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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.

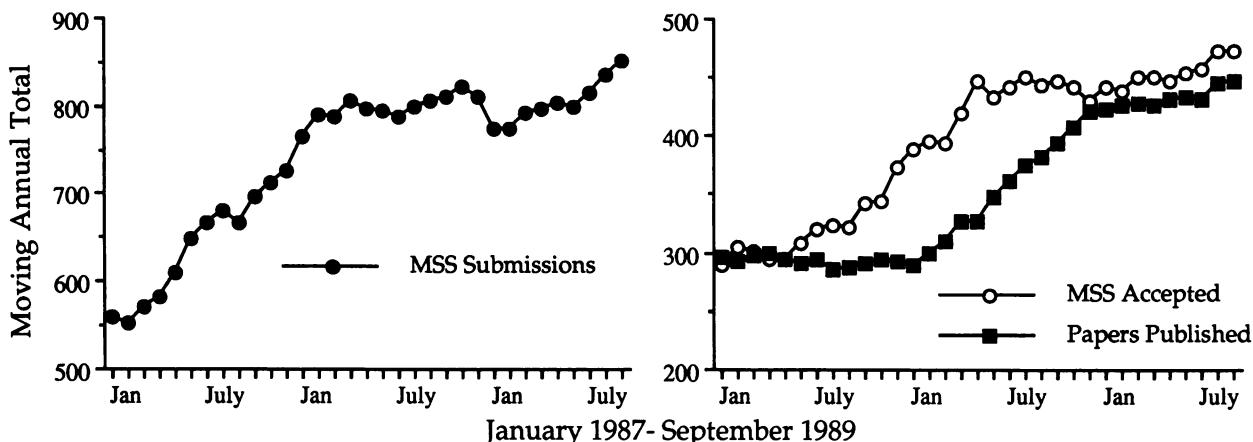


Figure 1. Numbers of submissions, acceptances and publications in consecutive twelve-month periods.

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 (Furchtgott, 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 (3×10^{-7} - 1×10^{-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 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 [³H]inositol at 1 μ Ci/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, preceded where appropriate by a 10 minute preincubation. Phorbol ester and related drugs were present both during the preincubation and incubation periods. The presence of 1 μ M 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 1 μ M, 4934±401; histamine 10 μ M, 26546±734; histamine 10 μ M plus TPA 1 μ M, 5494±637; (figures are d.p.m. of [³H]-IP, n=4±S.E.M.). This TPA effect could be seen at 10nM and was mimicked by mezerein (1 μ M) 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 (1 μ M) 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 (50 μ M R 59022 from Janssen and 10 μ M 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 H₁ 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-1 equivalents/mg wet weight of tissue respectively (n = 6).

Changes in CBF following topical application of 50 μ l 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: $\frac{CBF + \text{irritant (mV)}}{CBF + \text{vehicle (mV)}}$
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-1 β -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-1 β (rhIL-1 β) induced collagenase release by rabbit chondrocytes *in vitro* (Hunneyball et al., 1988). Other gold compounds but not ATM inhibit IL-1 α induced glycosaminoglycan (GAG) loss from bovine nasal septum (BNS) *in vitro* (Rainsford, 1989). We report the action of ATM on rhIL-1 β induced GAG loss and synthesis inhibition in rat femoral articular cartilage (RFC) *in vitro* and compared this to thiomolate (TM) and d-penicillamine (d-PEN).

RPC were cultured as previously described (Clay et al., 1989) in antibiotic supplemented DMEM with and without rhIL-1 β (3-1000ng/ml), or rhIL-1 β with drug for 5 days. Cartilages were pulsed with 0.25 uCi [35S]04 16h before washing and papain digestion. Media and cartilages were assayed for GAG content (Farndale et al., 1986) and counted by liquid scintillation. rhIL-1 β 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-1 β concentration-response curves at 100uM, 30uM and 10uM. An example of ATM action with or without rhIL-1 β 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-1 β (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-1 β , (*p<0.05, ***p<0.001) and rhIL-1 β to basal (+p<0.05, +++p<0.001 ANOVA RS-1, BBN Software).

	without rhIL-1 β GAG(ug/mg)	[35S]04(cpm)	with rhIL-1 β GAG(ug/mg)	[35S]04(cpm)
Basal	3.47 +/- 0.15	3826 +/- 117	3.61 +/- 0.12	4091 +/- 155
rhIL-1 β	-	-	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-1 β stimulated GAG loss and reversed rhIL-1 β inhibition of GAG synthesis in RFC *in vitro*. This is gold dependent as both thiomolate 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-1 β was kindly supplied by Roussel UCLAF, Romainville, France.

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162P COMPARATIVE STUDY OF THE PYROGENICITIES OF INTERLEUKIN 1 β AND INTERLEUKIN 6 IN CONSCIOUS RATS

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Interleukin 1 β (IL-1 β) causes fever in rats following both peripheral and central administration (Dascombe et al, 1989). IL-1 β 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-1 β and IL-6 on body temperature in rats were compared to determine whether IL-6 could mediate the febrile response to IL-1 β .

