, observed in rats pretreated with the alkaloid (Clarke 1987) is also associated with enhanced secretion of DA in the NAC. Extracellular DA levels were measured in samples collected from dialysis loops located in the NAC of freely moving rats treated acutely and chronically (6 daily injections) with nicotine (0.4 mg/kg) or amphetamine (0.5 mg/kg). Locomotor activity was measured in an activity box (Vale & Balfour, 1989). As can be seen in figure 1a, following pretreatment with nicotine, a challenge dose of the alkaloid caused a significant ($F(6,78) = 4.2$, $p<0.01$) increase in extracellular DA levels. This effect was not observed after acute nicotine administration. The magnitude and duration of the response were less than those obtained with acute ($F(6,54) = 8.8$, $p<0.01$) and chronic ($F(6,48) = 12.8$, $p<0.01$) amphetamine. Acute nicotine stimulated ($F(6,78 = 7.7$, $p<0.01$) locomotor activity, an effect which was enhanced ($F(6,78) = 3.2$ $p<0.01$) after pretreatment with the drug. The peak activity levels induced by chronic nicotine were comparable with those seen after acute ($F(6,54) = 15.4$, $p<0.01$) and chronic ($F(6,48) = 25$, $p<0.01$) amphetamine. The data suggest that chronic nicotine is associated with sensitisation of the mesolimbic DA system and enhanced locomotor activity, effects which do not occur in response to low, psychomotor stimulant doses of amphetamine.

FIGURE 1a

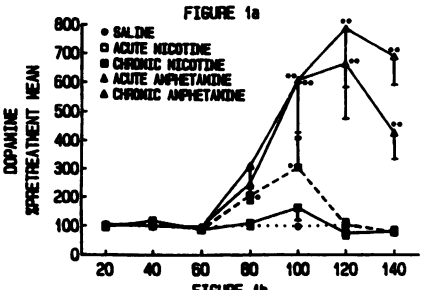
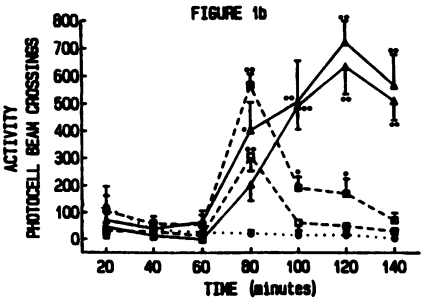


FIGURE 1b



The results are mean±SEM of 4-8 observations. Data were analysed by ANOVA followed by Tukey's test for post hoc analysis. *p<0.05, **p<0.01 significantly different from saline controls.

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260P DIFFERENTIAL SQUARE PULSE CONDITIONING VOLTAMMETRY: A NEW METHOD FOR ELECTROCHEMICAL ANALYSIS WITH MICRO-BIOSENSORS

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Differential pulse voltammetry (DPV) allows simultaneous sensitive and selective detection of dopamine, serotonin and their metabolites using Nafion coated or electrically pre-treated carbon fibre micro-electrodes (CFE) (Crespi et al., 1984 & 1988). This electrophysiological technique requires a delay of 2-6 mins between each scan in order to maintain the electrical characteristics of the biosensor (CFE) and a stable voltammogram. This can be avoided using other polarographic methods which allow more rapid and frequent measurements (scans) but show less sensitivity and selectivity (for a review see Marsden et al., 1987).

In order to obtain faster and more frequent DPV scans maintaining selectivity and sensitivity, we have developed a modified version of the methodology which we have called Differential Pulse Conditioning Voltammetry (DPCV). The modification consists of the application of 3 conditioning potentials to the CFE, immediately followed by a modified version of the DPV scan, called differential square pulse voltammetry (DSPV). This avoids the 2 to 6 mins wait between the normal DPV scans (lasting 1-2 mins each) without losing sensitivity and selectivity when dopamine and serotonin (or their metabolites) are simultaneously monitored in vitro with CFE. This contrasts with the loss of both sensitivity and selectivity encountered when normal DPV scans are performed without a 2 to 6 mins delay.

This new technique of Differential Square Pulse Conditioning Voltammetry (DSPCV) allows fast continuous measurements which should correlate better with neurotransmitter release and metabolism as well as behavioural variations in vivo.

Supported by the British Council and the MRC.

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261P OPPOSING EFFECTS OF DOPAMINE D_1 AND D_2 RECEPTOR STIMULATION ON THE PROPAGATION OF MOTOR SEIZURES IN MICE AND RATS

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Dopamine D-2 receptors mediating anticonvulsant effects have recently been pinpointed to the corpus striatum (Turski et al., 1988), but the role of D-1 receptors in the propagation of motor seizures remains obscure. In this study, the ability of D-1 and D-2 receptor-selective drugs to influence the production of motor seizures, was investigated in two separate seizure models using mice and rats.

Mice which had been injected with reserpine, 5 mg/kg, to deplete brain stores of monoamines, could be made to convulse when injected with the D-1 agonists SKF 38393 (15-30 mg/kg) and CY 208-243 (0.3-3 mg/kg) 24-48 hours (but not 3 hours) later. The convulsant action of SKF 38393, 15 mg/kg, could be prevented by coinjecting the D-2 agonists lisuride (5 mg/kg) and RU 24213 (5 mg/kg), the mixed D-1/D-2 agonist apomorphine (0.5 mg/kg), or the selective D-1 blocking drug SCH 23390 (0.1 mg/kg).

The high density of D-1 receptors in the substantia nigra pars reticulata (Dawson et al., 1988), suggested this nucleus was a possible site for the convulsant effects of D-1 stimulants, by attenuating the neuroinhibitory effects of GABA at striatonigral synapses (Waszczak & Walters, 1986). To test this hypothesis, rats were anaesthetised with halothane and microinjected stereotaxically in both nigras with vehicle (controls) or SKF 38393, 2.5 µg in 0.5 µl. Following recovery they were then given a dose of pilocarpine (400 mg/kg) previously determined to be subconvulsant. None of the control rats convulsed (0/9 rats) compared to 10/14 rats receiving the D-1 agonist. This effect of SKF 38393 was prevented by pretreatment with SCH 23390 (0.25 mg/kg). Similarly, SCH 23390, 1 µg per side, inhibited the seizures (2/8 rats convulsed) and deaths (1/8 rats died) induced by 800 mg/kg pilocarpine, compared to vehicle-injected controls (8/8 rats convulsed and died). These results provide evidence for a dual pro- and anti-convulsant action of dopamine in the brain mediated respectively by D-1 and D-2 receptors, and suggest D-1 receptors in the nigra can contribute to this proconvulsant effect.

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262P HALOPERIDOL-INDUCED BRADYKINESIA IN THE MARMOSET

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Neuroleptic treatment in man can produce extrapyramidal side-effects which resemble parkinsonian-like bradykinesia (Hornykiewicz, 1973). The same agents also induce bradykinesia in rodents which is commonly referred to as catalepsy (Stanley and Glick, 1976). Neuroleptic-induced catalepsy in rats has been proposed as a model of Parkinson's disease and has been used to identify compounds that might have potential therapeutic use for the treatment of this disorder. To date few primate models of neuroleptic-induced bradykinesia have been described. It was the purpose of this study to set up such a model and to illustrate that it too could be used to evaluate potential anti-parkinsonian agents.

Male and female common marmosets (350-450g; Glaxo, Ware) were used in these studies. Bradykinesia was induced after intraperitoneal (i.p.) administration of haloperidol (0.01 - 0.3mg/kg) and was assessed using a 7 point rating scale (Close et al. 1989) at various time intervals up to 6h post injection. Reversal of the drug-induced bradykinesia was attempted by administration of various agents and their vehicle controls 2h after the haloperidol injection.

Results show that bradykinesia can be induced in the marmoset by haloperidol, and that this effect is dose- and time-dependent. The directly acting dopamine D2 agonist, PHNO (10ug/kg; s.c.), and the muscarinic antagonist, scopolamine (0.3mg/kg; s.c.) both attenuated the effects of the neuroleptic.

The $5HT_{1A}$ full agonist, 8OHDPAT (up to 0.3mg/kg; i.p.) did not alter significantly the bradykinesia score induced by haloperidol, but did increase the alertness of the marmosets. The partial $5HT_{1A}$ agonist, BMY 7378 (up to 3.0mg/kg; i.p.) was also unable to reverse the haloperidol-induced bradykinesia. Finally, the NMDA antagonist, MK 801 (up to 0.03mg/kg; i.p.), was ineffective in the above animal model.

The data provided here illustrate that bradykinesia can be elicited in the marmoset by neuroleptics and that this behaviour can be reversed by drugs which are effective anti-parkinsonian agents in man (Lieberman et al 1988). We have previously shown that, in addition to PHNO and scopolamine, 8OHDPAT and BMY 7378 and MK 801 can attenuate fluphenazine-induced catalepsy in the rat (Elliott et al 1989). The exact reason why the present results in the marmoset differ from those in the rat is unknown, but might be due to anatomical or receptor differences.

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263P PREDICTING ACTIVITY AT THE D-2 DOPAMINE RECEPTOR USING THE [^{3}H]-SULPIRIDE/[^{3}H]-NO437 RATIO

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In the dopaminergic system radioligand binding assays have been used to define multiple receptors and affinity states. In order to estimate efficacy, however, detailed curve analysis, GTP shifts or functional assays are required. A simple biochemical method of estimating efficacy at the D-2 receptor is described using the antagonist [^{3}H]-sulpiride to label the low affinity state, and the agonist [^{3}H]-N0437 to label the high affinity state. Both were chosen for their D-2 receptor selectivity (Zahniser *et al.* 1983; Horn *et al.* 1985). Rat striatal membranes (0.03-0.05mg protein/tube) washed in 1mM EDTA were incubated with 10nM [^{3}H]-sulpiride for 10 min at room temperature in the presence of 100μM GppNHp. A similar concentration of untreated striatal membranes were incubated with 1nM [^{3}H]-N0437 for 60 min at 30°C. Both assays were carried out in 20mM HEPES/Krebs' + 10μM pargyline buffer at pH7.4, with 10mM EDTA added for the [^{3}H]-sulpiride assay. Non-specific binding was defined by 1μM (-)sulpiride. Incubations were terminated by 2 min on ice followed by filtration through GF/C filters soaked in 0.05% polyethyleneimine and washed with 10ml of ice cold buffer. The affinities for a number of standard dopaminergic compounds (George *et al.* 1985) in both assays are shown below The ratio between the affinities in the two assays was compared with the guanine nucleotide shift previously reported in [^{3}H]sulpiride binding.

Compound	[^{3}H]-Sulpiride Kapp (μM)	[^{3}H]-N0437 Kapp (μM)	Ratio	GppNHp Shift*
Dopamine	1.5 (1.2;1.8)	0.022 (0.018;0.028)	68	6.6**
Apomorphine	0.058 (0.054;0.062)	0.0024 (0.0021;0.0028)	24	4.4**
ADTN	0.026 (0.024;0.028)	0.0021 (0.0018;0.0025)	12	3.0**
Bromocryptine	0.00079 (0.00051;0.0012)	0.000087 (0.000074;0.0001)	9.1	1.2
(-)Sulpiride	0.0025 (0.0022;0.0030)	0.0065 (0.0051;0.0082)	0.38	1.3

Kapp: IC_{50} corrected for ligand occupancy, as geometric mean, numbers in parentheses are the low and high values. Each value is the mean of at least 3 independent experiments carried out in triplicate. Ratio: (Kapp in [^{3}H]-sulpiride)/(Kapp in [^{3}H]-N0437). *: Results from Freedman *et al.* (1981). ** Statistically significant shift $P < 0.05$.

Dopamine antagonists such as (-) sulpiride which had little GppNHp shift (1.3) gave very low ratios (0.38) . In contrast agonists such as dopamine which had a Gpp(NHp) shift of 6.6 gave rather higher binding ratios (68). The ratio does therefore appear to correspond with the efficacy of a compound, as defined by the guanine nucleotide shift at the D-2 receptor.

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264P EFFECTS ON CENTRAL DOPAMINERGIC FUNCTION ARE NOT INVOLVED IN THE PHARMACOLOGICAL ACTIONS OF THE NOVEL ANTIDEPRESSANT SIBUTRAMINE HCL

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The novel antidepressant sibutramine HCl (BTS 54 524; N-1-(1-[4-chlorophenyl]cyclobutyl)-3-methylbutyl-N,N-dimethylamine hydrochloride monohydrate) rapidly down-regulates rat cortical β-adrenoceptors and this is suggested to result from noradrenaline and 5-HT reuptake inhibition (Luscombe et al, 1989). We have used various techniques to determine whether enhancement of central dopaminergic function is also involved. Comparative experiments have been performed using the dopamine (DA) reuptake inhibitor bupropion (Soroko et al, 1977) and the DA releasing agent/reuptake inhibitor methamphetamine (Scheel-Kruger, 1971).

Male 150-300g CD rats (Charles River) or female 200-320g PVG rats (Olac) were used. Drugs were dissolved in distilled water for oral (po) and saline for intraperitoneal (ip) administration. DA release was measured by superfusion of striatal slices preloaded with [^{3}H]DA. Test substances were perfused for 8 min. Striatal 3-methoxytyramine (3-MT) was determined by HPLC-ECD 1h after treatment (Heal et al, 1988). In rats lesioned by a unilateral injection of 6-hydroxydopamine (8μg) into the substantia nigra, circling was measured at 10 min intervals for 1h. Generalisation to amphetamine was determined for 2.5 min, 15 min after treatment using rats trained to discriminate ip amphetamine (0.5mg/kg) from saline. Sibutramine HCl (10^{-7}-10^{-5}M) did not alter [^{3}H]DA release from striatal slices. Basal 3-MT levels (ng/g tissue ± s.e. mean) = 126 ± 6 (n=46) were not affected by sibutramine HCl (3mg/kg ip or 6mg/kg po). At 6mg/kg po, this antidepressant did not induce circling in nigrostriatal lesioned rats and, in drug-discrimination, it was recognised as saline at $\leq$ 3mg/kg ip, whereas doses $\geq$ 5mg/kg ip suppressed responding. Bupropion (10^{-7}-10^{-5}M) also had no effect on [^{3}H]DA release and bupropion (10mg/kg ip or 30mg/kg po) similarly did not alter 3-MT levels. At 10mg/kg po, bupropion did not induce circling, but produced weak ipsilateral circling (1.8 ± 0.4 turns/min, $P<0.01$) at 30mg/kg po. Bupropion was recognised as saline $\leq$ 10mg/kg ip, but generalised to amphetamine at 30mg/kg ip. Methamphetamine (10^{-8}-10^{-4}M) markedly and dose-dependently enhanced [^{3}H]DA release with increases between 27% ($P<0.05$) and 157% ($P<0.001$) at 10^{-8}M and 10^{-4}M. Methamphetamine enhanced 3-MT levels by 123% ($P<0.001$) at 3mg/kg ip and 92% ($P<0.001$) at 4.2mg/kg po. At 4.2mg/kg po, methamphetamine induced marked ipsilateral circling (8.4 ± 0.7 turns/min, $P<0.001$), and it generalised to amphetamine $\geq$ 0.3mg/kg ip.

Since sibutramine HCl rapidly down-regulates β-adrenoceptors at 1.8-3mg/kg and, at these doses, it does not mimic the biochemical and behavioural changes induced by bupropion or methamphetamine, inhibition of DA reuptake and/or stimulation of DA release are unlikely to contribute to the pharmacological actions of this antidepressant.

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265P EFFECTS OF L-GLUTAMATE (L-GLU) ON THE RELEASE OF ENDOGENOUS NORADRENALINE (NA) FROM THE RAT SUPRAOPTIC NUCLEUS (SON) *IN VITRO*

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Intracerebroventricular injection of L-GLU increases the arterial blood pressure (e.g. Lampa et al., 1988). This effect appears to be partially mediated by a rise in plasma vasopressin. The magnocellular neurons in the paraventricular nucleus and SON are innervated by noradrenergic nerve fibres which mediate an excitatory input (Day et al., 1984; Day & Renaud, 1984). In the present experiments it was tested whether L-GLU may facilitate the release of NA in the SON.

Hypothalamic slices of the SON region were prepared. Four slices were placed together into a teflon basket and incubated in 2 ml modified Krebs-HEPES solution which contained the neuronal amine uptake inhibitor desipramine (1 µM). In most experiments Mg^{++} was omitted from the medium. The incubation medium was changed every 10 min and the release of endogenous NA was determined by HPLC with electrochemical detection as described by Racké et al., (1989). L-GLU was added to the medium after 40 min of incubation for two subsequent incubation periods.

The spontaneous outflow of NA (determined between 30 and 40 min of incubation) was about 1.5 pmol/10 min and this corresponded to about 1 % of the tissue content determined at the end of the incubation experiments. In the absence of test substances, the outflow of NA declined by about 10 % during the following 70 min. L-GLU increased the outflow of NA in a concentration-dependent manner. At 0.3 mM, L-GLU evoked the release of 0.3 $\pm$ 0.1 pmol NA (mean $\pm$ s.e.m., n=3). At 1, 3, 5 and 10 mM, L-GLU caused the release of 1.1 $\pm$ 0.5, 4.1 $\pm$ 0.9, 10.1 $\pm$ 0.4 and 31 $\pm$ 9.1 pmol NA, respectively (each n=3-5). In the presence of 1 µM yohimbine the release of NA evoked by 0.3 and 1 mM L-GLU was enhanced to 1.9 $\pm$ 4 and 2.4 $\pm$ 0.5 pmol, respectively, whereas that evoked by 3 mM L-GLU was not significantly altered in the presence of yohimbine. The release of NA evoked by 3 mM L-GLU was not altered in the presence of tetrodotoxin (1 µM) and only slightly reduced after omission of calcium from the incubation medium or after addition of Mg^{++} to the incubation medium.

In conclusion, the noradrenergic nerve endings in the SON can be excited by L-GLU. The characteristics of the L-GLU-evoked release of endogenous NA in the SON appear to differ from those reported for the L-GLU-evoked NA release in cortical or hippocampal slice preparations.

Supported by the Deutsche Forschungsgemeinschaft; L.B. was supported by a followship of the C.N.R., Italy

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266P NICOTINE-STIMULATED NORADRENALINE RELEASE IN THE HIPPOCAMPUS OF FREELY MOVING RATS

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We have previously shown that acute nicotine stimulates noradrenaline synthesis in the hippocampus, determined ex vivo by measuring dihydroxyphenylalanine (DOPA) accumulation following inhibition of amino acid decarboxylase (Mitchell et al., 1989a and 1989b). Here we report the in vivo effect of acute nicotine on noradrenaline release in the hippocampus of freely moving animals using intracerebral dialysis.

Male Sprague Dawley rats (300-330 g), were chronically implanted with Carnegie Medicin dialysis guide cannulae just above the left lateral hippocampus. After at least 5 days recovery from surgery, a 3mm Carnegie dialysis probe was implanted via the guide into the hippocampus midway between CA1 and CA3 during brief anaesthesia (Brietal 10 mg). The probe was perfused with artificial C.S.F. containing 1.25 mM $CaCl_2$ and 5 µM nomifensine. After a 30 min recovery, samples were collected every 10 min and assayed for noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) using HPLC-ED; limit of detection 5-10 fmol/sample. Baseline samples were collected for 120 min and then animals received either two injections of nicotine (0.8 mg/kg free base, s.c.) or saline (1 ml/kg s.c.), with 150 min between injections. In another group of animals mecamylamine (5 mg/kg i.p.) was administered between successive nicotine challenges.

The first nicotine injection significantly increased extracellular NA (+253±21%, P<0.001, n=7-8), DOPAC (+400±38%, P<0.001, n=7-8) and HVA (+197±15%, P<0.001, n=6) compared to saline controls. DA levels were increased but this was not significant; 5-HIAA levels were not affected by nicotine. The second nicotine injection produced similar % changes, with no apparent desensitisation. In the animals pretreated with mecamylamine this second response was markedly attenuated. These results show that nicotine stimulates NA release in the hippocampus, an effect that may be mediated by central nicotinic receptors.

Supported by R.J. Reynolds Tobacco Co.

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267P SODIUM DEPENDENT BINDING OF [^{3}H]-RACLOPRIDE TO RAT STRIATAL MEMBRANES

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The substituted benzamide, [^{3}H]raclopride, binds with high affinity to dopamine D2 receptors in the presence of high concentrations of Na^+ ions (Kohler et al., 1985). However, it is not clear whether this binding is directly related to the Na^+ ion concentration. Therefore, we have studied the dependency of [^{3}H]raclopride binding to rat striatal membranes on the concentration of Na^+ ions.

Male CD rat (Charles River) striatal membranes were prepared as described by Kohler et al. (1985). The final pellet was resuspended in 50mM Tris-HCl (pH 7.4 at 25°C) containing 5mM KCl, 2mM $CaCl_2$, 10μM pargyline and varying concentrations of NaCl (0-120mM). Compensatory concentrations of choline chloride (120-0mM) were added to maintain the Cl^- concentration. Saturation binding was performed at six concentrations of [^{3}H]raclopride (0.3-10nM). Specific binding was defined by 1μM sulpiride. All incubations were carried out at pH 7.4 and 25°C; equilibrium was reached by 60 min. These parameters were determined from preliminary experiments. Equilibrium dissociation constants (Kd) and maximum number of binding sites (Bmax) were determined by non-linear regression analysis fitting to a one-site model (Munson and Rodbard, 1980).

In the absence of Na^+ ions the specific binding of [^{3}H]raclopride was less than 20% of total binding, thus Kd and Bmax values could not be determined. With increasing concentrations of Na^+ ions (30-120mM) there was a significant ($F(3,10)=144.22$, One-way ANOVA) concentration dependent increase in affinity (decreased Kd, Table 1). This was accompanied by an increase in Bmax (Table 1) which was not statistically significant ($F(3,20)=1.72$). These differences were not due to changes in non-specific binding (data not shown).

Table 1 The effect of Na^+ concentration on kinetic parameters of [^{3}H]raclopride binding

[Na^+] mM	30	60	90	120
Kd (nM)	10.5 ± 0.7	5.8 ± 0.1	3.6 ± 0.1	1.3 ± 0.1
Bmax (fmol/mg protein)	419 ± 36	493 ± 18	517 ± 46	522 ± 46

Values are means ± s.e. mean of 4-6 determinations

These data agree with the findings of Theodorou et al. (1980) that benzamide binding to D2 receptors, in contrast to the binding of other neuroleptics, is dependent upon the presence and concentration of Na^+ ions. The data also support the view that benzamides do not bind solely to the dopamine recognition site, but possibly to dopamine receptor ionophore sites controlling Na^+ conductance as well.

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268P TREATMENT WITH DSP-4 SELECTIVELY INCREASES β_1-ADRENOCEPTOR NUMBER IN RAT CEREBRAL CORTEX

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ß-Adrenoceptor number in rat brain can be modulated by various exogenous compounds or by changes in the level of the endogenous neurotransmitter noradrenaline. Depletion of the brain noradrenaline content following treatment with 6-hydroxydopamine or DSP-4 in the rat causes a gradual localised increase in ß-adrenoceptor number (Sporn et al, 1977; Dooley et al, 1983). Such changes are prominent in the cerebral cortex and hippocampus and absent in the cerebellum, suggesting that the effect of denervation supersensitivity is restricted to the β_1-adrenoceptor. In this study we set out to examine the effects of DSP-4 on β_1- and β_2-adrenoceptor sub-types in rat cerebral cortex by selective labelling of total and β_1-adrenoceptors using [^{125}I]-pindolol.

Male Wistar rats (250-350g) were pretreated with zimelidine (10mg/kg *i.p.*) then 30 min later received either 0.5ml saline or DSP-4 (100mg/kg *i.p.*). Animals were killed either 3 or 14 days after treatment and the brain immediately removed. The cerebral cortex was isolated, homogenised and frozen at -20C until assay. ß-Adrenoceptor number and affinity were characterised using [^{125}I]-pindolol essentially as described by Beer et al (1987). Specific binding of [^{125}I]-pindolol to total and β_1-adrenoceptors was defined by 100 uM isoprenaline and 100 nM CGP 20712A respectively. Binding data was analysed by non-linear regression analysis.

	TOTAL ß-ADRENOCEPTORS		β_1-ADRENOCEPTORS	
	CONTROL	DSP-4	CONTROL	DSP-4
3 DAYS	67.5 ± 5.5	68.8 ± 3.1	45.8 ± 2.9	46.3 ± 2.2
14 DAYS	57.5 ± 4.6	71.0 ± 5.8	43.6 ± 2.6	55.8 ± 4.7 *

Table 1: Binding capacity (fmol/mg protein) of total and β_1-adrenoceptors labelled by [^{125}I]-pindolol in rat cerebral cortex following treatment with DSP-4 or saline. Results are expressed as mean ± s.e.mean, n=4 in all groups. * $P<0.05$ unpaired t-test.

As shown in Table 1, treatment with DSP-4 did not alter total or β_1-adrenoceptor number when assayed 3 days later. However when tested 14 days after treatment both total and β_1-adrenoceptor number were increased by 23% and 28% respectively. Subtraction of the β_1- from the total ß-adrenoceptor capacity in each case indicated that β_2-adrenoceptor capacity was increased by 9%. This effect was not statistically significant ($P>0.3$). Our results indicate that the effect of denervation supersensitivity on ß-adrenoceptor number in the rat cerebral cortex is restricted to an increase in β_1-adrenoceptors. This confirms the suggestion by Dooley et al (1983) and reinforces the proposal that in the rat brain β_1-adrenoceptors are located close to noradrenergic nerve terminals whereas β_2-adrenoceptors are located some distance from such nerve terminals.

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269P IMMUNOBLOT ANALYSIS OF THE α SUBUNIT OF G PROTEINS IN CONTROL AND ALZHEIMER DISEASED BRAINS

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Numerous studies of Alzheimer brains have reported alterations in various neurotransmitters and their receptors (Roth & Iversen, 1986), whereas signal transduction systems have been the subject of minimal investigation. G proteins are part of many transduction systems and we have used polyclonal antibodies raised against specific peptide fragments of the α subunit of G_o, G_i and G_s (Milligan, 1988), to compare the amount of the subunits in frontal cortex from control and Alzheimer brains.

Membrane protein extracts from six control and six Alzheimer brains were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane and incubated with specific primary antibody. Immunoreactive proteins were detected using a peroxidase linked second antibody for G_i and G_o, and ^{125}I labelled second antibody for G_s. Bands were analysed on a Quantimet image analyser.

TABLE 1 Immunoblot analysis of 50μG of membrane extract

	(Age) (years)	(Plaques) (/mm²)	(optical density value, arbitrary units) G_o	G_s(H)	G_s(L)	G_i1	G_i2
Control	79 ± 18	1 ± 2.0	1.00 ± 10%	2.5 ± 19%	1.85 ± 13%	0.50 ± 28%	0.29 ± 24%
Alzheimer	86 ± 7	31 ± 15	1.02 ± 8%	2.9 ± 12%	2.0 ± 11%	0.45 ± 17%	0.32 ± 15%
P value		<0.005	0.69	0.13	0.32	0.61	0.34

Values are mean ± % standard deviation and P values determined by unpaired students t-test. G_s(H) and G_s(L) are the $G_{s\alpha}$ polypeptides with relative masses of 45 and 42 kDA respectively. Note: Comparisons can be made between G_i1 and G_i2 and also between G_s(H) and G_s(l), but not between subfamilies since different antibodies were used. The data suggests that there are no significant alterations in the amount of G proteins in the frontal cortex of Alzheimer brains.

This investigation was supported by the Scottish Home and Health Department and the Wellcome Trust.

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270P AN AUTORADIOGRAPHIC STUDY OF FORSKOLIN BINDING IN HUMAN HIPPOCAMPUS AND ITS ALTERATIONS IN SCHIZOPHRENIA

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The dopamine hypothesis for schizophrenia provides no potential for the development of novel therapeutic strategies in schizophrenia (Crow, 1987). Second messenger systems are well characterized and potential exists for their pharmacological manipulation (Worley, Baraban and Snyder, 1981). In view of the neuropathological changes now known to exist in the hippocampus in schizophrenia (Kerwin, 1989) we have performed a quantitative autoradiographic study, visualizing adenylate cyclase and inositol,1,4,5 triphosphate binding sites in slices from unfixed medial temporal lobe in control and schizophrenic brain.

Left and right hippocampal material was available from 6 age and sex matched controls and schizophrenic brains. Autoradiography was performed on section of unfixed material. Adenylate cyclase sites were visualized by incubation with ^3H-forskolin, (10-200 nM) and inositol triphosphate receptors visualized by incubation with ^3H-inositol,1,4,5 triphosphate; (up to 100 nM);non specific binding was determined with 1,000 fold excess cold ligand.

Quantification and visualization of autoradiographs was performed using an IBAS II Image analyzer.

The localization of forskolin binding is shown in Table 1.

Table 1

	Right Schizophrenic	Left Schizophrenic	Right Control	Left Control
Dentate gyrus	123.1 ± 22.7	133.9 ± 41.8	170.6 ± 38.2	85.7 ± 13.5
CA_4	87.9 ± 24.1	116.7 ± 24.1	97.8 ± 30.2	89.0 ± 8.8
CA_3	97.5 ± 16.4	116.8 ± 34.2	70.9 ± 51.0	74.3 ± 5.8
CA_2	127.9 ± 14.6	129.2 ± 45.4	103.5 ± 35.2	92.2 ± 18.6
CA_1	135.1 ± 22.0	138.7 ± 32.3*	107.7 ± 35.6	89.5 ± 8.2
Parahippocampal gyrus	134.9 ± 20.2*	208.7 ± 18.3**	101.9 ± 26.7	76.6 ± 14.6

*P<0.05
**P<0.001
Pmoles/g

We are grateful to Dr Gavin Reynolds (Nottingham) and Dr Clive Bruton (MRC, Runwell) for post-mortem material.

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271P SUB-CORTICAL β-ADRENOCEPTOR BINDING SITES IN POST-MORTEM BRAIN SAMPLES FROM DEPRESSED SUICIDE VICTIMS

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We have previously reported lower numbers of cortical β-adrenoceptors in depressed suicides compared to controls (De Paermentier et al., 1989a). We have now extended this study to sub-cortical areas from the same group of suicides (15M, 7F; mean age ± sem, 42±3 years; post-mortem delay, 35±4 h), in whom a firm retrospective diagnosis of depression was established and who had not recently been prescribed antidepressant drugs, and matched controls (15M, 7F; 43±3 years; 40±3 h). β-Adrenoceptor binding sites were measured by saturation binding of [^{3}H]CGP 12177 in well-washed membranes (De Paermentier et al., 1989b).

There were no significant differences in K_d or Bmax values for total β-adrenoceptor binding sites, or β-subtypes, between controls and depressed suicides in any of the brain regions (Table 1), although Bmax values in caudate and putamen were 10-12% lower in suicides. β-Adrenoceptor binding did not differ significantly in suicides who died by violent means (n=12) but β_1-adrenoceptors in caudate of those suicides who died by non-violent means (n=10) was significantly lower than matched controls (suicides, 70 ± 5, controls 86 ± 5 fmol/mg protein, p=0.03, Wilcoxon rank sum test).

Table 1. Bmax of β-adrenoceptor binding sites (fmol/mg protein, means ± sem)

	Total β-adrenoceptors		β_1-adrenoceptors		β_2-adrenoceptors	
	Control	Suicide	Control	Suicide	Control	Suicide
Caudate	103 ± 9	92 ± 4	89 ± 5	79 ± 3		
Putamen	134 ± 9	123 ± 7	120 ± 8	109 ± 6		
Hippocampus	61 ± 3	57 ± 3	28 ± 1	25 ± 1	36 ± 2	34 ± 2
Amygdala	44 ± 2	42 ± 3	22 ± 1	22 ± 2	22 ± 1	21 ± 2
Thalamus	44 ± 3	45 ± 2	22 ± 1	24 ± 2	23 ± 1	22 ± 2

Differences in the number of β-adrenoceptor binding sites between antidepressant drug-free depressed suicides and matched controls are largely restricted to cortical areas.

We thank the Sir Jules Thorn Charitable Trust for financial support.

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272P EFFECTS OF RS15385-197 IN *in vivo* PREPARATIONS

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RS-15385-197 is the most potent alpha$_2$-adrenoceptor antagonist thus far described (Clark et al., 1990). We have examined the effects of RS-15385-197 in *in vivo* preparations. RS-15385-197 was a potent antagonist of clonidine-induced mydriasis (300 µg/kg clonidine, s.c.) in pentobarbitone-anaesthetised rats with an ID50 of 7 µg/kg, i.v. and 95 µg/kg p.o. (n = >6). In conscious rats RS-15385-197 (1 and 10 mg/kg, p.o.) antagonized clonidine (100 µg/kg, p.o.)-induced depression of exploratory behaviour at 1 and 10 mg/kg, p.o. In pithed rats RS-15385-197 was a potent antagonist of pressor responses evoked by UK 14304 (ID50 15 µg/kg, i.v., n=5). RS-15385-197 (1 mg/kg, p.o.) did not affect exploratory behaviour of rats (activity meter), nor did it affect time spent on an accelerating rotarod.

In Sprague-Dawley rats, 0.5 mg/kg p.o. RS-15385-197 caused a significant increase in the cortical levels of the noradrenaline metabolite 4-hydroxy-3-methoxyphenyglycol (MHPG) ($p<0.001$, n=5) 1 h post dosing. The tissue level of noradrenaline was unaltered. This dosing schedule of RS-15385-197 did not change the tissue levels of 5-HT or its metabolite, 5-hydroxy-indole acetic acid (5-HIAA) indicating that RS-15385-197 selectively affected noradrenaline release. RS-15385-197 (0.5 mg/kg, p.o. daily for 14 days) reduced the number of beta-adrenoceptors in rat cerebral cortex without changing affinity (control Kd = 0.41 ± 0.03 nM, Bmax 70.7 ± 4.4 fmol/mg protein, n=9; RS-15385-197, Kd 0.34 ± 0.03, Bmax 51.2 ± 4.4, n=10). However, this dosing schedule of RS-15385-197 did not change the number (control Bmax, 103 ± 8 fmol/mg protein, and RS-15385-197, 97 ± 4, n=8) or affinity (control Kd 0.32 ± 0.02 nM ; RS-15385-197, 0.35 ± 0.01), of frontal cortical 5-HT$_2$ receptors. In conclusion, RS-15385-197 is a potent and selective alpha$_2$-adrenoceptor antagonist which selectively increases noradrenergic activity in rat cortex and down regulates beta-adrenoceptors without a change in the number of 5-HT$_2$ receptors, indicating that this compound will be a definitive tool to test the catecholaminergic theory of depression (Schildkraut, 1965).

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273P EFFECT OF CORTICOSTERONE AND STEROID ANTAGONIST RU38486 ON NORADRENALINE-STIMULATED CYCLIC AMP IN RAT CORTEX AND HIPPOCAMPUS SLICES

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Adrenalectomy has been shown to influence noradrenaline (NA) induced cyclic AMP accumulation in brain slices from rat cerebral cortex and hippocampus (Mobley and Sulser, 1980; Roberts et al., 1984). We have previously shown that this effect is expressed as an increase in the alpha adrenoceptor mediated component of the NA response, at least in cerebral cortex slices (Bharmal et al., 1989). However, it is not clear whether steroid hormones exert their effects directly via glucocorticoid receptors in brain or indirectly via the release of peripheral mediators. Since the hippocampus contains a high density of glucocorticoid receptors (McEwen et al., 1969) we have compared the effect of adrenalectomy on NA stimulated cyclic AMP in rat cortex and hippocampus. We have also studied the effect of corticosterone in vitro on the potentiation of isoprenaline stimulated cAMP in brain slices from adrenalectomised rats and the effects of steroid antagonist RU38486 both ex vivo and in vitro. Bilateral adrenalectomy was performed on male Sprague Dawley rats. For RU38486 ex vivo studies, rats were injected with RU38486 (5mg/100g body weight s.c.) or vehicle (50% propylene glycol in ethanol) 2h before being killed. Cyclic AMP formation in cerebral cortex slices or hippocampus slices was measured by the ^{3}H-adenine prelabelling method of Shimizu et al., (1969). Corticosterone or RU38486 were added to slices 15-30 minutes before agonists.

In cortex slices adrenalectomy had no effect on isoprenaline stimulated cyclic AMP but caused a significant increase in the response to NA from 282±21% to 407±51% of isoprenaline response ($P<0.05$, n=7). In hippocampus slices, isoprenaline stimulated cyclic AMP formation was reduced in adrenalectomised animals (4329±326 dpm in control slices; 3044±324 dpm in slices from adrenalectomised rats, $P<0.05$, n=4). In contrast, the alpha adrenoceptor mediated component was significantly increased ($P<0.05$) following adrenalectomy. (Control:334±47% isoprenaline response; adrenalectomy:696±145% isoprenaline response, n=7). Corticosterone (up to 100μM) tended to increase rather than decrease NA stimulated cyclic AMP in both hippocampus and cortex slices from adrenalectomised rats. RU38486 ex vivo and in vitro (up to 100μM) caused a small but not significant (10-20% over control) increase in NA stimulated cyclic AMP in rat cortex.

These results suggest that alpha adrenoceptor mediated cyclic AMP responses in cortex and hippocampus are both regulated by adrenal steroids but this control may not be exerted directly at the level of the CNS glucocorticoid receptor.

We wish to thank the SERC for financial support.

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274P NEUROCHEMICAL EFFECTS OF IDAZOXAN GIVEN CONTINUOUSLY BY OSMOTIC MINIPUMP IN THE RAT

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The α2-antagonist idazoxan, when given by minipump to rats, initially increased behaviour (Dickinson et al 1989), an effect which quickly returned to, and remained, normal for up to 10 days. Unpublished data from that study showed cortical ß-adrenoreceptor number to be reduced at 10 days (Bmax; vehicle 148±12, n=4; idazoxan 115±7, n=5, fmol mg^{-1} protein, $P<0.05$) although affinity was unchanged, as has been found with other antidepressant drugs. Using a similar protocol, the present study investigates the time course of biochemical changes of central and peripheral adrenergic function during idazoxan infusion.

Male Sprague-Dawley rats (Olac, 290g-410g), individually housed on a 12:12 hr lighting schedule at 20±2 °C were anaesthetised (isoflurane) and implanted subcutaneously with 'primed' osmotic minipumps (Alzet 2ML4) delivering saline (0.9%, 2.08 μl h^{-1}) or idazoxan (aprx. 0.50 mg kg^{-1} h^{-1}) and allowed to recover. In separate groups of rats, the following were measured after 1,3,7 or 10 days of infusion; i) Cortical ß-binding (saturation analysis using [I^{125}]-cyanopindolol ii) Brain, atrial & plasma catecholamine levels (by alumina extraction followed by HPLC with electrochemical detection). Data were analysed by ANOVA and Dunnett's test.

Table 1. mean±sem		Bmax fmol mg^{-1} pr.	Kd pM	Brain NA ng g^{-1}	Brain DA ng g^{-1}	Atrial NA ng g^{-1}
Saline day	1	168±11	98±7	294±27	982±33	445±28
n=6	3	167±10	116±26	284±18	879±74	632±35
	7	163±11	106±20	283±13	958±59	693±46
	10	153±8	108±13	320±15	1033±36	635±70
Idazoxan	1	144±14	69±14	270±19	845±27	383±26
n=6	3	142±7	95±19	256±7	962±28	627±45
	7	159±9	116±20	235±8*	948±36	787±62
*P<0.05	10	120±9*	103±14	266±14*	991±14	638±101

Idazoxan reduced [I^{125}]-CYP Bmax (but not Kd, table 1) on all days but, as in the prior study, only significantly by day 10. There was a reduction of brain NA (but not brain DA or atrial NA) which was significant, when compared to vehicle, by days 7 and 10. There were significant treatment effects of idazoxan on both plasma NA and adrenaline although, day by day, these were variable and yielded no clear differences. Both brain DA and atrial NA significantly varied over time but this was independent of treatment.

The data indicate that idazoxan selectively reduces brain NA (but not brain DA or peripheral NA). This may be a result of chronically enhanced NA release induced by blockade of pre-synaptic α2-receptors. Such increased release may similarly explain the observed reduction of brain ß-adrenoreceptors as this follows a similar time-course. It is interesting to note that these major neurochemical changes occur at a time when behaviour, in the previous study, had returned to normal.

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275P ARE HISTAMINE-H_2 ANTAGONISTS PROCONVULSANT?