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-1 β (DuPont, 5 μ g/kg, n=6) caused an increase in colonic temperature from 90 min after injection until 3h (thermal response index for 3h (TRI₃) saline -1.06 +/- s.e. mean 0.29 °Ch, IL-1 β 0.49 +/- 0.42 °Ch, P<0.05). Human IL-6 (ICI Pharmaceuticals, 5 and 25 μ g/kg, n=6) had no effect on colonic temperature following i.v. administration. I.c.v. IL-1 β (10ng in 2 μ l, n=6) caused a rise in body temperature 45 min after injection which lasted the study period (TRI₃ water 2.44 +/- 0.22 °Ch, IL-1 β 4.57 +/- 0.50 °Ch, P<0.05). IL-6 (100ng in 2 μ l, n=4) also caused an increase in colonic temperature (TRI₃ 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 (100 μ g in 4 μ l i.c.v. 15 min before cytokine) attenuated the response to 100ng IL-6 (P<0.05, n=6).

These results confirm that IL-1 β 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-1 β . 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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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 β -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 β_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 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free HEPES-buffered Krebs-Ringer bicarbonate buffer, pH 7.4 (KRB). Cells ($10^6/\text{assay}$) 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 μg 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 inhibitor, rolipram and denbufylline, for 2 min caused concentration-dependent inhibitions of OZ-stimulated H_2O_2 generation with maximal inhibitions of $71 \pm 5.5\%$ (mean \pm sem, n=3) and $67 \pm 3.7\%$ (n=4), and EC₅₀ 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 β_2 -agonist, salbutamol, also caused a concentration-dependent inhibition with maximal inhibition of $38 \pm 1.5\%$ and EC₅₀ 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 $64 \pm 7.9\%$ and the EC₅₀ 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 A₂ 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 \pm 1.4% with 3.0mgkg⁻¹, 16.4 \pm 1.8% with 0.1mgkg⁻¹; mean \pm s.e.m., n=6). Similar observations were seen in the total wet weight of the pituitary (21.4 \pm 3.1% and 14.6 \pm 3.5%), adrenal (50.0 \pm 3.1% and 33.9 \pm 4.5%), lung (49.2 \pm 2.3% and 33.4 \pm 4.4%) and spleen (64.5 \pm 1.5% and 46.7 \pm 2.4%) respectively. Dexamethasone induction of lipocortin 1 expression was observed in lung (327 \pm 9% with 3.0mgkg⁻¹, 88 \pm 25% with 0.1mgkg⁻¹; n=5) and adrenal tissue 83 \pm 46% and 45 \pm 20% respectively). Conversely, suppression was observed in spleen tissue (87 \pm 1% with 3.0mgkg⁻¹, 40 \pm 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 [³H]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 inhibitory 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 $\mu\text{g}/\text{ml}$) 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 $\mu\text{g}/\text{ml}$ 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-stimulated 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% CO_2 , containing 1 μM 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	Agonist	pre-synaptic	post-synaptic
Pempidine	0.08 ± 0.035 (4)	0.45 ± 0.25(4)	m-OH Ph(CH ₂) ₃ N ⁺ Me ₃	1.8 ± 0.5 (3)	5.6 ± 3.2 (3)
Mecamylamine	0.32	(2)	coryneine	10.7	(2)
Pentolinium	0.63	(2)	leptodactyline	9.5	(2)
Ph(CH ₂) ₄ NEt ₂	11.3 ± 0.4 (3)	7.4 ± 1.4 (3)	nicotine mono me-I	27	(2)
Ph(CH ₂) ₄ N ⁺ Et ₃	1.5	(2)	DMPP	29.9 ± 12.1(3)	23.4 ± 4.3 (3)
*agonist = 3 μM m-OH Ph(CH ₂) ₃ N ⁺ Me ₃			β -pyridyl CH ₂ N ⁺ Me ₃	31	(2)
			hordenine	50	(2)
			β -pyridyl(CH ₂) ₃ N ⁺ Me ₃	56.5	(2)
					25

We conclude that these compounds provide no evidence for a distinction between the pre- and post-synaptic receptors in the rat superior cervical ganglion.

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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 immuno-inflammatory 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 O₂/CO₂ 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 x 10⁻⁶ to 2 x 10⁻⁴ M) and to hydrogen peroxide (1 x 10⁻⁴ to 1 x 10⁻³ M) but not to histamine, 5-hydroxytryptamine or acetylcholine, in concentrations up to 1 x 10⁻³ M. Control strips did not respond to any agonist. Fibrotic lung preparations also responded to sodium chromate and sodium tungstate (both 1 x 10⁻⁵ to 1 x 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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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 \pm SD on 13 mucosal specimens. PC was the predominant PL present (19.3 ± 12.2 nmoles 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 $P<0.002$ cf with other FA; b $P<0.01$ cf with PC and SM; c $P<0.01$ cf with SM and PE; d $P<0.02$ 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.

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170P CHARACTERISATION OF CYSTEINYLM 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 $50 \mu\text{g ml}^{-1}$ goat anti-human IgE. Histamine release was measured using a radioenzymatic assay, prostaglandin D₂ by RIA and cysteinyl LTs by RIA after separation by HPLC. Mast cells at a final purity of $63 \pm 5.5\%$ ($n=3$) released a net $36.6 \pm 2.6\%$ histamine together with 33.9 ± 3.9 , 5.6 ± 0.5 and 6.5 ± 0.7 ng per 10^6 mast cells of PGD₂, LTC₄ and LTD₄/LTE₄ respectively, these being similar to values obtained in challenged unpurified cells containing $7 \pm 1\%$ mast cells.