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Initial studies of histamine-H_2 antagonists, developed for control of gastric acid secretion and treatment of peptic ulcers (Brimblecombe et al, 1975), suggested that these compounds did not readily penetrate the brain. However, recent reports indicate some cimetidine-related neurotoxicity in man (Van Sweden & Kamphuisen, 1984). The development of zolantidine (Calcutt et al, 1988) as a potent brain penetrating histamine-H_2 receptor antagonist provides a valuable pharmacological tool for investigating possible central physiological roles of histamine.

Groups of female albino mice (ex ICI strain, Triangle, Devon) were pretreated s.c. with histamine-H_2 antagonists 15 or 30 mins before induction of seizures using either pentylenetetrazol (leptazol) or picrotoxin. The frequency of the subsequent clonic convulsive activity was analysed using a Mann Whitney 'U' test and the incidence using Chi squared or Fisher's Exact Test.

At high doses of metiamide and cimetidine (400 mg kg^{-1}) and zolantidine (150 mg kg^{-1}) there were signs of toxicity, including seizures. None of the compounds potentiated leptazol-induced seizures at any dose tested but the picrotoxin-induced seizures were potentiated as follows.

Pretreatment	Dose(mg kg^{-1})	%increase in seizure incidence	Relative seizure frequency (control=1.0)
Cimetidine	200	90**	3.3****
Metiamide	100	180**	3.8****
Zolantidine	10	88*	2.7***
	3	88*	3.2****

* p <0.02
** p <0.05
*** p <0.005
**** p <0.001

Gerald and Richter (1976) also found inconsistent effects of metiamide and burimamide on leptazol-induced seizures in mice. Cimetidine can inhibit cytochrome p450 (Katzung, 1989) which might explain the effects observed in our experiments. The effects seen with zolantidine, however, are at levels reported to produce specific central histamine-H_2 antagonism in vivo and in vitro. They are thus consistent with possible H_2 involvement in the demonstrated histaminergic control of seizures (Fairbairn & Sturman,1989).

We wish to thank S.K.& F.Ltd. for the generous gifts of the H_2 antagonists.

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276P ACTIONS OF Bay K 8644 STEREOISOMERS ON THE CHANGES IN HIPPOCAMPAL FIELD POTENTIAL DURING ETHANOL WITHDRAWAL

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The neurophysiological basis of the ethanol withdrawal syndrome is uncertain. Field potentials in the isolated hippocampal slice show a complex pattern of changes on withdrawal from chronic ethanol treatment *in vivo* (Whittington & Little, 1989a). The calcium channel antagonist (+)PN 200-110 prevented these changes with no effect on control tissues (Whittington & Little 1989b). We have now examined the stereospecificity of the calcium channel activator BAY K 8644. In myocytes, (-)-BAY K 8644 activated calcium currents, but the (+) isomer acted as a calcium channel antagonist (Kass, 1987).

Male mice, C57 strain, 25-30g, drank ethanol, 24% v/v, as sole fluid, for fifteen weeks (ethanol intake 10-14 g/kg/day). Controls drank tap water. Hippocampal slices were made, without prior withdrawal of the animals from ethanol. Extracellular recordings were from CA1 area, with orthodromic stimulation. BAY K 8644 isomers were dissolved in DMSO, diluted in bathing medium (artificial CSF, final DMSO concentration 0.05%). Every 15 min, the thresholds for production of first and multiple population spikes and paired pulse potentiation (PPP, % 2nd/1st reponses; 70 msec interval,1.25 x threshold) were measured. The incidence of reverberative spiking (IRS) was noted for each treatment group. Results (mean ± s.e.m.) were compared by analysis of variance (n=5 each group).

After the first 2h of recording the thresholds for single population spikes were lower after ethanol treatment (Eth) than in controls. This change was significantly increased by 2 μM (-)BAY K 8644 ($P<0.03$), and decreased by 2μM (+) BAY K 8644 ($P<0.03$). Thresholds for multiple spikes were lower after ethanol treatment, an effect increased by 2 μM (-) BAY K 8644 ($P<0.03$), and prevented by the plus isomer ($P<0.001$). After ethanol treatment, paired pulse potentiation increased, compared with controls, from the beginning of recording, reached a peak at 2h, then returned to normal. Both isomers of BAY K 8644 decreased this change ($P<0.01$). On control tissues 2μM (+) BAY K 8644 had no effect but the minus isomer significantly decreased multiple spike thresholds ($P<0.001$). Reverberative spiking occurred in ethanol withdrawal with (-) BAY K 8644.

Thresholds, μA	Controls	Ethanol	Eth & (-)BK	Eth & (+)BK	Con & (-)BK	Con & (+)BK
Single spike (5h)	27±1	20±1	16±1	24±1	24±2	27±1
Mult. spikes (5h)	191±6	126±17	97±14	181±7	178±4	188±7
PPP, % (2h)	202±7	325±12	239±26	206±8	187±10	187±9
IRS	0/5	1/5	7/9	0/5	0/5	0/5

These results show that (-) BAY K 8644 increased the effect of ethanol withdrawal on population spike thresholds but not that on paired pulse potentiation, suggesting different neuronal bases for these two components of withdrawal.

We thank the Wellcome Trust and the Brewers' Society for financial support.

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277P RELEASE OF NEUROEXCITATORY AMINO ACIDS FROM RAT BRAIN FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION

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The neuroexcitatory amino acids, glutamate (glu) and aspartate (asp) have been implicated in the pathophysiological consequences of brain ischemia. The neuroprotective properties of N-methyl-D-aspartate (NMDA) receptor antagonists in animal models of global (Simon et al, 1984) and focal (Park et al, 1988) ischemia have been demonstrated. Moreover, a massive release of brain glu and asp in global ischemia models has been reported (Hagberg et al, 1985). In the present study, we have investigated whether neuroexcitatory amino acids are released from the striatum and cortex of anaesthetised rats following middle cerebral artery occlusion and whether the magnitude of glu/asp release correlates with the extent of ischemic neuronal injury.

Sprague-Dawley rats (male, 300-450g) were anaesthetised with halothane. Body temperature was maintained between 36-38°C by external heating. Dialysis probes (Sandberg et al, 1986) were stereotaxically implanted into the striatum and cortex and were secured in place using skull screws and dental cement. Probes were perfused with Krebs-Ringer bicarbonate buffer at 2.5µl/min. After a 60 min equilibrium period dialysates were collected in 15 min fractions and amino acid content was determined using HPLC with fluorescent detection. The middle cerebral artery (MCA) was then exposed and occluded by electrocoagulation at a point either proximal or distal to the lenticulostriate vessels. In control animals the MCA was exposed but not occluded. Dialysate were collected for 3-4 hours following MCA occlusion. Animals were then perfusion fixed and the extent of the ischaemic infarct determined histologically by an independent observer (D.I.G.). Basal efflux of glu and asp in striatum was 1.58 ± 0.04 pmol/min and 0.38 ± 0.13 pmole/min and in cortex was 1.83 ± 0.06 and 0.61 ± 0.15 pmol/min. Following MCA occlusion levels of aspartate and glutamate rose by 900-1100% and 2000-4000% in animals with large striatal infarcts (35-45% tissue volume). In animals with small striatal infarcts (10-20% of tissue volume; animals with distal MCA occlusion) smaller increases were observed (200-800% and 200-1200%). Maximal efflux was observed 30-60 min following MCA occlusion. Glu and asp efflux rose by 1500-1800% and 1000-3500% in the cortex of animals with cortical infarcts (20-40% of tissue volume). In this case maximal efflux was observed 15-30 min following MCA occlusion. Efflux of glu and asp was not affected in either the striatum or cortex of control animals.

These data demonstrate that the excitotoxic amino acids, glu and asp, are released from the cortex and striatum of MCA occluded rats. There appears to be a relationship between the amount of glu and asp released and the extent of MCA induced neuronal damage.

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278P THE ACTIONS OF ALPHAXALONE AND PENTOBARBITONE ON INHIBITORY AND EXCITATORY AMINO ACID RECEPTORS

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Anaesthetic steroids, such as alphaxalone, produce a potent and stereoselective potentiation of the actions of GABA on $GABA_A$ receptors and at relatively higher concentrations directly activate the $GABA_A$ receptor (Lambert et al., 1987). The present study investigates the actions of alphaxalone and pentobarbitone on inhibitory and excitatory amino acid receptors, in an attempt to determine their specificity of action.

Whole cell recordings (holding potential = -60mV) of agonist-evoked currents were made under voltage-clamp conditions on bovine chromaffin cells (GABA), mouse spinal neurones (glycine) and rat hippocampal neurones (GABA, NMDA, quisqualate and kainate) maintained in cell culture. All agonists were applied locally by pressure ejection from a modified patch electrode. For experiments on cation-conducting channels the internal (Cl^-) was reduced from 149mM to 15mM by substitution with potassium gluconate. NMDA-induced currents were recorded in a Mg^{2+}-free, glycine (1µM)-containing solution.

Alphaxalone (30nM-1µM) and pentobarbitone (10-300µM) produced a dose-dependent potentiation of GABA-evoked currents recorded from chromaffin cells and hippocampal neurones. In contrast alphaxalone (10µM, 97 ± 5% of control, n=7) and pentobarbitone (100µM, 113 ± 7% of control, n=6) had no effect on glycine-evoked currents. Pentobarbitone (10µM-1mM) produced a reversible, dose-dependent inhibition of quisqualate, kainate and NMDA-evoked currents with approximate IC_{50}s of 60, 69 and 690µM respectively (n=3-6). Alphaxalone (10µM) had no effect on currents evoked by these amino acids (n=5).

In contrast to pentobarbitone, alphaxalone is a highly selective modulator of the $GABA_A$ receptor. Enhancement of GABAergic transmission by alphaxalone may contribute to the anaesthetic properties of this steroid.

We thank the Scottish Hospitals Endowment Research Trust for support.

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279P ACUTE EFFECTS OF ETHANOL ON RAT HIPPOCAMPAL EXCITATORY AMINO ACID RECEPTORS

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Recently Lovinger (1989), reported that ion currents induced by the glutamate receptor agonist N-methyl-D-aspartate (NMDA) were inhibited by ethanol and other related alcohols. In these hippocampal neurones the inhibition of NMDA currents by ethanol appeared to be dose-dependent and fairly selective compared to non-NMDA induced currents. This report has prompted us to investigate the relationship between ethanol and excitatory amino acids on rat hippocampal neurones in vitro.

Using the grease-gap technique for rat hippocampal CA1 slices (Martin et al., 1989) the effects of ethanol (17-300mM) were examined on excitatory amino acid induced CA1 depolarisations. In the absence and presence of Mg^{2+} (0 and 1 mM) ethanol reduced the depolarising action of NMDA. The NMDA dose-response curves were shifted to the right by ethanol in a dose-dependent manner. Using the approximate EC_{50} value for NMDA (5 μM and 20 μM) in the absence and presence of Mg^{2+} (1 mM) ethanol had an apparent EC_{50} value of 125 and 60 mM respectively as an NMDA antagonist. It was noted that ethanol was more effective at lower NMDA concentrations. Thus the potency of ethanol as an NMDA antagonist appears to increase in the presence of physiological magnesium concentrations. Ethanol inhibits non-NMDA induced CA1 depolarisations but at substantially higher concentrations than that needed to affect NMDA responses. The threshold concentration for ethanol on inhibiting non-NMDA mediated CA1 depolarisations was 50 mM and was maximal at 300 mM.

Transverse rat hippocampal slices (625 μM) were prepared and extracellular recordings were made from stratum radiatum and pyramidale of CA1 in artificial cerebral spinal fluid (aCSF) containing 1 mM Mg^{2+} and 3 mM Ca^{2+}. The non-NMDA antagonist, 6,7,dinitro-quinoxaline-2,3-dione (DNQX, 10 μM), and the $GABA_A$ channel antagonist, picrotoxin (10 μM) were than added to the medium. This treatment allows the measurement of a greater than 90% pure NMDA-mediated synaptic potential in the presence of Mg^{2+}. Ethanol (20-100 mM) inhibited the synaptic activation of NMDA potentials. Antagonism of these responses by ethanol peaked at approximately 65% inhibition at 75 mM ethanol over the stimulus range tested (200-800 μA). These data, in conjunction with the aforementioned data strongly support an inhibitory action of ethanol against NMDA responses. Such effects of ethanol, at concentrations which elicit intoxication but not coma, suggest that the excitatory amino acid receptors and in particularly the NMDA-receptor complex may play an important role in the acute effects of ethanol.

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280P MODULATION OF NMDA-INDUCED CURRENTS RECORDED FROM RAT HIPPOCAMPAL NEURONES BY 1-HYDROXY-3-AMINOPYRROLIDONE-2 (HA-966)

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In the rat cortical wedge preparation, HA-966 non-competitively antagonises extracellularly recorded depolarizing responses evoked by NMDA. The ability of glycine and D-serine to reverse such blockade has led to the suggestion that HA-966 acts primarily as an antagonist at the strychnine-insensitive glycine recognition site associated with the NMDA receptor complex (Fletcher and Lodge, 1988; Foster & Kemp 1989). In the present study we have examined the effect of HA-966 upon NMDA-evoked currents recorded from voltage-clamped rat hippocampal neurones maintained in cell culture.

Neurones were dissociated and maintained in cell culture as previously described (Halliwell et al., 1989) and voltage-clamped using the whole-cell recording configuration of the patch-clamp technique. Inward currents evoked by the local application of NMDA (100 µM) were recorded in an Mg-free medium at a holding potential of -60 mV and room temperature (18-22°C).

Whole-cell currents evoked by NMDA were potentiated on average to 309 ± 41% of their control value (mean ± s.e. mean, n = 17) by the inclusion of glycine (1 µM) in the perfusate. Such potentiation was readily reversed on wash-out, unaffected by strychnine (3 µM; n = 3) and of variable magnitude (range = 120 - 865% of control), perhaps reflecting an inconsistent reduction of endogenous glycine levels by the bath perfusion system across recordings. HA-966 (3 - 300 µM) dose-dependently reduced NMDA-induced currents recorded in the presence of 1 µM glycine with an IC_{50} value of 26 µM. The antagonism produced by 100 µM HA-966 was completely reversed by a 10-fold increase in glycine concentration (from 1 to 10 µM; n = 3), whereas blockade by ketamine (10 µM) or DL-2-aminophosphonovaleric acid (30 µM) was little affected. The concentration-inhibition curve for HA-966 (3 - 300 µM) determined in the presence of 1 µM glycine was displaced to the right in a parallel fashion by a 3-fold increase in glycine concentration, suggesting a competitive interaction between HA-966 and glycine. In preliminary experiments, 1-aminocycloproprane carboxylic acid, which in radioligand binding assays acts as an agonist at the glycine recognition site of the NMDA receptor complex (Marizon et al., 1989), was found to mimic the effect of glycine upon NMDA-induced currents and their antagonism by HA-966.

In summary, the present results support the notion that HA-966 acts primarily as an antagonist at the glycine receptor associated with the NMDA-receptor complex.

We thank Organon Laboratories (Newhouse) for the gift of HA-966 and financial support. This work was funded in part by the MRC.

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281P THE EFFECTS OF POLYAMINES ON THE BINDING OF [^{3}H]-MK-801 TO RAT CORTICAL MEMBRANES

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The polyamine, spermidine, stimulates the binding of [^{3}H]-MK-801 to the N-methyl-D-aspartate (NMDA) receptor ion channel complex via a site distinct to those at which glutamate and glycine act (Ransom & Stec, 1988). In this study we have further characterized this polyamine interaction in rat cortical membranes.

P_2 membranes were prepared from rat cortex as previously described (Stirling et al, 1989). Assays were performed in 200µl Tris-HCl (5mM, pH 7.7) containing approximately 200µg protein, [^{3}H]-MK-801 (5nM) and other agents where appropriate. Non-specific binding was determined with MK-801 (10µM). Following incubation at 25°C for 15 min, assays were terminated by rapid filtration (Stirling et al, 1989).

In the absence of exogenous polyamines or amino acids, binding of [^{3}H]-MK-801 was typically 25 fmol/mg protein. This binding was stimulated by spermidine (100µM)(275 ± 3%, mean ± s.e. mean, n=4) or a combination of glutamate and glycine (both 10µM)(255 ± 15%, mean ± s.e. mean, n=3). A combination of spermidine, glutamate and glycine at the same concentrations gave no additional stimulation. Spermidine (100µM) produced a marked increase in the association rate of [^{3}H]-MK-801, comparable to the increase produced by a combination of spermidine (100µM), glutamate (10µM) and glycine (10µM). Spermidine and related compounds produced a concentration dependent increase in [^{3}H]-MK-801 binding (Table 1). Acrolein was inactive. In agreement with Carter et al (1989) ifenprodil reversed the spermidine (100µM) enhancement of [^{3}H]-MK-801 binding (IC_{50}, 15 ± 7µM, mean ± s.e. mean, n=3). Putrescine also reversed the effects of spermidine (100µM)(IC_{50}, 81 ± 16µM, mean ± s.e. mean, n=3).

Table 1 Effects of glutamate, glycine and polyamines on [^{3}H]-MK-801 binding (% Basal)

Drug concentration	Glutamate	Glycine	Spermidine	Spermine	N-acetyl Spermidine	N-acetyl Spermine	Putrescine	Ornithine	S-adenosyl-methionine	putreanine
10µM	229±15	119±9	139± 9	159±16	93± 4	150±15	91±2	111± 3	121± 7	107± 5
1mM	-	-	240±60	174±28	175±33	189±35	53±5	179±26	233±46	150±16

Values are mean ± s.e. mean, n=3-4.

This study shows that the NMDA receptor complex may be sensitive to a number of endogenous polyamines and related compounds. Such compounds may have stimulatory or inhibitory properties.

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282P PROPOFOL ANAESTHESIA IS ASSOCIATED WITH SEIZURE-LIKE BEHAVIOUR

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The Committee of Safety in Medicines recently reported 37 cases of convulsions occurring in association with propofol-induced anaesthesia (May 1989). However, there has been no report of seizures occurring in the pre-clinical pharmacological investigation of propofol. We report here the occurrence of seizure-like behaviour in mice in association with propofol-induced anaesthesia.

Male TO mice 25-30g were injected with propofol (1-200 mg kg-1 i.p.), and observed for up to 45 minutes. Anaesthesia was assessed by the loss of righting reflex. Each mouse was rolled onto its back every 5 minutes after propofol injection and given 60 seconds to regain the upright posture. Anaesthesia was produced by propofol at doses of 75 mg kg-1 and greater. Seizure-like behaviour, which was only seen at anaesthetic doses of propofol, predominantly at the onset and recovery from propofol anaesthesia, was characterized by four-limb clonus, facial grimacing and tongue clonus. All mice made full recoveries. Propofol 50 and 150 mg kg-1 had anticonvulsant effects against the GABA antagonist bicuculline. Bicuculline (0.05 mg ml-1, 1.4 ml min-1) was infused into tail veins of mice and the time recorded to onset of clonic seizures.

Drug	Time (min)	Bicuculline-Seizures Threshold (mg kg-1)	
Control	5	0.95±0.06 (n=5)	
Propofol 50 mg kg-1	5	1.35±0.08 (n=6)	*
Control	5&40	0.71±0.05 (n=6)	
Propofol 150 mg kg-1	5	1.52±0.16 (n=6)	*
Propofol 150 mg kg-1	40	1.04±0.19 (n=4)	

*p<0.05 Mann-Whitney U test, compared to controls

A subconvulsant dose of the glutamate agonist N-methyl-dl-aspartic acid (150mg kg-1 i.p.) given immediately prior to propofol 150 mg kg-1, did not alter the incidence of either loss of righting reflex or seizure-like behaviour as compared to controls. However, a subconvulsant dose of the glycine antagonist strychnine 0.3 mg kg-1 given immediately prior to propofol 150 mg kg-1 produced a significant increase in the incidence of seizure-like behaviour but did not alter the incidence of righting reflex.

Incidence of seizure-like behaviour

Drug	5	10	15	20	25	30	35 minutes
Control	2/7	1/7	3/7	1/7	0/7	0/7	0/7
Strychnine	7/7*	5/7	6/7	3/7	1/7	1/7	0/7

*p<0.05 Fisher's Exact Test

These results show that seizure-like behaviour seen at anaesthetic doses of propofol is potentiated by strychnine but not by other chemical convulsants. The seizure-like behaviour may originate at the level of the motoneuron where strychnine is known to inhibit glycine neurotransmission.

283P ANOXIA- AND HYPOGLYCAEMIA-INDUCED NEURODEGENERATION IN NEONATAL RAT HIPPOCAMPAL SLICES

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Neonatal rat hippocampal slices have been used previously to study the mechanisms underlying NMDA-and quisqualate-induced neurotoxicity (Garthwaite and Garthwaite, 1989). The aim of the present study was to determine if these slices could also be used to study the effects of anoxia and hypoglycaemia.

Hippocampal slices 400μm from 10 to 14 day-old rats (Garthwaite et al., 1989) were randomly distributed between conical flasks containing 20ml Krebs solution of the following composition (mM): NaCl 120, KCl 2, $CaCl_2$ 2, $NaHCO_3$ 26, $MgSO_4$ 1.19, KH_2PO_4 1.18, D-Glucose 11; equilibrated at 37°C with 95% O_2/5% CO_2. In some experiments $MgSO_4$ or $CaCl_2$ was omitted. Hypoglycaemia was produced by reducing the glucose concentration to 2 mM and anoxia was produced by equilibrating solutions with 95% N_2/ 5% CO_2. In all experiments slices were preincubated in normal Krebs for 90 min prior to exposure to anoxia, hypoglycaemia or NMDA, after which they were returned to normal Krebs for a further 90 min. Slices were then removed, fixed and 5μm wax sections cut and stained with haemotoxylin/eosin. Slices were then examined by light microscopy and the number of normal neurones counted in a defined area of the hippocampal CA1 pyramidal cell layer. In experiments investigating the effects of MK801, this antagonist was present throughout the incubation period.

In preliminary experiments concentration-dependent damage was produced by exposure (20 min) to NMDA; number of cells (mean±s.e. mean): control 72±3 (n=21), NMDA 10μM 63±8 (n=3), NMDA 30μM 18±4 (n=10), NMDA 100μM 9±2 (n=11). Pre-equilibration with MK801 (10μM) or Ca^{++}-free medium completely prevented NMDA-induced neurotoxicity; number of intact cells: NMDA + MK801 65±18 (n=4), NMDA + Ca^{++}-free 64±10 (n=7).

In Mg^{++}-containing Krebs, hypoglycaemia, alone or in combination with anoxia, failed to produce significant neuronal damage; no. cells: low-glucose 67±7 (n=7), 20-min anoxia + low-glucose 58±16 (n=4), 30-min anoxia + low-glucose 65±15 (n=6), 60-min anoxia + low-glucose 54±29 (n=3). In Mg^{++}-free Krebs, hypoglycaemia or anoxia alone was also ineffective; no. cells: low-glucose 67±7 (n=7), 60-min anoxia 63±6 (n=3). However, a combination of anoxia + low-glucose, in Mg^{++}-free conditions, produced marked neuronal damage. This effect was dependent on the time of exposure to anoxia; no. cells: low-glucose + 20-min anoxia 66±8 (n=3), low-glucose + 30-min anoxia 35±4 (n=4), low-glucose + 60-min anoxia 10±2 (n=9). The damage produced by 60-min anoxia/hypoglycaemia was prevented by MK801 (10μM); no. cells 58±12 (n=4).

This study shows that exposure of neonatal rat hippocampal slices to a combination of hypoglycaemia/anoxia produces marked neuronal damage. Preliminary pharmacological results indicate that this process is mediated by activation of NMDA receptors. This slice preparation may be useful to study the mechanisms underlying ischaemia-induced neurodegeneration.

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284P GLYCINE - A PUTATIVE CO-TRANSMITTER AT GLUTAMATE-OPERATED SYNAPSES: UPTAKE AND RELEASE BY RAT STRIATAL SLICES

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Glycine potentiates NMDA receptor-mediated responses in the mammalian CNS (Johnson and Ascher, 1987; Thomson et al. 1989) and indeed its presence may be mandatory for NMDA receptor activation (Kleckner and Dingledine, 1988). In the rat striatum, glycine modulates the well-established ability of NMDA to enhance the release of dopamine (Crawford & Roberts, 1989). In this study, we have investigated the uptake and release of [^{3}H]glycine from rat striatal longitudinal slices (400μm thick).

Striatal slices accumulated [^{3}H]glycine by a Na^+-dependent, high-affinity (K_m=35μM) process, with maximal uptake by 15 min. Interestingly, inclusion of NMDLA in the medium (1 or 10mM) significantly increased [^{3}H]glycine uptake (from 500 to 750 pmol/mg/15 min, at 10mM). Striatal kainate lesions carried out 3 weeks prior to assay did not influence subsequent [^{3}H]glycine uptake. In contrast, unilateral decortication significantly reduced glycine uptake by approx 25%, suggesting possible co-localisation within glutamatergic terminals.

Striatal slices which had been pre-loaded with 1μM [^{3}H]glycine and superfused with Krebs at 0.5ml/min, showed a robust release of glycine in the presence of K^+ (35-80mM) that was partially Ca^{2+}-dependent. Co-superfusion with L-glutamate (1-10mM) markedly potentiated the K^+-evoked release of glycine. This property was shared by 2-amino-4-phosphonobutyrate (AP4), while NMDLA was not found to influence [^{3}H]glycine release.

These data suggest that glycine may be released from striatal nerve terminals (probably including those of the cortico-striatal pathway) during depolarization and that an effective high-affinity uptake process for its subsequent removal is also present. Activation of excitatory amino acid receptors may also influence this release system, although the receptor type has not yet been defined. However, Weiss et al (1989) have recently reported that kainate can directly release glycine from striatal neurones in primary culture.

M. Crawford was an SERC research student.

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285P ROTENONE- AND IODOACETATE-INDUCED DEPOLARISATION OF NEONATAL RAT SPINAL CORD: AN *IN VITRO* MODEL OF ISCHAEMIA?

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Evidence suggests that release of excitatory amino acids and calcium influx plays an important role in ischaemia-induced neurodegeneration. The aim of the present study was to develop a model of ischaemia in vitro which would allow the mechanisms involved in this neurodegenerative process to be studied. For this the metabolic inhibitors, rotenone and iodoacetate, have been used (Kauppinen et al, 1988).

Hemisected spinal cords of 2 to 7 day-old Sprague-Dawley rats were set up for conventional grease-gap recording (Birch et al., 1988). Tissues were superfused (3ml/min) with oxygenated Mg^{++}-free Krebs-Ringer; composition (mM): KCl 4.7, NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1.3, KH_2PO_4 1.2, glucose 11.1; pH 7.4, at room temperature. After a 150-min pre-equilibration period, rotenone and iodoacetate, alone or in combination, were applied in a three-minute pulse. Other drugs were superfused for either 15 or 75 min prior to, and for 90 min after, exposure to rotenone or iodoacetate. At the end of the 90-min reperfusion period the morphology of spinal neurones was assessed histologically.

Rotenone (10^{-7} to 10^{-5}M; n=4) or iodoacetate (10^{-6} to 10^{-3}M; n=4) had no effect when added alone. However, a combination of rotenone (10^{-5}M) and iodeacetate (10^{-3}M) produced a large depolarising response. In the majority of tissues (n=27) this response was biphasic and consisted of a slow depolarisation (magnitude 4.0±0.5mV) upon which was superimposed a rapid spike-like depolarisation (magnitude 7.4±0.5mV). In other tissues (n=20) only a single peak was observed (magnitude 6.8±0.5mV). Tissues exposed to rotenone/iodoacetate failed to repolarise fully. Histological examination of the rotenone/iodoacetate-treated spinal cords revealed the presence of marked neuronal necrosis throughout the length of the cord. MK801 (10^{-5}M, 75-min pre-equilibration, n=8) or 3-((±)2-carboxypiperazine-4-yl) propyl-1-phosphonate (CPP,10^{-4}M, 15-min pre-equilibration, n=4) prevented all phases of the depolarising response and completely prevented neuronal damage. Pre-equilibration of tissues with the calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,-N',N'-tetraacetate (BAPTA, 10^{-3}M; Tsien, 1980), under calcium-free conditions and in the presence of 0.6mM Mg^{++}, prevented the rapid depolarisation but had little effect on the slow response (magnitude 2.8±0.7mV, n=7). Mg^{++} ions (0.6mM) alone did not affect the response to rotenone/iodoacetate (n=8).

In summary, exposure of the neonatal rat spinal cord to rotenone/iodoacetate produces a large depolarising response which leads to neuronal death. Preliminary pharmacological results indicate that these effects require activation of NMDA receptors and the presence of extracellular calcium.. This model may allow investigation of the neurodegenerative mechanisms initiated following a period of ischaemia.

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286P DIFFERENTIAL EFFECTS ON THE BEHAVIOURAL AND ANTICONVULSANT PROPERTIES OF MK-801 FOLLOWING REPEATED ADMINISTRATION IN THE MOUSE

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In addition to anticonvulsant and neuroprotective effects (Clineschmidt *et al*, 1982; Gill *et al*, 1987), the acute administration of the non-competitive NMDA receptor antagonist, MK-801, induces in rodents a motor syndrome consisting of stereotyped head weaving, hyperlocomotion and ataxia (Tricklebank *et al*, 1989). Although the anticonvulsant and motoric properties of MK-801 are thought to reflect blockade of NMDA receptors, we now show that repeated administration of the compound differentially alters the various components of the motor syndrome, but leaves anticonvulsant potency unchanged.

Head weaving, locomotion and ataxia were scored as previously described (Tricklebank *et al*, 1989) in male Swiss Webster mice during five 45 sec observation periods repeated at 4 min intervals starting 10 min after the subcutaneous injection of a submaximal dose of MK-801 (0.5mg/kg). Drug administration and behavioural observations were repeated at daily intervals for 6 consecutive days. For anticonvulsant testing, MK-801 (0.1-1mg/kg) was injected s.c. 15 min before administration of N-methyl-DL-aspartic acid (NMDLA, 500mg/kg, s.c.) and tonic seizures scored over the following thirty min period in animals given either saline or 0.5mg/kg MK-801 daily for 6 days. The ED_{50} dose (the dose protecting 50% of animals) was calculated by probit analysis.

The intensity of ataxia induced by MK-801 was not altered over the six-day administration period (mean score ± s.e.m. of 8 mice = 2.4 ± 0.1 and 2.5 ± 0.1 on days 1 and 6 respectively). In contrast, repeated treatment with MK-801 induced a marked decline in the frequency of head weaving (number of movements = 383 ± 31 and 162 ± 21 on days 1 and 6 respectively, $p < 0.05$), but significantly increased locomotion (number of areas of observation cage entered = 19 ± 8 and 90 ± 36 on days 1 and 6 respectively, $p < 0.05$). In animals given saline for 6 days, MK-801 dose-dependently inhibited seizures induced by NMDLA with an ED_{50} (95% confidence limits) of 0.24 mg/kg (0.16-0.34mg/kg) a value not significantly different from that of animals chronically treated with MK-801 (0.25mg/kg, 0.18-0.39mg/kg).

Thus, the results suggest that head weaving and hyperlocomotion induced by MK-801 may be mediated by different neuronal mechanisms to those subserving the ataxic and anticonvulsant properties of the compound. It remains to be seen whether this differentiation supports the possibility of obtaining non-competitive NMDA receptor antagonists devoid of stimulant effects.

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287P THE ROLE OF CHOLECYSTOKININ IN RAW SOYA FLOUR-INDUCED TROPHIC LESIONS IN RAT PANCREAS

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Feeding raw soya flour (RSF) to rats results in rapid hyperplasia and hypertrophy of the pancreas (McGuiness et al., 1984), with accompanying increase in synthesis, content and secretion of pancreatic enzymes (Folsch & Wormsley, 1974) These trophic changes can progress to pancreatic adenomas and carcinomas with continued feeding and have been ascribed to the effect on the pancreas of high circulating levels of cholecystokinin (CCK) resulting from the assumed inhibition of duodenal trypsin by RSF. L-364,718 is a novel nonpeptide CCK receptor antagonist derived from aspercillin and L-tryptophan. It exhibits extremely high potency and specificity both in vitro and in vivo for Type-A CCK receptors on pancreatic exocrine cells (Lotti et al., 1987).

Four groups of young male Wistar rats were fed powdered Rat and Mouse No. 1 Standard Maintenance Diet with or without RSF. L-364,718 5mg/kg was administered orally twice daily, a dose known to inhibit the secretory response to CCK (Wisner et al., 1988). Animals were autopsied after four days of treatment. Vincristine 1mg/kg was administered intraperitoneally two hours beforehand as a stathmokinetic agent. The pancreas was weighed and the mitotic index assessed in H & E stained sections of the body of the pancreas.

Group	Treatment	Mean Relative Pancreatic Weight/g (S.D.)	Mean Mitotic Index Counts/Field (S.D.)
1	Normal diet	0.53±0.06	1.34±0.77
2	Normal diet + L-364,718	0.46±0.07	1.22±0.74
3	50% RSF	0.76±0.10	2.29±1.44
4	50% RSF + L-364,718	0.51±0.09	0.31±0.34

The trophic effect of RSF is demonstrated by the significantly increased mean relative pancreatic weight obtained in Group 3 as compared with Group 1 ($P<0.01$). There was complete inhibition of this effect by L-364,718 in Group 4 as compared with Group 3 ($P<0.001$). The mitotic index was significantly reduced in Group 4 as compared with Groups 2 ($P<0.01$) and 3 ($P<0.001$), an effect that might reflect the putative role of CCK in the maintenance of normal pancreatic activity. The Student's t test was used. It is concluded that oral L-364,718 1mg/kg twice daily completely inhibited the trophic action of RSF on rat pancreas, lending support to the theory that peripherally-acting CCK is the prinicipal mediator of this effect.

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288P POSSIBLE INVOLVEMENT OF NEUROKININ-A, SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE IN PERIPHERAL HYPERALGESIA IN THE RAT

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Neurokinin-A (NKA) has been shown to be distributed in parallel with substance P (SP) in the dorsal horn of the spinal cord and its pharmacology has resembled SP in many ways (Vaught, 1988). A similar distribution is also described for calcitonin gene-related peptide (CGRP) (Gibson et al., 1984). The present study investigates the possible involvement of NKA and CGRP in peripheral pain as excitatory transmitters in the sensory nerve fibres, as has previously been proposed for SP (Nakamura-Craig & Smith, 1989). The possibility that NKA and CGRP produce sensitization in the rat paw by repeated administration of a sub-threshold dose, as has been recently shown for SP (Nakamura-Craig & Smith, 1989), has also now been investigated.

Hyperalgesia in the rat paw was measured by a modification of the Randall-Selitto test (Ferreira et al., 1978). Male Wistar rats (150-170g, n=5 per group) were pretreated with indomethacin (2 mg/kg p.o.) 30 min prior to the paw injections. NKA, SP and rat α-CGRP were injected into the paw in volumes of 0.05-0.1 ml. For the purpose of investigating the sensitization of the paw, 5 injections of the substance, at a sub-threshold dose, were given at 10 min intervals.

Intraplantar injections of NKA, SP and CGRP induced dose-dependent hyperalgesia, the ED50s and 95% confidence limits being 0.4 (0-4.1), 4.7 (3.46-6.9) and 5935 µg/paw, respectively at 30 min after administration. Furthermore, five injections of a sub-threshold dose (0.5 ng/paw at 10 min intervals) of NKA, SP or CGRP produced a substantial ($P<0.01$) increase in hyperalgesia, lasting for greater than 4h for NKA and SP and 2h for CGRP.

These results indicate that in the present system, NKA is 10 times more potent than SP in inducing hyperalgesia, which is 1000 times more potent than CGRP, suggesting that endogenous NKA and SP but not CGRP could have an important role in acute hyperalgesic conditions. Furthermore, the sensitization induced by several injections of sub-threshold doses of NKA, SP or CGRP suggest that these tachykinins could participate as mediators or modulators of chronic pain.

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289P SUSTAINED HYPERALGESIA IN RATS EVOKED BY 15-HYDROPEROXYEICOSATETRAENOIC ACID IS ATTENUATED BY THE PROTEIN KINASE INHIBITOR H-7

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Hyperalgesia, defined as a reduced pain threshold to stimuli which are normally non-painful, is evoked by some but not all inflammatory mediators. Fatty acid hydroperoxides cause pain when injected intradermally in man (Ferreira, 1972). Using a modification of the Randall-Selitto test, we have found that picogram quantities of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) rapidly evokes hyperalgesia (ED_{50}=2pg) that lasts a few hours. Moreover, repeated injections of 15-HPETE (1ng) into the same site, daily for 14 days, leads to **sustained** hyperalgesia that persists unchanged for at least 28 days after the last injection. This supports the observation of Ferreira *et al* (1987) who used different hyperalgesic stimuli. Sustained hyperalgesia due to 14 daily injections of 15-HPETE (1 ng) was associated with approximately 3-fold increase in electrical activity in C-fibres of the saphenous nerve stimulated by application of mustard oil to the dorsolateral surface of the paw, following the method of Heapy *et al* (1987).

Acute hyperalgesia was unchanged in rats dosed with indomethacin (10 mg/kg p.o.) to block cyclo-oxygenase or BWA4C (50 mg/kg p.o.) to block 5-lipoxygenase (Tateson *et al*, 1988) but was blocked by the analgesic dipyrone (100 mg/kg) given orally and by opioid analgesics (e.g. morphine 10-100μg) injected locally into the paw. It was also blocked by local injection (ED_{50}=5μg) of the protein kinase inhibitor H-7, described by Hidaka *et al* (1984). The **sustained** hyperalgesia due to 15-HPETE was also unchanged by either indomethacin or BWA4C but was transiently blocked by local injection of opioids, the effects of which lasted 24hr or less. In contrast, a single dose of dipyrone (100 mg/kg p.o.) abolished the sustained hyperalgesia, even when measured days later. This effect of dipyrone also supports the observations of Ferreira, *et al* (1987). Daily injection of H-7 (50μg) into the paw along with 15-HPETE (1ng) delayed development of sustained hyperalgesia. Furthermore, such treatment with H-7 completely blocked the 15-HPETE-induced increase in electrical response of C-fibres stimulated with mustard oil.

Thus, repeated exposure of sensory nerve endings to 15-HPETE provokes hyperalgesia that becomes sustained, even when the 15-HPETE is discontinued. This sustained phase is associated with increased electrical activity in sensory afferent fibres that may follow activation of protein kinases, suggesting similarities with long-term potentiation of synaptic transmission in the hippocampus where protein kinase activation has also been implicated (Hu *et al* 1987).

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290P BEHAVIOURAL PROFILE OF THE δ-OPIOID RECEPTOR ANTAGONIST NALTRINDOLE IN MICE

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Recently, naltrindole, a stable non-peptide antagonist has been developed and shown to act selectively at δ-opioid receptors in vitro and to antagonise the antinociception induced by δ- but not μ- or k-agonists in mice (Portoghese et al., 1988). Since there have been no other reports of the effects of naltrindole in vivo we have determined its behavioural profile in mice using a variety of measures.

Naltrindole (Reckitt & Colman) dissolved in pH3 saline was assessed in male TO mice (20-30g; n=10) using the following paradigms: behavioural analysis (Jackson & Kitchen, 1989); body temperatures; rotarod performance; and seizure thresholds after electroshock (Jackson et al., 1989) or i.v. infusion of bicuculline (a GABA antagonist).

Naltrindole (0.1-10 mg/kg s.c.) had no effects on locomotor activity, wall-climbing, rearing, grooming, gnawing, sniffing, yawning or stereotyped mouthing. The highest dose produced a small increase in scratching (score of 6.3±1.4 vs. 1.3±0.6 for vehicle; maximal score of 60; P<0.005) although this was mainly around the injection site. These doses of naltrindole had no effect on rectal body temperatures (e.g. mean temperatures of the vehicle group were 37.7±0.3 °C vs. 37.5±0.3 for the 10 mg/kg group at 30 min following drug treatment and 37.7±0.3 vs. 37.6±0.3 at 60 min). Naltrindole (0.3-10 mg/kg i.p.) did not impair the ability of mice to stay on a rotating rod (90% remaining on the rotarod 15 and 30 min after 10 mg/kg) and had no effect on the amount of bicuculline required to produce clonic and tonic seizures in these animals (0.38±0.05 and 0.55±0.06 mg/kg for the vehicle group vs. 0.39±0.07 and 0.57±0.1 for the 10 mg/kg group after 30 min pretreatment; values represent clonic and tonic seizure thresholds respectively). Conversely, electroshock seizure thresholds were significantly (*P<0.05) lowered by naltrindole (Table 1). These results show that naltrindole, in doses of up to 10 mg/kg, does not produce any overt behavioural effects, change body temperature or alter seizure threshold to a chemical convulsant. Its proconvulsant activity against electroshock could be related to blockade of δ-receptors as the non-selective opioid antagonist naloxone has no effect in this model except at high doses (unpublished observations; Puglisi-Allegra et al., 1985).