In order to investigate the possible interaction between mast cells and other cell types, $250\mu\text{l}$ aliquots of fractions containing the highest purities of mast cells were added to $250\mu\text{l}$ 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 (LTC₄, LTD₄ and LTE₄) 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 LTC₄ 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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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 EC₅₀ of 2 nM, and produces the same maximal response as iloprost. ZK119817 displaces [3 H]-iloprost binding to platelet membranes with an IC₅₀ 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 of [3 H]-iloprost; this appeared to be due to a decrease in the [3 H]-iloprost B_{max} (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 a lipophilic ligand.

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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 ID₂₅ 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 PGE₂ content, gastric mucosal 6-keto-PGF_{1α} 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 PGE₂ 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_{1α} 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 TXB₂ FROM DIFFERENTIATED U937 MONOCYTE CELLS

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Human lung macrophages release thromboxane B₂ (TXB₂) 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 TXB₂ and several lysosomal 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 TXB₂ in response to 1 μ M fMLP, 0.1 μ M PAF or 2 μ M 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 (γ IF). The release of TXB₂ under these conditions was rapid, transient and complete within 5 min. In these cells PMA also triggered TXB₂ 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 γ IF induces expression of CD23/FcE-R11, and using an anti-CD23 monoclonal antibody we have confirmed similar induction by HTB5637 conditioned medium. However, no TXB₂ 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 TXB₂ 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 TXB₂. The competence of these cells to respond to PAF or fMLP appears independent from their capacity to express receptors for IgE since IL4 and γ IF induce expression of CD23/FcE-RII, but inhibited release of TXB₂, whether triggered by fMLP, PAF or PMA.

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174P INCREASED EXPRESSION OF FC ϵ RII/CD23 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, Fc ϵ RII/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 Fc ϵ RII/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 μ g/ml) or lyso-PAF (500 μ g/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 μ M). The expression of Fc ϵ RII/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 Fc ϵ RII/CD23 on AM from non-sensitized BN rats, challenged or not with OA, was observed. In contrast, a maximum of 74 % of AM expressed Fc ϵ RII/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. Pretreatment of BN rats with BN 52021 markedly reduced (-82 %) the expression of Fc ϵ RII/CD23 on AM induced by OA. Aerosol administration of PAF (500 μ g/ml) to SD rats induced after 24 h the expression of Fc ϵ RII/CD23 on 79 % of the AM. This is to be compared to the 20 % cells expressing of Fc ϵ RII/CD23 after aerosol administration lyso-PAF (500 μ g/ml). *In vitro*, PAF induced a concentration-and time-dependent increase of Fc ϵ RII/CD23 expression on AM from SD rats, which was maximum at 1 μ M and after 24 h. In contrast, lyso-PAF was inactive. These results demonstrate that administration of the antigen in sensitized BN rats induces Fc ϵ RII/CD23 expression on AM, in a process inhibited by BN 52021. The fact that PAF induces Fc ϵ RII/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-Stenzel 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 α₁-adrenoceptors on prostatic stromal cells (James et al. 1989) and α₁-antagonists may be therapeutically useful in BPH. We have isolated and grown stromal cells from the human prostate in order to examine intracellular calcium, [Ca²⁺]_i, responses, partly to establish whether functional α₁-receptors are present on these cultured cells.

For measurement of [Ca²⁺]_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 10⁵ cells/ml with an external calcium concentration of 1mM. Fluorescence measurements were performed in a PTI Deltascan dual wavelength spectrofluorimeter and [Ca²⁺]_i calculated according to Grynkiewicz et al. (1985). Elevation of [Ca²⁺]_i was observed under these conditions in response to a variety of agonists. Large changes in [Ca²⁺]_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 α₁-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 EC₅₀ values (nM) ± s.e.mean or 95% confidence limits for neurokinin-induced bladder contraction.

Agonist	<i>In Vitro</i>	<i>In Vivo</i>	
		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 [³H]-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 [³H]-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 [³H]-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.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 [³H]-NA stimulation through ring electrodes of the bottom 1/5 of the vas which was not incubated with [³H]-NA evoked a fractional overflow of $0.76\pm0.17\times10^{-3}$ whereas, when the whole vas was incubated with [³H]-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 [³H]-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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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% O₂, 5% CO₂ 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 myometria; 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 x 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 ISOPRENALEINE