Table 1 Current inducing tonic seizures in 50% of sample (95% fiducial limits)

Vehicle i.p.	12.7 (12.0-13.4)
Naltrindole 0.3 mg/kg i.p.	11.0 (10.1-11.9)*
Naltrindole 1 mg/kg i.p.	11.0 (10.1-11.9)*
Naltrindole 3 mg/kg i.p.	10.2 (9.7-10.7)*
Naltrindole 10 mg/kg i.p.	10.2 (9.7-10.6)*

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291P D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 IS A HIGHLY SELECTIVE μ-LIGAND WITH LOW *IN VITRO* ANTAGONIST ACTIVITY

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Although several highly selective μ-agonists are available, there are no highly selective μ-antagonists. However, several analogues of somatostatin have been shown to have antagonist activity at μ-receptors (Pelton et al, 1986). In rat brain homogenates, the cyclic somatostatin analogue D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) had the highest selectivity for the μ-binding site (Hawkins et al, 1989). We have tested CTOP in both binding and pharmacological assays.

The potency of CTOP to displace the binding of selective opioid ligands was measured in homogenates of guinea-pig brain at 25°C. The μ-sites were labelled with [^{3}H]-[D-Ala^2, MePhe^4, Gly-ol^5] enkephalin (1nM), the δ-sites with [^{3}H]-[D-Pen^2, D-Pen^5] enkephalin (1.5nM) and the k-sites with [^{3}H]-U-69,593 (1.5nM). CTOP was tested for agonist and antagonist activity in the guinea-pig ileum myenteric plexus.

In binding assays, CTOP was a highly selective μ-ligand with a K_i value of 1.69±0.32nM (n=4) whereas 1μM CTOP caused less than 20% inhibition of δ- or k-binding. [^{3}H]-CTOP labelled a single class of binding site with a K_d of 0.12±0.01nM (n=3). The binding of [^{3}H]-CTOP was readily displaced by [D-Ala^2, MePhe^4, Gly-ol^5] enkephalin and naloxone whereas selective δ- and k-ligands displayed low potencies.

In the myenteric plexus, the antagonist dissociation constant (K_e) for CTOP against the μ-ligand [D-Ala^2, MePhe^4, Gly-ol^5] enkephalin was 16.1±1.9nM (n=5) and against the k-ligand U-69,593 was 444±78nM (n=3).

Thus, in binding assays it was confirmed that CTOP is a highly selective μ-ligand. However, CTOP has a low antagonist potency at μ-receptors in vitro.

Supported by grants from MRC and US NIDA

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292P INCREASED FORMATION OF DIACYLGLYCEROL METABOLITES FOLLOWING MUSCARINIC CHOLINERGIC STIMULATION OF SYNAPTOSOMES

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Synaptosomes were isolated from cerebral cortices of 2-4 rats by centrifugation on discontinuous percoll fradients (Dunkley et al, 1986) and labelled by incubation with [2-^{3}HH]-glycerol (6μCi/mg protein) for 20 min. at 37°C. The labelled synaptosomes were then incubated with carbachol (10μM-1mM) in the presence or absence of atropine (10μM) for 10 min. at 37°C before termination of reactions by addition of trichloro-acetic acid to give a final concentration of 6% (w/v). After centrifugation, lipids were extracted from the pellet as previously described (Brammer et al, 1988) and analysed by thin layer chromatography on silica gel "G" plates using hexane-diethyl ether-formic acid (80:20:2 vy vol) as the developing solvent. Lipids were visualised using iodine vapour, identified by comparison with authentic standards applied to the same plates, scraped and their radioactivity determined by liquid scintillation counting.

It was found that carbachol did not significantly change the labelling of either 1,2-diacylglycerols (1,2 DAG) or 1,3-diacylglycerols (1,2 DAG) but did increase the labelling of monoacylglycerols (MAG) and of total phospholipids (maximal increases 50% and 90% respectively over control values at 1mM carbachol). The increases in labelling were significant at all carbachol concentrations greater than 10μM (p 0.05), dose-dependent (EC_{50} values approx 20μM for both MAG and phospholipids), and were antagonised by atropine (no significant increases in the presence of 10μM atropine).

We conclude that occupancy of synaptosomal muscarinic receptors causes a rapid, dose-dependent increase in the flux of glyycerol through synaptosomal glycerophospholipids. this probably results from very rapid turnover or diacylglycerol (1,2 DAG) formed by receptor-mediated polyphosphoinositide breakdown. Enhanced 1,2-DAG labelling was not detected, but can be inferred from the increase in radioactivity in its two major metabolic products (MAG and phospholipids).

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293P THE INHIBITION OF ACETYLCHOLINE RELEASE FROM HUMAN TEMPORAL CORTEX BY ANGIOTENSIN II

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In the present study we investigated the action of angiotensin II (ATII) on 'in vitro' K^+ stimulated [^{3}H]acetylcholine (Ach) release from fresh human temporal cortex tissue, having previously demonstrated that ATII is potent to inhibit the release of K^+-stimulated [^{3}H]Ach from rat entorhinal cortex.[1]

Temporal cortex from the subdominant hemisphere was obtained during surgical removal of a deep-seated tumour from a 47 year old woman. Within 1hr the cortical tissue was cross-chopped to produce slices (0.35mm x 0.35mm x thickness of cortical ribbon). The tissue was pre-stimulated in an elevated K^+ (37.5mM) Krebs buffer and then incubated for 40 min in a Krebs buffer containing 0.1μM [^{3}H]choline. The slices were thoroughly washed and loaded into each of 20 Swinnex perfusion chambers, each constituting a separate channel. After a 30 min washout period with Krebs buffer containing 1.0μM hemicholinium-3, 4 min fractions were collected for 80 min. Two 4 min K^+ pulses were elicited by a 20mM K^+ Krebs buffer applied at 12 min (S1) and 48 min (S2). The tritium content of the fractions and that remaining in the tissue was assayed by liquid scintillation spectroscopy. Disintegrations per min were converted to fractional release and the S2/S1 ratio calculated. ATII recognition sites were quantified by saturation analysis with [^{125}I]ATII (1988 Ci mmol^{-1}) using the method of Bennett and Snyder.[2] [1-sarcosine-8-threonine]ATII (final concentration 10.0μM) was used to define non-specific binding. Angiotensin converting enzyme (ACE) was fluorometrically determined, using the artificial substrate hippuryl-L-histidyl-L-leucine[3] at an excitation wavelength of 360nm and an emission wavelength of 500nm. The Coomassie blue method was used to measure the protein content of the tissues, with bovine serum albumin used as standard. ATII (1.0μmolL^{-1}) significantly inhibited K^+ stimulated [^{3}H]Ach release from slices of human temporal cortex. This could be antagonised by [1-sarcosine,8-threonine]ATII (0.1μM) which alone failed to modify [^{3}H]Ach release.

ACE activity in human temporal cortex was 1.03nmoles min^{-1} mg^{-1} protein (mean of a triplicate determination). Scatchard analysis of [^{125}I]ATII saturation data identified a homogenous population of high affinity [^{125}I]ATII binding sites. (B_{max} = 8.6 fmol mg^{-1} protein; k_d = 1.02 nmol dm^{-3}, correlation coefficient (r) = 0.88).

The finding that ATII can inhibit Ach release from human temporal cortex, with the knowledge that ATII formation can be inhibited by the ACE inhibitors, lends support to the development of the ACE inhibitors as cognitive enhancing agents.

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294P REPETITIVE FIRING OF THE ISOLATED RAT PHRENIC NERVE-HEMIDIAPHRAGM PREPARATION: EFFECTS OF HIGH CALCIUM AND (+)-TUBOCURARINE

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Nicotinc receptors present on motor nerve terminals mediate a positive feedback loop on transmitter release (Bowman, 1986; Wessler, 1989) and repetitive nerve firing (Clark & Hobbiger, 1983). The nicotinic receptors mediating repetitive nerve firing are thought to be localized far from the release zones (Bowman, 1986). In the present experiments the compound action potentials of both the muscle fibres (MAP) and phrenic nerve (NAP) were recorded before and after partial blockade of cholinesterase to investigate the effects of extracellular calcium and tubocurarine (TC) on the repetitive nerve firing.

Rat phrenic nerve-hemidiaphragms were superfused in 15 ml organ baths with a physiological salt solution. The phrenic nerve was stimulated via two platinum electrodes placed 2 mm proximal to the insertion. For extracellular recording two stainless steel needles were placed in the muscle fibres and two platinum electrodes onto the phrenic nerve outside the organ bath at a part of the nerve that was embedded in vaseline. Developed tension of the hemidiaphragm was also recorded. The phrenic nerve was stimulated continuously (0.2 Hz) and at 5 min intervals intermittently by a single pulse and trains of 9 pulses (1 - 50 Hz).

Exposure to neostigmine (2 min, 3 μM) caused a nearly 2-fold increase in muscle tension and repetitive potentials in the nerve and muscle. During repetitive nerve stimulation a characteristic pattern of MAP was observed: a sharp decline of the second MAP (decrement) and a gradual increase during the subsequent MAPs (increment). Decrement-increment was positively related to the stimulation frequency. Both repetitive nerve firing and decrement-increment disappeared after 20 min due to a spontaneous recovery from cholinesterase inhibition. High calcium (3.8 mM), low magnesium (0.2 mM) intensified markedly the repetitive nerve firing and the decrement. Low concentrations of TC (10 - 100 nM) did not reduce MAPs of a train but abolished the repetitive nerve firing and the decrement of MAP.

After partial blockade of the cholinesterase repetitive NAPs, MAPs and the decrement-increment of MAP was observed. Decrement was strongly related to the duration and amplitude of repetitive nerve firing. Presumably, repetitive nerve firing reduces transmitter release after the first pulse of a train either by interferring with the impulse propagation at the unmyelinated part of the axon or by the exhaustion of the ready-releasable transmitter pool. The high potency of TC suggests either different pharmacological properties of the nicotinic receptors or a low agonist concentration, i.e. a location of these receptors far from the release zones.

Supported by NMFZ University of Mainz and the Deutsche Forschungsgemeinschaft

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295P *IN VIVO* CARDIOSELECTIVITY OF THE ANTI-MUSCARINIC DRUG AQ-RA 741

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AQ-RA 741 (11-[[4-[4-(diethylamino)butyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido [2,3-b][1,4]benzodiazepin-6-on) is a recently synthetized M2-selective muscarinic antagonist with an improved affinity and selectivity towards cardiac-M2-receptors compared to AF-DX 116 (Eberlein et al. 1989). In the present study the in vivo selectivity of AQ-RA 741 was investigated, using anaesthetized rats, guinea-pigs and cats.

In pithed rats the dose (ED50,iv) of AQ-RA 741 necessary to antagonize the bradycardia due to stimulation of the right vagus nerve (20 Hz,2 ms, supramaximal voltage) amounted to 0.029 mg/kg.Approximately 43-fold higher doses were required to block the McN-A-343 (0.3 mg/kg,iv) induced increase in blood pressure (ED50=1.23 mg/kg). Doses up to 10 mg/kg were ineffective to influence the pilocarpine (1.4 g/kg,ip) induced salivary secretion in rats. The administration of acetylcholine (50 µg iv and ia) in guinea-pigs elicited a decrease in heart rate, an increase in airway resistance and contraction of bladder smooth muscle. The doses of AQ-RA 741 (iv) to inhibit these responses by 50% were 0.027, 0.24 and 1.04 mg/kg, respectively. Similar experiments were performed in the cat. However beside heart rate, airway resistance and bladder pressure, also the effect of methacholine (50 µg iv and ia) on duodenum pressure and salivary secretion were measured. The ED50 value of AQ-RA 741 (iv) to antagonize the bradycardia was 0.015 mg/kg, whereas 13-67 fold higher doses were necessary to antagonize the effects on duodenal pressure (0.47 mg/kg), bladder pressure (0.57 mg/kg), airway resistance (0.20 mg/kg) and salivation (1.0 mg/kg).

It can be concluded that in the three species investigated AQ-RA 741 antagonizes muscarinic agonist induced bradycardia in doses that do not affect other muscarinic responses elicited by these agonists. Between 13- and more than 100-fold higher doses are needed to antagonize responses like salivation or intestinal smooth muscle contraction. Accordingly, AQ-RA 741 showed a marked in vivo cardioselectivity.

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296P LACK OF EFFECT OF THE ANTIMALARIAL AGENT HALOFANTRINE ON HEPATIC DRUG METABOLISM

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Several quinoline antimalarials have been shown to inhibit cytochrome P-450 dependent mixed function oxidase activity (Murray, 1987). Halofantrine (Hf) is a structurally related phenanthrene methanol effective against multidrug resistant P. falciparum (Horton, 1988) whose potential to influence hepatic drug metabolism has not been examined. In view of the relevance of this to drug interactions, we chose to investigate the effect of Hf on hepatic drug oxidation in the rat (in vitro and in vivo) and in the mouse (in vivo). Using rat liver microsomes, Km and Vmax for aminopyrine N-demethylase (0.47 ± 0.20mM and 2.6 ± 0.2nmoles/min/mg protein) and ethoxycoumarin O-deethylase (35.0 ± 19.0nM and 90.2 ± 31.7pmoles/min/mg protein) were unchanged after incubation with increasing concentrations (0.01-0.1mM) of Hf ($P>0.05$; One way ANOVAR). Additionally, the values of these same parameters were not significantly different when determined using microsomes prepared from livers of rats dosed chronically with Hf (20mg/kg for four days) when compared to vehicle dosed controls ($P>0.05$ unpaired t-test). To complement these studies, the disposition of antipyrine (2.5mg) was investigated in the isolated perfused rat liver system following administration of bolus doses of Hf (0.5 and 5.0mg). There were no differences in the values of the pharmacokinetic variables clearance, (Cl) apparent volume of distribution (Vd) or elimination half-life (T1/2) for antipyrine compared with controls (Table 1; mean (s.d.)).

Table 1 Pharmacokinetic variables for antipyrine in the isolated perfused rat liver

	Cl(ml/min)	Vd(ml)	T1/2(min)
Control	0.51(0.11)	189(14)	265(50)
0.5mgHf	0.48(0.12)	177(33)	275(80)
5.0mgHf	0.40(0.08)	188(19)	338(59)

A potential selective effect of Hf was examined in male Wistar rats in vivo. Urinary recoveries of antipyrine, norantipyrine, 3-OH antipyrine and 4-OH antipyrine in a control group (0-24h; n=6) after a 2.5mg oral dose of antipyrine (142 ± 64, 729 ± 117, 76 ± 79 and 506 ± 131µg) were unchanged (152 ± 44, 763 ± 121, 85 ± 77 and 476 ± 82µg; $P>0.05$; one way ANOVAR) following pre-dose with Hf (5.0mg; n=6). Finally, using female CBA mice, mean weight = 30g, (n=12) control pentobarbitone sleeping times (70 ± 17min) were not significantly changed following the acute administration of increasing (2,10 and 20mg/kg) of Hf ($P>0.05$; one way ANOVAR). Likewise, chronic dosage with Hf (20mg/kg for four days; n=15) produced no significant change in the control value (118 ± 71min; $P>0.05$ unpaired t-test). Taken together, these observations strongly suggest that Hf, in contrast to the aminoquinoline antimalarials, is not a potent or specific inhibitor of hepatic drug metabolism.

K.A.M. is supported by a Postgraduate Studentship from the Research Development Fund of the University of Liverpool. S.A.W. is a Wolfson lecturer.

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297P SYNAPTOSOMAL STUDIES OF THE REGULATION OF TOTAL AND FREE CALCIUM CONCENTRATIONS

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Ca^{2+} is intimately involved in neuronal processes such as neurotransmitter release. We have examined the relationships between the concentration of external Ca^{2+}($[Ca^{2+}]_o$), intrasynaptosomal total calcium(Ca_T), intrasynaptosomal free Ca^{2+}($[Ca^{2+}]_i$) and calcium accumulation, in rat cortical synaptosomes. Ca_T, assayed by atomic absorption spectrometry(Gitelman,1967), was estimated to be a maximum of 10.7mM(n=8) at 1mM $[Ca^{2+}]_o$. $[Ca^{2+}]_i$ was measured by fluorescence spectrophotometry(Adamson et al,1987) using Fura-2. Basal $[Ca^{2+}]_i$ was 118nM(n=10) in 1mM $[Ca^{2+}]_o$. This agrees with earlier reports that $[Ca^{2+}]_i$ is a small fraction (1/10,000th) of the Ca_T(Gibson and Peterson,1987). However, at various $[Ca^{2+}]_o$ (0-5mM), there are approximately linear relationships between $[Ca^{2+}]_i$, Ca_T and $[Ca^{2+}]_o$.

^{45}Ca accumulation into synaptosomes was linearly dependent on $[Ca^{2+}]_o$. Under physiological conditions(1mM $[Ca^{2+}]_o$ and 5mM $[K^+]_o$), ^{45}Ca(1mM) accumulation was rapid(80% of the equilibrium level was attained in 2min) and reached equilibrium in 5min. The initial rate of accumulation was 3.4 nmol/mg protein/min: thus, if all intracellular calcium was freely exchangeable, total exchange would occur in approximately 10min. At equilibrium, the specific activities of ^{45}Ca inside and outside synaptosomes allowed estimation of the exchangeable pool size: under physiological conditions, the rapidly exchangeable Ca^{2+} pool in synaptosomes is only 10% of Ca_T. ^{45}Ca accumulation into synaptosomes during a 2min incubation was dependent on $[Ca^{2+}]_o$ (2.8, 4.1, 10 and 13 nmol/mg protein at 0.1, 1, 10 and 60mM $[K^+]_o$, respectively), i.e., as would be expected, the process is depolarisation dependent. However, at physiological $[K^+]_o$, there was considerable ^{45}Ca accumulation (7nmol/mg protein). As Ca^{2+} entry is a passive process, mainly occurring via voltage-dependent Ca^{2+} channels, it appears that specific Ca^{2+} channels on synaptosomes show a range of voltage sensitivities.

In conclusion, we have shown, a) Ca^{2+} accumulation into synaptosomes is rapid, depolarisation dependent and substantial even under resting membrane conditions; b) Ca_T is approximately 10,000 times higher than $[Ca^{2+}]_i$; c) only 10% of Ca_T is freely exchangeable at 1mM $[Ca^{2+}]_o$; d) the freely exchangeable pool is higher(x1,000) than the free pool; e) varying external Ca^{2+} alters Ca_T, $[Ca^{2+}]_i$ and accumulation in a linear fashion, suggesting that the $[Ca^{2+}]_i$ is not highly conserved and that it is maintained by simple equilibria between the various pools.

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298P SPERMINE TOXICITY IN NORMAL AND TRANSFORMED BABY HAMSTER KIDNEY CELLS IN CULTURE

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The polyamines, spermidine and spermine and their precursor, putrescine, are found in all mammalian cells. Although their exact function within the cell is not known they are essential for normal cell growth and differentiation (Pegg, 1986). High concentrations of these amines inhibit cell growth in a number of different cell lines (Alarcon *et al.*, 1961). Therefore they can act as both positive and negative regulators of cell growth. The toxic effect produced by polyamines is believed to be a result of their oxidation to reactive aldehydes, although the exact mechanisms involved are not known. The aim of this study was to investigate the intracellular mechanisms involved in spermine toxicity in two mammalian cell lines: a normal fibroblast cell line derived from baby hamster kidney cells (BHK-21/C13) and their sister polyoma virus transformed cell line (P_yY). Both cell lines are routinely grown in Dulbecco's medium supplemented with 10% (v/v) horse serum in a humidified atmosphere of 95% air/ 5% CO_2. Protein content was used as a measure of cell growth. Protein content, intracellular polyamines and reduced glutathione were determined as descibed previously (Brunton *et al.*, 1989).

Spermine produced a dose-dependent inhibition of cell growth in both cell lines, with the P_yY cells being 3-4 fold more resistant than BHK cells. Such a difference in sensitivity may result from a) reduced uptake and accumulation of spermine, b) increased excretion of spermine or c) more efficient intracellular protective mechanisms.

Both cell types contain a specific polyamine uptake system. However the rate of spermine uptake into the transformed cells was approxiametly 50% of that into the normal cells. Following exposure to toxic concentrations of spermine, the intracellular spermine concentration of both cell lines was increased. In BHK cells the intracellular concentration was greater than 6 times that in the medium whereas in P_yY cells the intracellular concentration was only twice that of the medium.

Table 1

Exposure Time (h)	Spm Added (mM)	Protein Content (mg)	Intracellular Spermine (nmoles/mg protein)	Intracellular Glutathione (nmoles/mg protein)	Spm Added (mM)	Protein Content (mg)	Intracellular Spermine (nmoles/mg protein)	Intracellular Glutathione (nmoles/mg protein)
BHK Cells					P_yY Cells			
4	-	1.29 ± 0.03	6.21 ± 0.53	6.68 ± 0.44	-	1.97 ± 0.03	3.35 ± 0.98	20.34 ± 0.31
	2	1.24 ± 0.05	62.70 ± 5.82	1.69 ± 0.05	7.5	0.68 ± 0.03	75.48 ± 0.40	20.26 ± 0.60
12	-	1.62 ± 0.07	9.96 ± 0.85	6.74 ± 0.12	-	1.97 ± 0.03	7.27 ± 0.83	17.18 ± 1.79
	2	0.63 ± 0.05	72.47 ± 1.47	n.d.	7.5	0.68 ± 0.03	114.40 ± 2.59	20.65 ± 2.68

Reduced glutathione plays a major cytoprotective role within the cell either by direct conjugation with reactive electrophilic compounds or through the prevention of redox cycling of reactive oxygen species. In control cells the glutathione content of P_yY cells is around 3 fold higher than in BHK cells (Table 1). Following exposure of BHK cells to toxic concentrations of spermine, there was a rapid early loss of glutathione, which occurred before any effect on cell growth. In P_yY cells there was no loss of glutathione until 24h after exposure to toxic concentrations of spermine, although an effect on cell growth was seen between 6-8h.

Transformed cells are able to withstand exposure to excess spermine better than the normal BHK cells, by reducing the uptake of the amine and possibly by increasing its excretion. The high levels of glutathione in P_yY cells may also protect these cells from spermine induced damage.

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299P COMPARISON OF SURFACTANT-MEDIATED CHANGES IN MICROBIAL MEMBRANE PERMEABILITY

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Surfactants are able to alter permeability of biological membranes; for example, previous work has studied the effects of one such group of compounds, the n-octyl phenyl polyoxyethylene ethers (Tritons), on microbial and other membrane systems (Al-Assadi et al., 1987). One question arising from this previous work is the extent to which concentration and purity are important determinants of surfactant effects. To answer these questions, we have compared the effects of Triton X-100 and different fractions of the poloxamer surfactant, Pluronic F-68, on uptake of fluorescein diacetate (FDA) into yeast.

Cultures of Saccharomyces cerevisiae (X 2180 1B) were grown at 30°C for up to 330 mins; cell samples were removed, washed, and re-suspended in 4 ml CP medium. 50 µl of this cell suspension was then incubated in a fluorescence spectrometer with 2.95 ml of CP medium containing 50 µl FDA (0.5 mg/ml in acetone). The rate of fluorescence increase with time (Fr) was measured using an excitation wavelength of 485 nm and emission wavelength of 515 nm. Once a baseline Fr was established during a pre-assay period of ca. 10 min, a final concentration of 0.05 - 5.0 % (w/v) of one of the following was added: (i) commercial grade Pluronic F-68 (Atochem/ICI, U.K.); (ii) commercial grade Pluronic F-68 (BASF-Wyandotte, U.S.A.); (iii) purified ICI pluronic (Bentley et al., 1988); (iv) purified BASF pluronic; and (v) 0.1 - 0.5% (v/v) Triton X-100 (BDH, U.K.); changes in Fr were monitored for a further 10 min period.

Fr increased up to 2.5 fold above baseline rate following addition of 1.0-5.0% unpurified BASF pluronic while the purified preparation had no comparable effect. However, purified ICI pluronic had an inhibitory effect on Fr which increased with increasing surfactant concentration: Fr decreased by 78% as the pluronic concentration was increased from 0.1 - 5.0%. Unpurified ICI pluronic did not affect Fr. None of the pluronic preparations had any effect on the rate of FDA hydrolysis by cell-free extracts, polypeptide profiles of crude membrane extracts or cell structure. Fr was also reduced by up to 88% following the addition of 0.1 - 0.5% Triton X-100. No significant change in culture pH occurred during the experiments.

These results show that different surfactants have variable effects on FDA uptake into yeast. Pluronic F-68 can alter the rate of dye uptake without causing any changes in the activity of enzymes directly responsible for its hydrolysis. The variation in response to different pluronic fractions shows that both source and purity of compound are important determinants of their effects on dye uptake. We speculate that unidentified contaminants in some commercial grade pluronics can perturb membrane transport/permeability processes in yeast. The inhibition of dye uptake by Triton X-100 at higher concentrations was in accord with its established role as a membrane-disrupting agent (Helenius et al., 1979).

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300P EFFECTS OF EMULSIFIED PERFLUOROCHEMICALS ON RAT LIVER ARYL ESTERASE ACTIVITY

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Perfluorochemicals (PFC) and their emulsions are attracting interest as vehicles for respiratory gas transport and as contrast agents for diagnostic tissue imaging (Lowe, 1988). PFCs can accumulate in the liver (Lowe, 1988) but their effects on hepatic enzyme systems have not been studied in detail. We have therefore examined the effects of different PFC emulsions on liver aryl esterase (LAE) activity in rats.

Male or female Wistar rats (body weight (b.w.): 150-250g; n = 95) were used. They were injected intravenously (i.v.) via a tail vein with 10ml/kg b.w. of one of the following: (i) saline (0.9% w/v NaCl); (ii) Fluosol-DA 20% (F-DA; Green Cross, Japan); (iii) Oxypherol (FC-43; Green Cross); and (iv) a novel 20% (w/v) perfluorodecalin (FDC) emulsion containing 1% (w/v) of a C-16 oil additive and 4% (w/v) Pluronic F-68 (Sharma et al., 1987). At 24 hr, 72 hr and 7 days after injection, groups of animals were killed and their livers removed. Tissues were homogenized in 1.15% (w/v) KCl solution buffered with Tris/HCl. LAE activity was measured spectrophotometrically using 25 mM indoxylacetate as substrate (Bosmann, 1982).

Mean LAE activity in male rats increased progressively following injection of the novel emulsion and after 7 days was 48% greater ($P < 0.05$) than that measured in saline-injected controls (1.75 ± 0.22 µmol/min/mg protein; n = 4). LAE activity in male rats was unaffected by injection of either F-DA or FC-43 with overall mean values of 2.13 ± 0.15 µmol/min/mg protein (n = 12) and 2.09 ± 0.14 µmol/min/mg protein (n = 12) respectively recorded throughout. In contrast, mean LAE activity in female rats at 72 hr after injection of FC-43 was 35% lower ($P < 0.05$) than in controls (2.08 ± 0.19 µmol/min/mg protein; n = 4); a similar trend towards a decrease in LAE activity also occurred in female rats injected with both F-DA and the novel emulsion although in both cases these changes were not significant. Overall mean LAE activities in female rats injected with either F-DA or the novel emulsion were generally lower than corresponding values measured in male rats (F-DA: 1.64 ± 0.14, n = 12; novel emulsion: 1.59 ± 0.14, n = 11).

These results show that there are marked variations in LAE responses to components of different PFC emulsions between male and female rats. The induction of LAE by the novel emulsion in males was consistent with previous observations on the hepatic microsomal cytochromes P-450 complex (Armstrong & Lowe, 1988). The decrease in LAE in female rats injected with FC-43 emulsion suggests a specific transient inhibitory effect of its perfluorotributylamine oil component but this needs to be clarified.

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301P USE OF A COMMERCIALLY AVAILABLE SIGNAL PROCESSING CENTRE FOR 'ON-LINE' ANALYSIS OF HAEMODYNAMIC DATA

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The Modular Instruments Inc. M3000 Signal Processing Centre (MI^2) is a commercially-available device for data collection and analysis. It comprises a personal computer, an eight channel thermal printer, an interface system consisting of a number of electronic modules that effect analogue to digital conversion and signal processing, and controlling software referred to as the 'XYZ-Real-time Spreadsheet'.

The MI^2 system replaces pen-recorders, pre-amplifiers and analogue signal processing units, and provides tabulated output of the numerical values of selected haemodynamic and respiratory variables. It can also provide an 'in-depth' ECG analysis of a single lead.

The MI^2 unit contains a 'scroller', which functions as a storage oscilloscope for high-resolution monitoring of signals. It can archive data in digital form, either in excerpted portions onto the computer's disc, or continuously onto a high capacity digital storage device. Data collected by the MI^2 can be transferred to other computers for further processing, and files have successfully been re-processed using the widely available statistics package known as RS/1 (BBN Software Products).

The operation of the MI^2 system is controlled by a computer programme in the form of a 'spreadsheet'. We have evaluated the performance of the equipment using a spreadsheet we have prepared for analysis of experiments designed for the determination of ventricular fibrillation threshold in open-chested, pentobarbitone anaesthetised dogs.

Ventricular fibrillation threshold was determined at 20 minute intervals during the course of each experiment using a programmable stimulator described by Butcher *et al.* (1980). The values of haemodynamic variables recorded at fixed intervals between determinations were tabulated by the MI^2 device, and subsequently compared with values recorded simultaneously on a traditional chart-recorder and measured manually.

Correlations between manual and automatic measurements were generally good, and it is concluded that the device could effect a substantial saving in time and effort for the analysis of cardiovascular data.

Butcher, R.A., Dodds, M.G. & Twissell, D.J. (1980). *Br. J. Pharmac.* 73, 316-317P

302P COMPUTER PROGRAMME INCLUDING ANIMATED SEQUENCES TO ILLUSTRATE THE PRINCIPLES OF MECHANISMS BY WHICH DRUGS MAY ALTER INTESTINAL PROPULSION

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This computer programme was designed to supplement a practical class for second year pre-clinical medical students in which the effects of various autonomic and gastrointestinal drugs on a rat isolated colon preparation are demonstrated. In the "live" experiment longitudinal muscle responses and flow through the colon are recorded and circular muscle activity is observed and noted. As part of their assignment, students are asked to consider the ways in which drugs, by influencing circular or longitudinal muscle activity, can alter propulsion through the colon; the animated sequences of the computer programme illustrate the principles involved.

The programme, run on Apple IIe PCs, occupies most students for 10-15min. There are two main phases of the programme. In the first, the apparatus is depicted diagrammatically and by stages the various parts are labelled and their functions are explained. In the second, the effects of certain substances (0.9% NaCl solution, acetylcholine in moderate and high doses and adrenaline) are in turn simulated in animated sequence, then explained in a page of text. An example of the explanatory text, in this case for the intraluminal injection of a moderate dose of acetylcholine (ACh 200 μg in 0.2ml flushed in by 0.1ml of 0.9% NaCl solution), is as follows:

"ACh diffuses across the mucosa to reach the circular and longitudinal muscle layers which contract due to activation of muscarinic receptors. The sequence of contraction is coordinated by the myenteric plexus to cause propulsion of luminal contents. The ACh might also reach sufficiently high concs. to stimulate nicotinic receptors within the plexus and this could contribute to the enhanced response to the reflex test. The action of ACh is rapidly terminated by cholinesterases present in the tissue."

We thank Professor J.B.E. Baker for his advice and encouragement during the development of this programme.

COMMUNICATIONS

The author who intended to present the work
is indicated by an asterisk (*)

1P **Drieu La Rochelle C, Riou B, Berdeaux A* & Giudicelli JF**
K^+ channel openers dilate both large and small coronary arteries in conscious dogs

2P **Duty S* & Weston AH**
Analysis of the mechanisms involved in the contractile response to cromakalim in isolated strips of rabbit aorta under Ca^{2+}-free conditions

3P **Longmore J*, Bray KM & Weston AH**
The relationship between the effects of cromakalim and diazoxide on K^+ and Rb^+ efflux, membrane potential and tension in bovine tracheal smooth muscle

4P **Lawson A***
Differential effècts of ouabain on K^+-induced contractions of guinea-pig tracheal and aortic smooth muscle

5P **Wickenden AD* & Grant TL**
2-deoxy-D-glucose, an inhibitor of glycolysis, inhibits cromakalim but not sodium nitroprusside induced vasorelaxation

6P **Quast U*, Blarer S, Manley PW, Cook NS, Pally C & Fozard JR**
The cardiovascular effects of SDZ PCO 400, a novel K^+ channel opener

7P **Fozard JR*, Menninger K, Cook NS, Blarer S & Quast U**
The cardiovascular effects of SDZ PCO 400 *in vivo*: comparison with cromakalim

8P **Richer C*, Mulder P & Guidicelli JF**
Interactions between K^+ channel openers and the sympathetic nervous system in pithed spontaneously hypertensive rats

9P **Tomita T & Brading AF***
The effect of cromakalim in guinea-pig stomach cannot be ascribed solely to opening K^+ channels

10P **Downing SJ* & Hollingsworth M**
Antagonism of relaxin by glibenclamide in the uterus of the rat *in vivo*

11P **Kentish JC* & Palmer S**
Evidence that the sensitising action of caffeine on myofibrils is not due to a direct effect on the Ca^{2+} affinity of troponin C

12P **Himmel HM* & Ravens U**
The putative intracellular calcium antagonist TMB-8 nonselectively reduces the membrane conductances for Ca^{2+}, Na^+ and K^+ ions

13P **Ravens U* & Liu G**
Mioflazine and lidoflazine protect atrial muscle against calcium overload damage: a study of ultrastructure, cytochemistry and function

14P **Wainwright CL*, Parratt JH & Van Belle H**
The effects of a nucleoside transport inhibitor, R75231, on ischaemic arrhythmias in anaesthetised pigs

15P **Gwilt M, Milne AA*, Solca AM & King RC**
UK-68,798, a potent and selective class III anti-arrhythmic agent, reduces dispersion of repolarisation in canine hearts *in situ* induced by rapid pacing

16P **Forster C* & Armstrong PW**
Coronary vascular responsiveness to angiotensin in pacing-induced heart failure: effect of enalapril

17P **Carr RD*, Killingback PG, Higgs L, Mitchell PD, O'Connor SE, Robson A & Wells E**
Differences in tissue ACE-inhibitory profile between FPL 63547 and enalapril after chronic dosing to spontaneously hypertensive rats

18P **Gristwood RW*, Llenas J, Bou J, Puig C & Berga P**
Flosequinan (BTS 49465) behaves as an inhibitor of phosphodiesterase type III

19P **Vegh A, Szekeres L & Parratt JR***
Preconditioning of the ischaemic myocardium: role of the cyclooxygenase pathway

20P **Hugtenberg J*, Belsner K, Moses A, Scheufler E & Wilffert B**
Effect of nifedipine, mioflazine and dipyridamole on interstitial adenine nucleotide catabolites during global ischaemia in guinea-pig hearts

21P **Nakamura M & Bakhle YS***
Nafazatrom and Ph CL 28A inhibit prostaglandin E_2 catabolism and prostanoid synthesis in rat isolated heart

22P **Kirkman E*, Marshall HW, Heyworth J & Little RA**
Ethanol potentiates the reflex bradycardia elicited by stimulating cardiac C-fibre afferents in the anaesthetised dog

23P **Anderson SMP*, Christodoulou MS & Grahame-Smith DG**
The effects of phorbol esters on 5-HT release from rat hippocampal slices after chronic lithium treatment

24P **Hunter JC*, Leighton GE, Horwell DC, Rees DC & Hughes J**
CI-977, a novel and selective agonist for the kappa opioid receptor

25P **Sleight AJ* & Peroutka SJ**
Effect of central and peripheral injections of sumatriptan on extracellular levels of 5-HT in guinea-pig frontal cortex

26P **Sizer AR* & Roberts MHT**
5-HT attenuates glutamate-evoked responses of rat entorhinal cortical neurones *in vitro*

27P **Grasby PM*, Sharp T & Grahame-Smith DG**
The effect of gepirone, ipsapirone and buspirone on local cerebral glucose utilisation in the rat

28P **Bull DR*, Sheehan MJ & Hayes AG**
8-OH-DPAT acts at D_2 receptors to inhibit firing rate of substantia nigra zona compacta cells maintained *in vitro*

29P **Manahan-Vaughan D, O'Connor JJ, Rowan MJ* & Anwyl R**
Comparison of the effects of buspirone analogues and 1-(2-pyrimidinyl)-piperazine on synaptic transmission in the rat hippocampus *in vivo*

30P **Dreteler GH***
Cardiorespiratory effects of microinjections of 5-HT_{1A} agonists into the raphé obscurus of the anaesthetised rat

31P **Gartside SE* & Cowen PJ**
Evidence that prolactin and ACTH responses to 5-HTP in the rat are mediated by 5-HT_2 receptors

32P **Kilpatrick GJ*, Butler A & Oxford AW**
m-chloro-phenylbiguanide is a potent high affinity 5-HT_3 receptor agonist

33P **Lummis SCR*, Kilpatrick GJ, Nielsen M & Martin IL**
Molecular target size of 5-HT_3 receptors in N1E115 neuroblastoma cells and brain differ by a factor of two

34P **Rodgers RJ & Shepherd JK***
Stereoselective effect of 5-HT_{1A} ligand, MDL 72832, on non-opioid defeat analgesia in mice

35P **Lawrence JA*, Dawson IM, Olverman HJ, Goodwin GM, Wilson NH & Kelly JS**
Binding characteristics of 8-OH-DPAT analogues suitable for iodination

36P **Villalón C*, Bom AH, Heiligers J, den Boer MO & Saxena PR**
Reduction of porcine carotid arteriovenous shunting by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype

37P **den Boer M*, Villalón C, Heiligers J, Saxena PR & Humphrey PPA**
The craniovascular effects of sumatriptan in the pig

38P **Bouhelal R* & Mir AK**
Evidence that the cardiovascular and the hypoinsulinaemic effects of 8-OH-DPAT are mediated through different mechanisms

39P **Chaouloff F*, Baudrie V & Laude D**
Adrenaline-releasing effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT, buspirone and ipsapirone in the conscious rat

40P **Bishop LA* & Williams BC**
Pharmacological characterisation of the aldosterone response to 5-HT in rat zona glomerulosa cells

41P **Choudry NB, McEwan J*, Lavender E, Williams A & Fuller RW**
The effect of BRL 43694A (granisetron) on capsaicin-induced cough in man

42P **Toothill VJ* & Pearson JD**
Neutrophil-endothelial cell interactions in the acute inflammatory response: detection of novel adhesive ligands

43P **Gouw MAM*, Wilffert B, Wermelskirchen D & van Zwieten PA**
Noradrenaline-induced Ca^{2+} influx in the isolated guinea-pig aorta

44P **Thorin-Trescases N, Oster L, Atkinson J* & Capdeville C**
Cytosolic free calcium and vasoconstriction in the normotensive rat tail artery

45P **John GW*, Fabrègues E & Massingham R**
Calcium antagonists differentially inhibit caffeine-induced contractions in vascular smooth muscle

46P **Kraneveld AD* & Trevethick MA**
12(s)-hydroxyeicosatetraenoic acid induced relaxation of dog isolated coronary artery

47P **Guc MP*, Furman BL, Gray GA & Parratt JR**
Endotoxin depresses vascular reactivity to vasodilators in the pithed rat

48P **Muller F, Janlan P, Bray L, Atkinson J*, Scalbert E, Devissaguet M & Capdeville C**
Effect of perindopril on cerebral blood flow in awake renovascular hypertensive and normotensive rats

49P **Feng Q*, Hedner T, Hedner J & Pettersson A**
Clonidine restores the renal response to atrial natriuretic peptide in rats with chronic ischaemic heart failure

50P **D'Souza GA*, Caldwell J, Smith RL, Horner M & Moss MS**
The effect of frusemide on the metabolism and detectability of theophylline in the horse

51P **Fox AJ* & Morton IKM**
Comparison of opioid inhibition of 5-HT_3 and NK-3 evoked release of [^{3}H]-ACh from the guinea-pig ileum myenteric plexus

52P **Boeckxstaens GE*, Pelckmans PA, Ruytjens IF, Rampart M, Herman AG & Van Maercke YM**
$GABA_A$-receptor mediated non-adrenergic non-cholinergic relaxations in the canine ileocolonic junction