J.K. Clayton, K. Marshall, R Sangha, J. Senior & P.J. Gardiner¹, School of Pharmacology, University of Bradford, Bradford BD7 1DP and ¹Bayer U.K. Research Department, Stoke Court, Stoke Poges, Slough SL2 4LY. We have previously reported that butaprost, a selective EP₂ 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 PGE₁ 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 (Masseele and Senior 1981) and were superfused with Krebs solution (37°C, 95% O₂/5% CO₂) 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 10⁻⁶M. Isoprenaline in doses greater than 5 x 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 EP₂ 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 TR4752 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 E₂ caused maximal inhibition of activity, after initial contraction, of 15 ± 10 mins duration (2 x 10⁻⁸ m non-pregnant tissue; 6 x 10⁻⁸ m tissue from pregnant donors). In summary, the rank order of potency of the uterine spasmolytics tested TR4752 > butaprost > prostaglandin E₂ > isoprenaline. The advantage of butaprost and TR4752 over existing tocolytic therapy would be the lack of adrenoceptor stimulant activity; the specificity for the EP₂ receptor avoids the stimulant effect of PGE₂ 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 isometrically in 1 ml organ baths between two platinum electrodes in Krebs-Henseleit solution containing 10 μ M indomethacin at 37°C and bubbled with 95% O₂ + 5% CO₂ 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 μ M, then to physostigmine 1 μ M and finally to tetrodotoxin 0.5 μ M. 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 μ M. In Protocol 1 experiments: The lowest concentration of atropine that produced a maximal inhibition ranged from 0.5-1 μ M. Atropine at no concentration fully blocked EFS-evoked responses (% maximum inhibition by atropine Mean \pm SEM = 72 \pm 3.5). The atropine-resistant responses were not potentiated by 15 min exposure to physostigmine but were readily abolished by tetrodotoxin, 0.5 μ M. 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-HT₃" 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 1 μ M), whereas the response to high concentrations of 5-HT involved 5-HT₃ 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%O₂/5%CO₂ and containing ondansetron (GR38032F; 10 μ M), spiperone(1 μ M) and eserine(0.01 μ M). 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-HT₁-like, 5-HT₂ and 5-HT₃ receptors, 5-HT produced monophasic contractile responses with an EC₅₀ value of 50(11-210)nM (95% confidence limits,n=4). This response was completely blocked by tetrodotoxin(0.3 μ M) and atropine(1 μ M) 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 10 μ M
α -Methyl-5-HT	95(20-432)	80±16	Metoclopramide	-	25±12 at 30 μ M

In addition, the following compounds were inactive both as agonists and antagonists up to a concentration of 100 μ M; 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 pD₂' values of 5.0 and 7.2 respectively. The responses to dimethylphenylpiperazine(4 μ M) were not inhibited by high concentrations of metoclopramide or cisapride (10 μ M and 1 μ M respectively). In summary, the present study confirms the presence of a 5-HT receptor in guinea-pig ileum which cannot be designated 5-HT₁-like, 5-HT₂ or 5-HT₃, and which can be stimulated by various benzamides.

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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 μ M). Potency comparisons were made by calculating mean ED₅₀ 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 μ M) 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 (ED₅₀, μ M) 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, ED₅₀ 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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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% O₂, 5% CO₂ and isometric contractions were recorded via a FT03 transducer linked to a Grass polygraph. Cumulative dose response curves to carbachol (0.1 - 100 μ M) and ET-1 (0.01 - 10 μ M) 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 EC₅₀s in both upper and lower portions of 1-2 μ M, (ii) flat dose response curves reaching a plateau at 1 μ M 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 0.01 μ M ET-1 >50% of the carbachol response. Prior incubation of the tissues (30 min) with bestatin, phosphoramidon, captopril or thiorphan (10 μ M) 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 10 μ M	EC50 (nM)	Tension (g) induced by 100 μ M
RAT	Upper	315 ± 69	0.20 ± 0.049	728 ± 76
	Lower	269 ± 67	0.26 ± 0.064	643 ± 110
GUINEA-PIG	Upper	975 ± 286	0.53 ± 0.069	555 ± 67
	Lower	1813 ± 713	0.41 ± 0.068	607 ± 85

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% CO₂ 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 ip. 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 (EC₅₀ 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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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% O₂/5% CO₂) 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 C₁₈ 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), veratrididine (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 (Ca²⁺) 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 (IC₅₀ = 0.67 μM). Under identical conditions, RR did not significantly affect SP-LI release to 60 mM K⁺, 25 μM Ver or 1 μM BK.

We conclude that RR does not inhibit transmembrane Ca²⁺ 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% O₂ and 5% CO₂ 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 (·O₂) by guinea pig AM after stimulation. The AM were stimulated by either the allsurface receptor-independent, soluble phorbol ester phorbol myristate acetate (PMA) or by the allsurface receptor-dependent particle zymosan. ·O₂-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 cultured 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 ·O₂-generation after PMA-stimulation but not after zymosan-stimulation. PMA-induced ·O₂-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 ·O₂-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 ·O₂-generation. Furthermore we demonstrated that azelastine reduces the ·O₂-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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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_{2α} as substrate for 15-hydroxyprostaglandin dehydrogenase (PGDH; E.C. 1.1.1.141), PGD₂ for 11-ketoreductase (11-KR; E.C. 1.1.1.188) and PGE₂ for 9-ketoreductase (9-KR; E.C. 1.1.1.189). Starting prostanoid substrate concentrations were 2 µg 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 Δ¹³-reductase.

These data show that in contrast to lung parenchyma, the airways mucosa does not exhibit PGDH activity, despite being a source of PGE₂ which is a potential substrate for this enzyme (Herbert & Robinson, unpublished). Interestingly, PGD₂ 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 PGD₂) 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 assessment 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 × 10⁶/µl vs 8.2 ± 0.2 × 10⁶/µl in control rats, p < 0.05), an increase of platelets (1284 ± 81 × 10³/µl vs 1081 ± 42 × 10³/µl, p < 0.05) and blood eosinophils (0.69 ± 0.18 × 10³/µl vs 0.14 ± 0.03 × 10³/µl, 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/mm² vs 28.1 ± 4.3 cells/mm² 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. Despite 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 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 (O_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 O_3 ($3 \pm 0.5\text{ppm}$) 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 artificially 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 - 3000 $\mu\text{g}/\text{ml}$) for 10s and measuring the increase in PIP. The SP concentration provoking a 20cmH₂O rise in PIP (PC₂₀) was determined by interpolation. The SP PC₂₀ ($\mu\text{g}/\text{ml}$) in control animals was 1011 ± 260 (n=7) but after O_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 PC₂₀ produced by 30 min O_3 , suggesting that the BHR to SP induced by O_3 was mediated through oxidant damage.

In a separate set of control animals, threshold airway responses were obtained using a 10s nebulisation of SP (100 $\mu\text{g}/\text{ml}$). The enkephalinase inhibitors (EI) phosphoramidon, thiorphphan 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 $40.3 \pm 1.1\text{cmH}_2\text{O}$ ($p<0.001$, n=4) by EI. However, following O_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 O_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 $418 \pm 37\%$ (mean \pm s.e.mean, n=6), from a resting value of $4.0 \pm 0.5 \text{ cm H}_2\text{O ml}^{-1} \text{ s}^{-1}$, and a decrease in C_{dyn} of $28 \pm 5\%$ from a resting value of $8.8 \pm 0.9 \text{ ml cm H}_2\text{O}^{-1}$. 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 $52 \pm 3\%$ (n=5) reversal of the 5-HT-induced increase in R_{aw} , and a $24 \pm 8\%$ (n=5) reversal of the decrease in C_{dyn} . Onset of bronchodilation 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 \pm 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 ($sGaw$, 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 $\log_{10} sGaw = -0.717 \pm 0.013$. The loading dose of methacholine (2.1 [1.7, 2.7 mean - s.e.m., mean + s.e.m.] mg) reduced $\log_{10} sGaw$ to -1.136 ± 0.020 (62% reduction in baseline $sGaw$) and in the no drug control experiment the maintenance dose rate of 0.025 ± 0.002 of the loading dose min^{-1} produced a $\log_{10} sGaw$ of -1.179 {-1.158, -1.120 95% C.L.} (66% reduction in baseline $sGaw$) with a slope of $0.000357 \pm 0.000275 min^{-1}$ ($R^2 0.02$). 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 $t_{1/2}$ of the drug at its site of action after inhalation ≈ 25 min.

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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% O₂/5% CO₂) 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 (0.1-100μM) and compared to the maximal relaxation produced by isoprenaline (1μM). The effects of glibenclamide (GLIB 1-10μM), procaine (PRO 0.1-10mM), tetraethylammonium (TEA 0.1-10mM), apamin (APA 0.1μM) and phentolamine (PHEN 10μM) were assessed against the relaxation produced by CROM.

CROM produced concentration-related relaxation of the tissue (EC₅₀ 2.73 \pm 0.52μM) and was antagonised by GLIB and PHEN (concentration-ratios 8.89 \pm 2.36 & 22.33 \pm 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 5μM produced graded relaxation of the tissues. This effect was insensitive to either nitrendipine or propranolol (0.5 & 1μM respectively). PRO, TEA (0.1mM) & APA (0.1μM) 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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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.

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196P CROMAKALIM INCREASES THE MEMBRANE PERMEABILITY OF FROG SKELETAL MUSCLE *IN VITRO*

D.C.Benton and D.G.Haylett, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT. 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 ⁸⁶Rb⁺ (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 ⁸⁶Rb⁺ efflux. With 100µM the increase was 88±12% (means.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 IC₅₀ between 10 and 30 nM. (Diazoxide which increases the opening of ATP-sensitive K⁺ channels in pancreatic β-cells, had no clear effect on ⁸⁶Rb⁺ 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.

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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 $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\beta 2$ 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 $\alpha 2$ -subunit probe gave a hybridisation signal only in small ($<12\mu\text{m}$) and medium-sized (12-20 μm) cells in the ventral horn. The $\alpha 3$ -subunit produced a signal in laminae II, IV and X, while some motoneurones also contained the $\alpha 3$ transcript. The $\alpha 4$ transcript was found in many cells in all laminae, as was the $\beta 2$ transcript. The majority of motoneurones were found to contain the $\alpha 4$ and $\beta 2$ 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.

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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 ($>1\text{ mM}$) 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, α -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 μM) or by raising the extracellular K^+ concentration (20-100 mM). Isometric tension was measured in strips 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 μM), which inhibits cyclase activation. When caged cGMP (1-100 μM) 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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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 respiration 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* O₂ (mmHg): 83.3 ± 2.4 to 60.1 ± 2.9) and hypercapnia (*P* CO₂ (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⁻¹min g (saline treated group) to 366 ± 57 mmHg ml⁻¹min 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 B₁ 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 B₁ 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% CO₂/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 ³H-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 ³H-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 ³H-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 Arg¹⁰-kallidin, a selective B₁ 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 Arg¹⁰-kallidin (10⁻⁹-10⁻⁷ M) were antagonised > 50% by a selective B₁ antagonist [Leu⁹]-des Arg¹⁰-kallidin (5 × 10⁻⁷ M). Higher concentrations of des Arg¹⁰-kallidin (10⁻⁶-10⁻⁴ M) caused smaller effects than lower concentrations and were not significantly antagonised by [Leu⁹]-des Arg¹⁰-kallidin, 5 × 10⁻⁷ M. We conclude that kinins stimulate proliferation of rabbit coeliac artery smooth muscle cells, and that B₁ receptor activation is particularly effective in this regard.