53P **MacDonald A*, Daly CJ, Wilson VG & McGrath JC**
Effects of prejunctional α-adrenoceptor activation on neurotransmission in the rabbit saphenous artery

54P **Wessler I*, Hellwig H & Racké K**
Stimulation-induced overflow of [^{3}H]-phosphorylcholine and [^{3}H]-acetylcholine from the isolated guinea-pig trachea: inhibitory role of the epithelium

55P **Green AC* & Dowdall MJ**
The presynaptic muscarinic autoreceptor of *Torpedo* electric organ exhibits the M_1 subtype pharmacology: evidence from direct binding studies

56P **Eglen RM*, Cornett CM & Whiting RL**
Anomalous pA_2 values for parafluorohexahydrosiladifenidol (pFHHSiD) at tracheal muscarinic receptors

57P **Tschirhart E* & Miller R**
Airway epithelium actively metabolises endothelin and modulates endothelin-induced contraction in guinea-pig trachea

58P **Conroy DM*, Samhoun MN & Piper PJ**
Relaxations of guinea-pig trachea induced by platelet-activating factor are epithelium-dependent and are antagonised by WEB 2086

59P **McKenniff MG, Norman P* & Gardiner PJ**
Bay u3405, a novel thromboxane receptor antagonist: activity on isolated airway tissues

60P **Francis HP*, Greenham S, Patel U, Thompson AM & Gardiner PJ**
Bay u3405 antagonises U46619-induced bronchoconstriction in the anaesthetised guinea-pig

61P **Norel X, Labat C, Gardiner P & Brink C***
An evaluation of the inhibitory activity of Bay u3405 on the U46619 response in human isolated bronchial muscle preparations

62P **Hagan RM*, Ireland SJ, Jordan CC, Beresford IJM, Stephens-Smith ML, Ewan G & Ward P**
GR71251, a novel, potent and highly selective antagonist at neurokinin NK-1 receptors

63P **Ireland SJ*, Hagan RM, Bailey F, Jordan C & Stephens-Smith ML**
Receptors mediating neurokinin-induced contraction of the guinea-pig isolated trachea

64P **Sanjar S* & Morley J**
The effect of peptides on tracheal mucus velocity (TMV) in the anaesthetised ferret

65P **Imaizumi A*, Lefort J & Vargaftig BB**
Pertussis toxin induces bronchopulmonary hyperreactivity to histamine, 5-HT and antigen in guinea-pigs, but suppresses responses to fMLP

66P **Chapman I*, Mazzoni L & Morley J**
An anomalous effect of salbutamol in sensitised guinea-pigs

67P **Plummer CJ*, Halket J & Phillipson OT**
Identification and distribution of N-methyladrenaline in the central nervous system of the rat

68P **Cheetham SC*, Viggers JA, Slater NA, Luscombe GP, Heal DJ & Buckett WR**
Important methodological considerations in reuptake experiments using [^{3}H]-monoamines

69P **Saura Martí J, Kettler R, Da Prada M & Richards JG***
Selective inhibition of MAO-B in rat brain by Ro 19-6327: correlation with binding *in vivo*

70P **Lavian G, Di Bona G & Finberg JPM***
Inhibition of sympathetic nerve activity by tricyclic and monoamine oxidase-inhibitor antidepressants

71P **Robinson JP* & Kendall DA**
Niguldipine differentiates between α_1 adrenoceptor subtypes linked to inositol phospholipid hydrolysis and potentiation of cyclic AMP formation in rat brain

72P **Shah K, Bradshaw CM & Szabadi E***
Interaction between antidepressants and d-amphetamine on operant behaviour

73P **Tricklebank MD*, Olès RJ & Singh L**
Reversal by inositol of the proconvulsant action of lithium in pilocarpine-treated mice

74P **Heal DJ*, Prow MR & Buckett WR**
Effects of antidepressants including sibutramine HCl and elector-convulsive shock on pre- and post-synaptic α_2-adrenoceptors assessed by clonidine hypoactivity and mydriasis

75P **Smith KM* & Joseph MH**
Contrasting effects of chronic nicotine on tyrosine hydroxylase activity in noradrenergic and dopaminergic neurones in the rat brain

76P **O'Boyle KM* & Waddington JL**
Interaction of CY 208-243 with D_1 dopamine receptors: influence of Gpp(NH)p

77P **Collins P*, Broekkamp CLE, Jenner P & Marsden CD**
Purposeless chewing and facial tremor in rats: an electro-myographical investigation

78P **Ongini E*, Trampus M, Ferri N & Monopoli A**
Blockade of D_1 dopamine receptors by SCH 23390 leads to enhancement of REM sleep in the rat

79P **Turner JD*, Schneider HH, Marten P & Fritz S**
In vivo binding of the reportedly selective D_2 agonist N-0437 is not displaced by dopaminergic ligands

80P **Rose S*, Jenner P & Marsden CD**
Carbidopa pretreatment prevents the alterations in L-DOPA pharmacokinetics after chronic trreatment of rats with L-DOPA plus carbidopa

81P **Dexter DT*, Wells FR, Javoy-Agid F, Agid Y, Marsden CD & Jenner P**
Alterations in brain iron and ferritin levels in multiple system atrophy and Huntington's chorea

82P **Silva P*, Martins MA, Cordeiro R & Vargaftig BB**
Generation in the PAF-injected rat pleural cavity of eosinophilin, a chemoattractant for eosinophils

83P **Hultkvist-Bengtsson U*, Anderson GP & Morley J**
PAF-induced intrathoracic neutrophil accumulation in anaesthetised guinea-pigs

84P **Desquand S*, Ruffié C, Lefort J & Vargaftig BB**
Interference of a novel PAF antagonist compound, PCA 4248, with active anaphylactic shock *in vivo* and hyperresponsiveness to PAF in perfused guinea-pig lungs

85P **Cunningham FM*, Foster AP, Andrews M & Lees P**
WEB 2086 inhibits PAF-induced platelet aggregation and plasma protein extravasation in the horse

86P **Foster AP*, Cunningham F & Lees P**
The inflammatory effects of PAF in equine skin

87P **Smither RL* & Fan T-PD**
PAF antagonists inhibit angiogenesis in a rat sponge model

88P **Safrany ST*, Wojcikiewicz RJH, McBain J, Cooke AM, Potter BVL & Nahorski SR**
Evaluation of calcium-mobilising properties of phosphorothioate-containing analogues of inositol (1,4,5) trisphosphate in SH-SY5Y human neuroblastoma cells

89P **Alexander SPH*, Hill SJ & Kendall DA**
$[Ca^{2+}]$ elevations differentially affect receptor-stimulated inositol phosphates accumulation in mouse and rat cortex

90P **Gibbons SJ* & Roberts PJ**
2-amino-4-phosphonobutyrate (AP4) has non-specific stimulatory effects on guinea-pig brain phosphoinositide turnover

91P **Owen PJ* & Boarder MR**
Evidence that bradykinin stimulation of phosphatidic acid production in adrenal chromaffin cell cultures may be by direct activation of phospholipase D

92P **Nunn DL* & Taylor CW**
Properties of inositol (1,4,5) trisphosphate receptors in liver and cerebellum

93P **Ney P* & Schrör K**
Stimulus-dependent inhibition of human polymorphonuclear leukoctye (PMN) activation by the PGD_2 mimetic ZK 110.841

94P **Buckley TL*, Brain SD & Williams TJ**
Oedema formation induced by IL-1 *in vivo*: interactions with the neuropeptide, calcitonin gene-related peptide (CGRP) in rabbit skin

95P **Priestley T*, Horne AL, McKernan R & Kemp JA**
Glycine site antagonists protect against hypoxia-induced injury to rat cortical cell cultures

96P **Bagetta G*, Nistico G & Bowery NG**
Evidence that the hippocampal damage produced by tetanus toxin can be prevented by the NMDA receptor antagonist, MK 801

97P **Kerwin R*, Meldrum BS & Patel S**
The glutamate receptor system in schizophrenic hippocampal formation: a post-mortem receptor autoradiographic study

98P **Rogers H, Birch PJ & Hayes AG***
The gerbil hippocampal slice: an *in vitro* model of ischaemia?

99P **Warner TD**
A new method to compare the responses of the arterial and venous vessels within the isolated perfused superior mesenteric vascular bed of the rat

100P **D'Orleans-Juste P*, Wood EG, Mitchell JA & Vane JR**
Endothelin, EDRF and prostacyclin are released from bovine venous as well as arterial endothelial cells

101P **Mitchell JA*, Hecker M, Katsura M, Änggård E & Vane JR**
Relationship between L-arginine concentration and EDRF release in bovine aortic endothelial cells

102P **Buchan K* & Martin W**
Bradykinin-induced elevations of cytosolic calcium in bovine aortic endothelial cells: role of intracellular and extracellular calcium

103P **Gardiner SM*, Compton AM, Bennett T, Palmer RMJ & Moncada S**
Haemodynamic effects of prolonged infusions of N^G-monomethyl-L-arginine (L-NMMA) in conscious rats

104P **Gray DW* & Marshall I**
Calcitonin gene-related peptide (CGRP) endothelium-dependent relaxation in rat aorta is inhibited by L-NMMA

105P **Nunez DJ*, Taylor EA, Schofield JP, Oh VMS & Brown MJ**
Endothelin-1 mRNA in the rat kidney

106P **De Meyer GRY*, Bult H, Van Hoydonck A-E & Herman AG**
Early atherosclerosis induced by a perivascular collar impairs muscarinic endothelium-dependent relaxation in the rabbit carotid artery

107P **Gray GA*, Fleming I, Julou-Schaeffer G, Parratt JR & Stoclet J-C**
N^G monomethyl-L-arginine restores pressor responses to noradrenaline in endotoxin-treated rats

108P **Cattell V, Cook HT* & Moncada S**
Glomeruli synthesise nitrite in experimental nephrotoxic nephritis

109P **Cairns HS*, Rogerson ME, Neild GH & Westwick J**
Effect of endothelium-dependent and -independent vasodilators on renal function in the anaesthetised rabbit

110P **Schröder H*, Barann M & Schror K**
Vasopressin increases cyclic GMP in LLC-PK_1 kidney epithelial cells *via* the L-arginine/nitric oxide pathway

111P **Hutcheson IR*, Whittle BJR & Boughton-Smith NK**
Endotoxin-induced intestinal damage in the rat is enhanced by L-NMMA, an inhibitor of nitric oxide formation

112P **Pike NB*, Watts IS & Lumley P**
Effects of guanylate cyclase activation and type I phosphodiesterase inhibition upon human platelet aggregation

113P **Armstrong RA*, Lumley P & Humphrey PPA**
Reduction in the number of thromboxane P-receptors (B_{max}) on human platelets after exposure to GR 32191

114P **Davenport AP*, Nunez DJ & Brown MJ**
Comparison of binding sites for [^{125}I]-endothelin-1, 2, 3, big endothelin, sarafotoxin and mouse vasoactive intestinal conttractor in rat, pig and human tissues

115P **van Tits LJH*, Grosse-Wilde H & Brodde O-E**
Effect of chronic β-adrenoceptor-antagonist treatment on isoprenaline-evoked changes in function and subset composition of circulating mononuclear leukocytes

116P **Deighton NM*, Motomura S, Sohlmann W, Zerkowski H-R & Brodde O-E**
β-adrenoceptor-mediated inotropic effects of dopamine and epinine in human isolated right atrium

117P **Böhm M*, Gierschik P, Jakobs KH, Schnabel P & Erdmann E**
Differential alterations of adenylate cyclase regulation by G_i in human dilated and ischaemic cardiomyopathy

118P **Sun X*, Dahlöf C & Hedner T**
Does endothelin 1 or 3 facilitate pressor responses mediated by postjunctional α-adrenoceptors?

119P **McLaughlin DP* & MacDonald A**
'Atypical' β-adrenoceptors in the rat gastric fundus

120P **Nials AT*, Butchers PR, Coleman RA, Johnson M & Vardey CJ**
Salmeterol and formoterol: are they both long-acting β_2-adrenoceptor agonists?

121P **Coleman RA*, Johnson M, Nials AT & Sumner MJ**
Salmeterol, but not formoterol, persists at β_2-adrenoceptors *in vitro*

122P **Forster C*, Wanes D & Armstrong PW**
Vascular contractions to isoprenaline are mediated by an α_2-adrenoceptor

123P **Clark R, Spedding M* & Macfarlane CB**
RS-15385-197, a potent and selective α_2-adrenoceptor antagonist

124P **Docherty JR***
Peripheral actions of pertussis toxin pretreatment in the rat

125P **Nixon GF*, Hamilton CA & Reid JL**
Inositol phosphate formation in developing and established perinephritis hypertension in the rabbit

126P **Norman J-C*, Gurney AM & Gillham B**
Type-2 glucocorticoid receptors differentially regulate the release of adrenocorticotrophin variants from anterior pituitary cells

127P **Davenport AP*, Ashby MJ, Easton P, Ella S, Bedford J, Dickerson C, Nunez DJ, Capper SJ & Brown MJ.**
Endothelin-like immunoreactivity in human plasma: development of a radioimmunoassay and comparison of levels in hypertension

128P **Ashforth EIL*, Chenery RJ & Houston JB**
Prediction of *in vivo* disposition from hepatic microsomal and hepatocyte data

129P **Huston E* & Dolphin AC**
The effect of the isomers of 202-791 and the $GABA_B$ agonist baclofen on glutamate release from depolarised cultured rat cerebellar neurones

130P **Ebenezer IS* & Baldwin BA**
Does pharmacological stimulation of central $GABA_A$ receptors stimulate feeding in satiated pigs?

131P **Picardo A, Gent JP & Feely M***
Anticonvulsant tolerance and recovery during repeated administration of the GABA uptake inhibitor NO-328

132P **Morgan GP*, & Simmonds MA**
The peripheral-type site for benzodiazepines may be associated with the uptake carrier for adenosine

133P **Piot O*, Bardone MC, Zundel JL, Stutzmann JM, Boireau A & Doble A**
RP 59037, a novel partial agonist at the GABA-benzodiazepine receptor: binding and behavioural studies in the rodent

134P **Laurie DJ* & Pratt JA**
Local cerebral glucose utilisation during spontaneous and "flumazenil-precipitated" withdrawal from diazepam

135P **Lee C* & Rodgers RJ**
Influence of diazepam on the analgesic effects of elevated plus-maze exposure in mice

136P **Marshall FH*, Pinnock RD & Hughes J**
Characterisation of cholecystokinin octapeptide stimulated endogenous dopamine release from rat nucleus accumbens *in vitro*

137P **O'Neill MF, Kitchener SJ, Dourish CT* & Iversen SD**
The CCK antagonists devazepide and L-365,260 promote weight gain in weanling rats

138P **Hendrie CA* & Dourish CT**
Anxiolytic profile of the cholecystokinin antagonist devazepide in mice

139P **Hendrie CA* & Dourish CT**
Evidence for the involvement of CCK-A receptors in the mediation of opioid social conflict analgesia in mice

140P **Whitham EM*, Challiss RAJ & Nahorski SR**
Muscarinic receptor mediated phosphoinositide metabolism in cerebellar granule cells

141P **Sebastiao AM* & Ribeiro JA**
Xanthine affinities for the adenosine receptor mediating inhibition of transmission at the rat hippocampus

142P **Barnes NM, Costall B, Horovitz ZP, Naylor RJ & Williams TJ***
Characterisation of [^{3}H]-SQ29,852 recognition sites in rodent brain

143P **Lambert DG* & Nahorski SR**
Effect of pertussis toxin on carbachol-stimulated rises in $[Ca^{2+}]_i$ and inositol phosphate accumulation in SH-SY5Y neuroblastoma cells

144P **Wojcikiewicz*, Safrany ST, Challiss RAJ, Strupich J & Nahorski SR**
Intracellular stores of Ca^{2+} in electrically permeabilised SH-SY5Y cells are discharged following muscarinic receptor and G-protein activation

145P **Keen M*, Kelly E & MacDermot J**
Guanine nucleotide sensitivity of [^{3}H]-iloprost binding sites

146P **Antunes E, Cicala C, Cirino G* & de Nucci G**
Polycations induce rat hind-paw oedema

147P **O'Neill LAJ*, Bird TA & Saklatvala J**
Interleukin-1 activates a G-protein in EL4.NOB1 murine thyoma cells: a possible signal transduction mechanism

148P **Cruwys SC* & Pettipher ER**
Interaction between interleukin 1 and the fibrinolytic system in the degradation of collagen by rabbit chondrocytes

149P **Ford AJ*, Green DJ & Longridge DJ**
Effects of dosing regimen of tissue plasminogen activator on the extent and dynamics of fibrinolysis

150P **Peers SH***
5-HT and bradykinin pleurisy in the rat is insensitive to dexamethasone pretreatment

151P **McLeod JD* & Piper PJ**
The effects of endothelin-1 on guinea-pig isolated working hearts

152P **Le Monnier de Gouville A-C*, Thiry C, Gaudibert A & Cavero I**
Hypotension produced by endothelins undergoes crossed tachyphylaxis in both anaesthetised and conscious rats

153P **Lopez-Belmonte J & Whittle BJR***
Inhibition of endothelin-1-induced rat gastric damage by calcitonin gene-related peptide

154P **Gardiner SM, Compton AM & Bennett T***
Effects of indomethacin on regional haemodynamic responses to endothelins and sarafotoxin-S6b in conscious rats

155P **Barnes JC* & Roberts FF**
Effects of muscarinic antagonists on arecoline-induced hippocampal theta rhythm and hypertension in the anaesthetised rat

156P **Lange KW*, Wells FR, Rossor MN, Jenner P & Marsden CD**
Cortical and hippocampal muscarinic receptors are differentially affected in Alzheimer's disease and Parkinson's disease

POSTER COMMUNICATIONS

157P **Lees GM**
Coping with rapid changes in the rate of submissions to the British Journal of Pharmacology

158P **Leff P, Harper D, Dainty IA & Dougall IG**
A test to detect possible errors in agonist affinity estimation

159P **Jones JA, Owen PJ & Boarder MR**
Influence of phorbol ester and other protein kinase C-related drugs on agonist-stimulated inositol phosphate accumulation in cultures of adrenal chromaffin cells

160P **Moore HD & Cunningham FM**
Mediators of primary irritant dermatitis in miniature swine

161P **Seed MP & Cheung CY**
Sodium aurothiomalate inhibits recombinant human interleukin-1β-induced degradation of rat femoral head articular cartilage *in vitro*

162P **Redford EJ & Dascombe MJ**
Comparative study of the pyrogenicities of interleukin 1β and interleukin 6 in conscious rats

163P **Dent G, Giembycz MA & Barnes PJ**
Inhibition of eosinophil oxygen radical production by type IV- but not type III-selective cAMP phospho diesterase inhibitors

164P **Forder RA, Sudlow AW, Stone MA, Haworth D & Carey F**
Glucocorticoid induction of the anti-phospholipase protein, lipocortin I, in the rat

165P **Michel MC, Polizzi S & Motulsky HJ**
Inhibition of lymphocyte proliferation and activation by ketotifen and cyclosporin A

166P **Caulfield MP, Barlow RB & Brown DA**
Pharmacological studies of pre- and post-synaptic neuronal nicotinic receptors

167P **Hambleton P & McMahon S**
Site-dependent differences in inflammatory oedema formation in the rat

168P **Olufsen AS & Hicks R**
Myofibroblast-like contracture of fibrotic rat lung-strip preparations provoked by oxidising agents

169P **Wilson G & Hillier K**
Phospholipid fatty acid profiles in human colon mucosa

170P **Campbell AM, Holgate ST & Robinson C**
Characterisation of cysteinyl leukotriene release in human lung mast cells

171P **Krane A, Keen M & MacDermot J**
Characterisation of a putative photoaffinity label of the prostacyclin receptor

172P **Melarange R, Blower PR, Gentry C & O'Connell C**
Nabumetone, an effective anti-inflammatory agent, lacks the gastric irritancy potential of piroxicam or ibuprofen

173P **Storch J, Edwards RJ & MacDermot J**
f-Met-Leu-Phe or platelet activating factor, but not IgE-antigen complexes, release TXB_2 from differentiated U937 monocyte cells

174P **Lagente V, Dugas B, Bolchot E, Picquot S, Capron M, Mencia-Huerta JM & Braquet P**
Increased expression of FcεRII/CCD23 on rat alveolar macrophages by PAF and antigen