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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% CO₂ in O₂ (pH 7.4). Amplitude was calculated as a percentage of the maximum noradrenaline (NA, 3x10⁻⁶M)-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 (2.7x10⁻⁶M), or by tetrodotoxin (3x10⁻⁷M), (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 (Keatinge, 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 EP₂-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 EP₂-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 EC₅₀ ± 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)

PGE₁(0.03nM-3μM) and 6-keto-PGE₁(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 (?EP₂) which is not present in significant concentrations on HWP, and the second phase through the IP-receptor. PGE₂ (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 EP₁ 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 EP₁ or FP-receptors.

RJV contains IP-, DP, ?EP₂ 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 $^{45}\text{Ca}^{2+}$ 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 $^{45}\text{Ca}^{++}$ uptake in intact vessels, using different compounds which interfere with either Ca^{++} influx or intracellular processes.

For the measurement of slowly exchanging $^{45}\text{Ca}^{++}$ (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 $^{45}\text{Ca}^{++}$ -containing K-H solution (1 $\mu\text{Ci}/\text{ml}$) 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 $^{45}\text{Ca}^{++}$ -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 $^{45}\text{Ca}^{++}$ uptake in the lower part of the guinea-pig aorta. In the upper parts A II elicited a concentration-dependent increase in slowly exchanging $^{45}\text{Ca}^{++}$ 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 $^{45}\text{Ca}^{++}$ uptake. CoCl_2 (3×10^{-4} M), TMB-8 (10^{-4} M) and chlorpromazine (10^{-4} M) also entirely inhibited the increase in slowly exchanging $^{45}\text{Ca}^{++}$. Basal $^{45}\text{Ca}^{++}$ 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 alpha₂-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 pA₂ 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 alpha₂-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 alpha₁-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.

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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 100 μ M 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 \pm S.E.M. (n=3-6). In GPA (+)-niguldipine fully inhibited NA (100 μ M) induced HPI (K_i : 27.1 \pm 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 \pm 0.01 and 3.4 \pm 0.3 nM). In RA the pseudo-Hill coefficient was lower than 1. In RA, similar to darodipine (1 μ M), (+)-niguldipine (1 μ M) non-competitively inhibited contraction by NA and reduced Emax by about 30%. (-)-Niguldipine was less effective than (+)-niguldipine. In GPA and RPH (-)-niguldipine (1 μ M) displaced the CR curve to NA in a parallel manner to the right (K_B : 6.39 \pm 0.11 and " K_B ": 7.78 \pm 0.09). (+)-Niguldipine (1 μ M) inhibited NA induced vasoconstriction in a non-competitive manner and decreased Emax by 49 \pm 4 and 72 \pm 4%, respectively. In GPA and RPH darodipine and nitrendipine lacked inhibitory effect. In RPH niguldipine (1 μ M) 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 inhibitory component. The differential results from radioligand binding and functional studies suggest that a DHP sensitive calcium channel is an integral part of α_{1B} -adrenoceptors. This idea is strongly supported by the complete dependence 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 μ g/kg) or GTN (30 μ g/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 μ g/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 μ g/kg) but not GTN (up to 30 μ g/kg) shortened ECLT at 30 min after administration by 34 \pm 5 % (mean \pm 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 μ g/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.

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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 [$\text{Trp}^6, \text{Leu}^7$]- and [$\text{Thr}^2, \text{Phe}^4, \text{Thr}^5, \text{Tyr}^6, \text{Lys}^7, \text{Tyr}^{14}$]-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, [$\text{Ala}^{1,3,11,15}$]-, [$\text{Ala}^{1,15}$]-, [$\text{Cys}(\text{Acm})^{3,11}$]-ET-1 and sarafotoxin S6b (S6b; [$\text{Lys}^4, \text{Asp}^5, \text{Met}^6, \text{Thr}^7, \text{Leu}^{12}, \text{Gln}^{17}, \text{Val}^{19}$]-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
[$\text{Ala}^{1,3,11,15}$]-ET-1	200	10
[$\text{Ala}^{1,15}$]-ET-1	150	13
[$\text{Cys}(\text{Acm})^{3,11}$]-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 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 \pm 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 EC₅₀ 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 EC₅₀ 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.

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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	$28.65 \pm 3.91^*$	7
ET-1	SNP	$32.69 \pm 4.07^*$	7
Sarafotoxin	SNP	$24.65 \pm 2.24^*$	6

*Significantly different to control (no SNP; $P < 0.05$).

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 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 (μl 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).

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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 [¹²⁵I]-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 contraction, 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 [¹²⁵I]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 [¹²⁵I]endothelin-1 binding sites are the preoptic 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 preoptic nucleus (AP) 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 AP 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).