175P **Ortega MP, Fernandez-Gallardo S, Garcia MC, Fau de Casa-Juana M, Priego JG, Sunkel C & Sanchez Crespo M**
1,4-dihydropyridines, a new class of PAF receptor antagonists

176P **Patel MK, Schachter M & Sever PS**
Preliminary pharmacological characterisation of cultured human prostatic stromal cells

177P **Pietra C, Ziviani L, Ceserani R, Gaviraghi G, Hagan RM & Jordan CC**
Effects of selective neurokinin agonists on rat bladder motility *in vitro* and *in vivo*

178P **El-Mas M & Hughes IE**
Influence of cocaine on electrically evoked [^{3}H]-NA overflow from noradrenergic nerves: tissue dependent effect

179P **Iravani MM, Samtaney N & Zar MA**
Pharmacological actions of neuropeptide Y (NPY) on human myometrium *in vitro*

180P **Clayton JK, Marshall K, Sangha R, Senior J & Gardiner PJ**
Inhibition of the spontaneous activity of myometrial strips *in vitro* by prostaglandin E analogues and isoprenaline

181P **Luheshi GN, Webb RJ & Zar MA**
Presence of an atropine resistant component in the motor transmission of the isolated human detrusor muscle

182P **Hill JM, Bunce KT & Humphrey PPA**
Investigation of the neuronal "non-5-HT_3" receptor mediating contraction of guinea-pig ileum

183P **Burleigh DE**
Regional sensitivity of human colon to neurohumoral agents

184P **Peachey JA & Kitchen I**
Comparison of endothelin responses in rat and guinea-pig trachea and their modulation by peptidase inhibitors

185P **Thorne JR & Broadley KJ**
Examination of the effect of adenosine on superfused trachea from normal and sensitised guinea-pigs

186P **Ray NJ, Jones AJ & Keen P**
Ruthenium red: selective, reversible inhibition of capsaicin-stimulated substance P release from primary afferent neurons in rat trachea

187P **Spina D & Page CP**
Lack of an effect of peptidases on vascular responses to epithelium-derived inhibitory factor

188P **Schmidt J & Szelenyi I**
Inhibition of guinea-pig alveolar macrophage superoxide anion generation by azelastine

189P **Herbert CA & Robinson C**
Comparative regional distribution of lipid mediator metabolisng enzymes in bovine lung

190P **Touvay C, Vilain B, Lejeune V, Carré C, Soulard C, Menica-Huerta JM & Braquet P**
Hypereosinophilia and bronchial reactivity in the rat

191P **Yeadon M, Wilkinson D & Payne AN**
Ozone induces bronchial hyperreactivity to inhaled substance P by functional inhibition of enkephalinase

192P **Drewett IJ & Rodger IW**
Assessment of bronchodilator effect of rolipram in the anaesthetised cat

193P **Foster RW & Rakshi K**
Bronchodilator potency, effectiveness and time course of inhaled nebulised AH 21-132 in normal human subjects

194P **Lewis PE, Growcott JW & Wilson C**
Potassium channel blockade: effects on cromakalim-induced relaxation of the guinea-pig tracheal chain

195P **Johns EJ & Palmer RE**
Effect of cromakalim on regional blood flow through the kidney

196P **Benton DC & Haylett DG**
Cromakalim increases the membrane permeability of frog skeletal muscle *in vitro*

197P **Morris BJ, Wisden W, Hunt SP & Barnard EA**
Localisation of mRNAs encoding subunits of the nicotinic acetylcholine receptor to distinct cell groups in the rat spinal cord

198P **Allerton-Ross G, Clapp LH & Gurney AM**
Direct relaxation of rabbit pulmonary arterial strips by flash photolysis of caged cyclic GMP

199P **MacLean MR & Hiley CR**
Effect of neuropeptide Y on the mesenteric arterial bed of the pithed rat: effect of low ventilation volume

200P **Ritter JM, Doktor HS & Stratton P**
Effect of bradykinin B_1 receptor selective peptides on thymidine uptake by rabbit coeliac artery smooth muscle cells

201P **Leathard HL, Eccles NK & Yeats DA**
Rhythmical contractility *in vitro* of human uterine blood vessel circular muscle strips

202P **Giles H, Bolofo ML & Leff P**
Rabbit jugular vein contains three different relaxant prostaglandin receptors

203P **Van Heiningen PNM, Batink HD & Van Zwieten PA**
Angiotensin II-induced increases in $^{45}Ca^{2+}$ uptake and contractile responses in guinea-pig aorta

204P **Blue DR & Clarke DE**
Characterisation and modulation of vascular α-adrenoceptor responses in rat kidney

205P **Beckeringh JJ & Korstanje C**
Enantiomers of niguldipine differentially inhibit α_1-adrenoceptor-mediated hydrolysis of phosphoinositides and contraction in rat and guinea-pig vascular tissue

206P **Korbut R, Lidbury PS, Thiemermann C & Vane JR**
Nitrovasodilators modulate blood fibrinolytic activity

207P **Zreika M & Pelton JT**
Radioimmunoassay for endothelin-1: antibody cross-reactivity with endothelins, some analogues of endothelin-1 and sarafotoxin S6b

208P **Huggins JP, Topouzis S & Miller RC**
Rat aortic rings denuded of endothelium are more sensitive to sodium nitroprusside after precontraction by sarafotoxin S6b than by endothelin-1

209P **Gardiner SM, Compton AM, Kemp PA & Bennett T**
Effects of endothelin-1 and sarafotoxin S6b on cardiac output in conscious rats

210P **Moody CJ, Dashwood M, Sykes R, Chester M, Jones SM, Yacoub M & Harding SE**
Endothelin receptors on human and rat cardiac myocytes: a comparison with isolated smooth muscle cells

211P **Moser PC**
Effect of endothelin-1 on pupil diameter following injection into the anterior pretectal nucleus of the rat

212P **Martin de Aguilera E, Vila JM, Irurzun A, Cruz Martinez M, Flor B & Lluch S**
Endothelin-induced contraction of human omental arteries

213P **Clapham JC, Hamilton TC & Trail BK**
Comparison of the relaxant profiles of BRL 38227 and dazodipine in rat isolated aorta precontracted by endothelin

214P **Clarkson PN, Growcott JW & Cox B**
Lack of effect with ICI 170809 at the 5-HT_3 receptor in the isolated rabbit heart

215P **Craig DA & Clarke DE**
5-methoxytryptamine and 2-methyl-5-hydroxytryptamine as discriminative tools for excitatory neuronal receptors (5-HT_3 and novel site) in guinea-pig ileum

216P **Eglen RM, Swank SR, Dubuque LK & Whiting RL**
Characterisation of 5-HT receptors mediating contractions of guinea-pig ileum, *in vitro*

217P **Growcott JW, Cox B, Clarkson PN & Blackburn TP**
ICI 170809: apparent antagonist affinity differences for 5-HT between rabbit aorta, rat caudal artery and pig coronary artery

218P **Clark AH & Garland CJ**
5-HT stimulates diacylglycerol production in rabbit isolated cerebral arteries

219P **Racké K, Bähring A & Wessler I**
Effects of 5-HT, metitepine and ketanserin on the release of endogenous noradrenaline (NA) from the rat trachea *in vitro*

220P **Plane F, Bruckdorfer KR & Jacobs M**
The effect of LDL on the contractile response to 5-HT in rabbit aorta

221P **MacLennan SJ & Martin GR**
Comparison of the effects of methysergide and methylergometrine with GR43175 on feline carotid blood flow distribution

222P **Ramos EG, Esplugues JV, Gil L & Esplugues J**
Involvement of capsaicin-sensitive afferent neurons in the acid secretory responses of the rat stomach

223P **Canfield P & Harbord M**
Effect of luminal NH_3 on alkaline secretion by guinea-pig stomach *in vitro*

224P **Sharma SC**
Oestradiol increases histamine levels and acid content of mouse stomach

225P **Canfield P & Gillen D**
Colloidal bismuth subcitrate and arachidonic acid protect guinea-pig isolated gastric cells from damage by ammonia

226P **Baxter GF, Jones KB & Sturman G**
An *in vitro* investigation of the pepsin inhibitory properties of several bismuth compounds

227P **Correia-de-Sá P, Fougo JL, Sebastiao AM & Ribeiro JA**
Evidence that phrenic nerve endings possess inhibitory and excitatory adenosine receptors

228P **Monopoli A, Forlani A, Ongini E, Lagrasta C, Ricci R & Olivetti G**
Effects of long-term administration of dilevalol and pindolol on blood pressure and myocardial hypertrophy in young spontaneously hypertensive rats

229P **Heijnis JB, Mathy M-J & Van Zwieten PA**
Effects of various calcium antagonists and of calcium ions in isolated hearts of diabetic and normal rats

230P **King RC, Milne AA, Packman F, Gwilt M & Higgins AJ**
Estimation of synchrony of cardiac repolarisation by simultaneous on-line acquisition of 31 local electrocardiograms from the dog heart *in situ* using a microcomputer

231P **Tsuchihashi K & Curtis MJ**
Tedisamil possesses direct defibrillatory activity during myocardial ischaemia and during reperfusion

232P **Tamargo J, López-Sendón J, Delpón E, González MA & De Miguel E**
Cardiovascular effects of elgodipine, a new dihydropyridine derivative

233P **Delpón E, Tamargo J, Valenzuela C & Delgado C**
Effects of the combination of mexiletine and flecainide in guinea-pig papillary muscles

234P **Spiers JP, Harron DWG, Wilson R & Allen JD**
Effect of α_1-adrenoceptor modulation and UK-52,046 on atrio-ventricular conduction in the anaesthetised dog

235P **Wall FA**
Comparative effects of atracurium and lignocaine at the rat neuromuscular junction

236P **Wootton R & Hazelwood R**
8-OH-DPAT labels two sites in the raphé nucleus area of rat brains

237P **Lawrence KM, De Paermentier F, Cheetham SC, Crompton MR, Katona CLE & Horton RW**
Symmetrical hemispheric distribution of brain [^{3}H]-paroxetine binding sites in post-mortem samples from depressed suicides and controls

238P **Van der Hoek GA & Cooper SJ**
Microstructural analysis of the effects of the selective 5-HT_3 antagonist, ondansetron, on feeding and other behavioural responses

239P **Wang H & Grahame-Smith DG**
Effects of rubidium on 5-HT-mediated behaviour in rat and mouse

240P **Sarna GS, Whitton P, Curzon G & Leathwood P**
Effect of 5-HT_1 agonists on carbohydrate/protein dietary selection of rats

241P **Kennett GA, Whitton P & Curzon G**
ID_{50} values of antagonists *versus* mCPP-induced hypophagia and 5-HT_2-mediated head shakes indicate 5-HT_{1C} sites mediate the hypophagia in rats

242P **Redfern PH & Singh A**
Inhibition of 5-HT_{1B} autoreceptors by methiothepin does not reveal a circadian variation in autoreceptor sensitivity in the cerebral cortex of the rat

243P **Leathley M, Taylor MAI & Goudie AJ**
Effects of the 5-HT_3 antagonist GR38032F on benzodiazepine withdrawal in rats

244P **Higgins GA & Elliott PJ**
Differences in open-field behaviour of rats after injection of 5-HT_{1A} selective compounds into dorsal and median raphé nuclei

245P **Mitchell EA, Pratt JA & Balfour DJK**
Aversive environmental stimuli do not evoke behavioural responses to GR38032F following chronic treatment and withdrawal

246P **Murray TK, Hunter AJ, De Souza RJ & Cross AJ**
Manipulation of central 5-HT function disrupts performance of a delayed-non-match-to-sample task in rats

247P **Rudd JA & Naylor RJ**
Preliminary assesment of feverfew (*Tanacetum Parthenium*) to antagonise cisplatin-induced emesis in the ferret

248P **Moser PC**
The effect of novel anxiolytics on the head-twitch response in mice

249P **Costall B, Kelly ME & Tomkins DM**
An automated dark/light box to assess anxiolytic potential

250P **Pearson HA, Lees G & Wray DW**
Lack of effect of avermectin on calcium channels in muscle fibres from moth larvae

251P **Ekwuru MO & Cunningham JR**
Phaclofen increases GABA release from valproate treated rats

252P **Bagust J, Gardner CR, Walker RJ & Ward RA**
Studies on the efficacy of low affinity benzodiazepine receptor ligand on rat cerebellar slice activity

253P **Colquhoun L, Holden-Dye L & Walker RJ**
The pharmacology of cholinoceptors on the somatic muscle cells of the parasitic nematode *Ascaris suum*

254P **File SE & Toth E**
Characterisation of spatial learning in a helical maze in the absence of intra- or extra-maze clues

255P **Perrault G, Morel E, Sanger DJ & Zivkovic B**
Comparison of the pharmacological profiles of four hypnotics with different chemical structures: quazepam, brotizolam, zopiclone and zolpidem

256P **Eigenhuis JJ, Leeuwin P, Mantel P & Van Wilgenburg H**
Pre- and post-synaptic effects of midazolam on respiratory skeletal muscle

257P **Zharkovsky A**
Flumazenil suppresses, and CGS8216 enhances, physical signs of diazepam withdrawal in rats'

258P **Trout SJ, O'Connor JJ, Wieczorek W & Kruk ZL**
Regional differences in evoked striatal dopamine overflow measured using fast cyclic voltammetry in brain slices

259P **Benwell MEM & Balfour DJK**
The effects of nicotine and amphetamine on nucleus accumbens dopamine secretion: a microdialysis study

260P **Crespi F, Baumeyer T, Moblus C & Dittrich J**
Differential square pulse conditioning voltammetry: a new method for voltammetric analysis with micro biosensors

261P **Al-Tajir G, Starr MS, Chandler CJ & Starr BS**
Opposing effects of dopamine D_1 and D_2 receptor stimulation on the propagation of motor seizures in mice and rats

262P **Close SP & Elliott PJ**
Haloperidol-induced bradykinesia in the marmoset

263P **Harley EA, Post JA & Woodruff GN**
Predicting activity at the D-2 dopamine receptor using the [^{3}H]-sulpiride/[^{3}H]-NO437 ratio

264P **Heal DJ, Frankland ATJ, Gosden J, Hutchins LJ, Luscombe GP & Buckett WR**
Effects on central dopaminergic function are not involved in the pharmacological actions of the novel antidepressant sibutramine HCl

265P **Berrino L, Hering B, Marmo E and Racké K**
Effects of L-glutamate (L-GLU) on the release of endogenous noradrenaline (NA) from the rat supraoptic nucleus (SON) *in vitro*

266P **Mitchell SN, Brazell MP & Gray JA**
Nicotine-stimulated noradrenaline release in the hippocampus of freely moving rats

267P **Martin KF, Phillips I, Cheetham SC & Buckett WR**
Sodium dependent binding of [^{3}H]-raclopride to rat striatal membranes

268P **Payvandi N & Elliott JM**
Treatment with DSP-4 selectively increases β_1-adrenoceptor number in rat cerebral cortex

269P **Mclaughlin M, Ross BM, Knowler JT, Milligan G & McCulloch J**
Immunoblot analysis of the α subunit of G proteins in control and Alzheimer diseased brains

270P **Beats B & Kerwin RW**
An autoradiographic study of forskolin binding in human hippocampus and its alterations in schizophrenia

271P **De Paermentier F, Cheetham SC, Crompton MR, Katona CLE & Horton RW**
Sub-cortical β-adrenoceptor binding sites in post-mortem brain samples from depressed suicide victims

272P **Brown CM, Clague RU, Kilpatrick AT, MacKinnon A, Martin AB & Spedding M**
Effects of RS15385-197 in *in vivo* preparations

273P **Robinson JP & Kendall DA**
Effect of corticosterone and steroid antagonist RU38486 on noradrenaline-stimulated cyclic AMP in rat cortex and hippocampus slices

274P **Dickinson SL, Gadie B, Hayne MJ, Lane AC, Walter DS & Waltham K**
Neurochemical effects of idazoxan given continuously by osmotic minipump in the rat

275P **Freeman P & Sturman G**
Are histamine-H_2 antagonists proconvulsant?

276P **Whittington MA & Little HJ**
Actions of Bay K 8644 stereoisomers on the changes in hippocampal field potential during ethanol withdrawal

277P **Butcher SP, Bullock R, Graham DI & McCulloch J**
Release of neuroexcitatory amino acids from rat brain following middle cerebral artery occlusion

278P **Sturgess NC, Peters JA, Hales TG & Lambert JJ**
The actions of alphaxalone and pentobarbitone on inhibitory and excitatory amino acid receptors

279P **Martin D, Morrisett RA, Swartzwelder HS & Wilson WA**
Acute effects of ethanol on rat hippocampal excitatory amino acid receptors

280P **Halliwell RF, Lambert JJ & Peters JA**
Modulation of NMDA-induced currents recorded from rat hippocampal neurones by 1-hydroxy-3-aminopyrrolidone-2 (HA-966)

281P **Robertson C, Robinson TN, Cross AJ & Green AR**
The effects of polyamines on the binding of [^{3}H]-MK-801 to rat cortical membranes

282P **Smith MB & Dolin SJ**
Propofol anaesthesia is associated with seizure-like behaviour

283P **Harrison SM, Rogers H, Hayes AG & Birch PJ**
Anoxia- and hypoglycaemia-induced neurodegeneration in neonatal rat hippocampal slices

284P **Roberts PJ, Crawford M & Strike C**
Glycine - a putative co-transmitter at glutamate-operated synapses: uptake and release by rat striatal slices

285P **Fernandez L & Birch PJ**
Rotenone- and iodoacetate-induced depolarisation of neonatal rat spinal cord: an *in vitro* model of ischaemia?

286P **Olés RJ, Singh L & Tricklebank MD**
Differential effects on the behavioural and anticonvulsant properties of MK-801 following repeated administration in the mouse

287P **Sundaram S & Dayam AD**
The role of cholecystokinin in raw soya flour-induced trophic lesions in rat pancreas

288P **Nakamura-Craig M & Gill BK**
Possible involvement of neurokinin-A, substance P and calcitonin gene-related peptide in peripheral hyperalgesia in the rat

289P **Follenfant RL, Nakamura-Craig M & Garland LG**
Sustained hyperalgesia in rats evoked by 15-hydroperoxyeicosatetraenoic acid is attenuated by the protein kinase inhibitor H-7

290P **Jackson HC, Ripley TL & Nutt DJ**
Behavioural profile of the δ-opioid receptor antagonist naltrindole in mice

291P **Kosterlitz HW & Paterson SJ**
D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 is a highly selective μ-ligand with low *in vitro* antagonist activity

292P **Balasubramanian MK, Boess F, Brammer MJ & Campbell IC**
Increased formation of diacylglycerol metabolites following muscarinic cholinergic stimulation of synaptosomes

293P **Barnes JM, Barnes NM, Costall B, Horovitz ZP, Ironside JW, Naylor RJ & Williams TJ**
The inhibition of acetylcholine release from human temporal cortex by angiotensin II

294P **Besser R, Vogt T & Wessler I**
Repetitive firing of the isolated rat phrenic nerve-hemidiaphragm preparation: effects of high calcium and (+)-tubocurarine

295P **Doods HN**
In vivo cardioselectivity of the anti-muscarinic drug AQ-RA 741

296P **Milton KA, Ward SA & Edwards G**
Lack of effect of the antimalarial agent halofantrine on hepatic drug metabolism

297P **Xiang J-Z, Morton J, Brammer MJ & Campbell IC**
Synaptosomal studies of the regulation of total and free calcium concentrations

298P **Brunton VG, Russell SN, Grant MH & Wallace HM**
Spermine toxicity in normal and transformed baby hamster kidney cells in culture

299P **King AT, Mulligan BJ & Lowe KC**
Comparison of surfactant-mediated changes in microbial membrane permeability

300P **Armstrong FH, Shaw ST & Lowe KC**
Effects of emulsified perfluorochemicals on rat liver aryl esterase activity

DEMONSTRATIONS

301P **Gardner CJ & Twissell DJ**
Use of a commercially available signal processing centre for 'on-line' analysis of haemodynamic data

302P **Leathard HL & Higman M**
Computer programme including animated sequences to illustrate the principles of mechanisms by which drugs may alter intestinal propulsion

British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

Institute of Education

(United Medical and Dental Schools of
Guy's and St Thomas's Hospitals)

3rd–5th January 1990