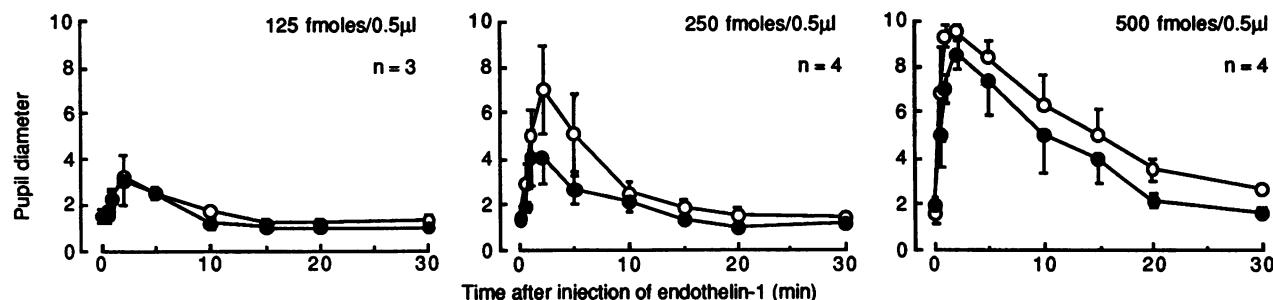


Figure 1. The effect of endothelin-1 injected into the right APT on pupil diameter. Pupil diameter was measured in arbitrary units (2.5 units = approximately 1mm). Left eye (open symbols); right eye (filled symbols). All values are mean ± s.e. mean.

These results demonstrate that small quantities of endothelin-1 injected into the APT, a brain area with a high density of [¹²⁵I]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 AgNO₃ to visualize the endothelium (Caplan and Schwartz, 1973). Functional integrity of the endothelium was demonstrated by the presence of relaxation induced by acetylcholine (10⁻⁶M).

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 EC₅₀ value was 5.4 x 10⁻⁹M. Removal of endothelium did not affect these responses. Removal of extracellular calcium or addition of the calcium antagonist nifedipine (10⁻⁶M) reduced maximal response of endothelin to about 50% without significant changes in EC₅₀.

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.

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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 (0.1 μM), 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; 3 μM) 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 (0.1 μM) elicited a slowly developing and well maintained contraction. In endothelium-denuded RIA, lemakalim (0.3-30 μM) evoked concentration dependent relaxation of ET and NA contractions (IC_{50} 5.3 μM and 4.3 μM 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 1 μM). BRL 38226 had weak relaxant activity (-27±14% at 100 μM) against ET-induced contractions. GLIB (3 μM) 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 (0.1 μM) 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 30 μM) 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-HT₃ RECEPTOR IN THE ISOLATED RABBIT HEART

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We have previously shown that ICI 170809 possesses potent 5-HT₂ 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-HT₁-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-HT₃ 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-HT₃ 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 (10⁻⁷M), ketanserin (10⁻⁷ to 10⁻⁶M), methiothepin (5x 10⁻⁸M), MDL 72222 and GR 38032F (5x 10⁻¹⁰ to 5x 10⁻⁸M). All compounds were allowed a 15 min contact time prior to addition of 5-HT.

MDL 72222 and GR 38032F (5x10⁻⁸M) 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-HT₂ and 5-HT₁ respectively) these compounds had no significant antagonist effects at 5x10⁻⁸M, (MDL 72222 concentration ratio's = 1.15 and 1.52 respectively; GR38032F concentration ratio's = 1.40 and 1.68 respectively). ICI 170809 (1x10⁻⁷M) 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 (pA₂ value = 9.78). Ketanserin (10⁻⁷M) 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 5x10⁻⁸M (concentration ratio = 1.00).

These results demonstrate that ICI 170809 possessed no significant 5-HT₃ 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-HT₃ 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-HT₄ 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-HT₃ 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 manoeuvre was taken from the original observation by Fozard (1985). 5-HT₃-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 (1×10^{-10} to 3×10^{-5} M) or renzapride (BRL 24924; 3×10^{-9} to 3×10^{-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 (1×10^{-5} M) given 1.5 h previously. Tissues were incubated with 5-MOT (1×10^{-5} M) or 2-M-5-HT (1×10^{-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-HT₃ 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-HT₃ 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-HT₄ receptor described by Dumuis *et al.* (1989).

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216P CHARACTERISATION OF 5-HT RECEPTEORS 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-HT₃ 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 μ M methysergide (to antagonise 5-HT₁-like and 5-HT₂ receptors) and 1 μ M GR 38032F (to antagonise 5-HT₃ 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 \pm s.e. mean from 6-10 preparations. Concentration-dependent contractions were observed to 5-HT, 5-methoxytryptamine, BRL 24924, and zacopride (-log EC₅₀ values were 7.5 ± 0.8 , 7.0 ± 0.03 , 7.0 ± 0.08 and 6.3 ± 0.12 , respectively). Zycopride 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 μ M) and no agonist responses were observed with BRL 43694, ICS 205-930 or GR 38032F (1 nM - 10 μ M). The responses to 5-HT, 5-methoxytryptamine and zycopride were abolished in the presence of atropine (1 μ M) or tetrodotoxin (0.1 μ M) and were enhanced in terms of maximal response, but not the potency, in the presence of 0.1 μ M physostigmine. The responses to all three agonists were unaffected in the presence of 1 μ M GR 38032F, BRL 43694, quipazine or n-methylquipazine. ICS 205-930 (3 μ M) antagonised responses to 5-HT, 5-methoxytryptamine and zycopride (-log K_B values = 6.3 ± 0.12 , 6.0 ± 0.09 and 6.5 ± 0.14 , respectively). ICS 205-930 (10 μ M) did not affect responses to carbachol either in terms of potency (control -log EC₅₀ = 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-HT₁-like, 5-HT₂ or 5-HT₃ 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 (Dumuis *et al.*, 1988a,b) and may be a peripheral example of a 5-HT₄ 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-HT₂ 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% CO₂. After obtaining control concentration-response curves to 5-HT, ICI 170809, 1x10⁻⁹-1x10⁻⁷M, 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 pA₂ values.

In the rat caudal artery ICI 170809 produced potent antagonism of the 5-HT-induced contractions, the Schild-plot analysis yielding a pA₂ 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 pA₂ 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 ³²P-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.

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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 known 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 μ M) and, in addition, yohimbine (1 μ M), in order to prevent the autoinhibition of NA release, and tyrosine (10 μ M), 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 carried 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 ± 3.5 pmol/g ($n=53$). In control experiments the ratio S2/S1 was 0.94 ± 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 μ M, respectively. Metitepine (1 μ M) alone had no effect on the release of NA, but shifted the concentration response curve for 5-HT to the right (apparent pA₂-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 μ M, respectively. 0.1 μ M ketanserin, present 40 min before S2, reduced the release of NA by 45 %. When 0.1 μ M 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 by interfering 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.

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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).

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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-HT₁-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-HT₁-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 distension (20 cm H₂O).

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. Distension 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 distension.

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 distension.

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Helicobacter (formerly *Campylobacter*) pyloris infection of the stomach is associated with increased luminal NH₃ concentration and NH₃ has been reported to damage the mucosa of rat stomach in vivo (Murukami et al, 1988). We have examined the effect of luminal NH₃ 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 suppressed by SCH 28080 (50 uM) and the serosal side buffered by HCO₃⁻/CO₂. Measurement of AS is complicated by the alkalinising effect of NH₃ and no attempt was made to measure AS during the 15 min exposure to NH₃. Subsequent measurements were contaminated by NH₃ washout from the tissues so control experiments were performed where the serosal side was buffered with Hepes/O₂ which abolishes AS in this tissue. NH₃ is expressed as % (v/v) dilution of a solution containing 28-30% NH₃ (Aldrich Chemical, Poole). AS is expressed as the change from preceding basal values (Δ AS) in $\mu\text{mole cm}^{-2}\text{h}^{-1}$ and are mean and s.e. mean (n = 5 or 6 for all). A typical basal value for AS was $1.1 \pm 0.21 \mu\text{mole cm}^{-2}\text{h}^{-1}$.

Below 0.35% NH₃ there were no significant differences between HCO₃⁻ and Hepes buffered tissues except for the first 15 min period after washout when HCO₃⁻ was greater. Increasing NH₃ up to 2% lead to a sustained increase in AS of 2-4 $\mu\text{mole cm}^{-2}\text{h}^{-1}$ in HCO₃⁻ tissue whereas in Hepes tissue AS fell to basal levels within 75 min. Hepes tissues treated with 0.75% NH₃ and allowed to recover to basal value were then exposed to HCO₃⁻/CO₂ buffer. There was a rapid increase in AS to values not significantly different from those seen in tissues in HCO₃⁻/CO₂ throughout. Prior exposure to 0.1% NH₃ had no effect on subsequent exposure to 0.75% compared with control tissues exposed only to 0.75% (AS test 2.49 \pm 0.32, control 2.16 \pm 0.43). Luminal exposure to either NaOH or NH₄Cl (1 mM) had no effect on AS.

Luminal exposure for 15 mins to NH₃ above 0.35% in HCO₃⁻/CO₂ buffered tissues lead to a sustained increase in AS. In view of the ability of NH₃ 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 HCO₃⁻ 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. H₁ 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 dstd.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 \pm SEM			
	At 3 h		At 24 h	
	Control	Injected	Control	Injected
5	6.39 \pm 0.81	9.48 \pm 1.17*	7.11 \pm 0.70	11.38 \pm 0.97**
10	7.85 \pm 0.71	11.86 \pm 1.01**	6.47 \pm 0.61	13.39 \pm 1.08**
20	6.14 \pm 0.70	14.03 \pm 1.12**	7.06 \pm 0.85	12.80 \pm 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 persistent even at 24 h after ED injection. This is unlike the rat uterus which 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 NH₃ concentration (Yoshida et al, 1989). We have examined the effect of NH₃ 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 Ca²⁺-Mg²⁺-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 Ca²⁺ and Mg²⁺ (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 10⁷ cell/ml). Average yield per stomach was 1.1 ± 0.11 × 10⁸ 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. NH₃ is expressed as % (v/v) dilution of a solution of NH₄OH containing 28-30% NH₃ (Aldrich Chemical, 16.5M).

Exposing cells to NH₃ 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% NH₃ cell survival was 50% of control and at 1% NH₃ it was 30%. Treating cells with KOH or NH₄Cl at concentrations equivalent to 1% NH₃ gave survival rates of 90% and 68% respectively. 1%NH₃ 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% NH₃. Pretreatment of cells for 30 min, prior to exposure to 0.5% NH₃, 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 NH₃ 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 cyclooxygenase 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 dicitratabismuthate (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 (IC₅₀) 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 Dicitratabismuthate	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 [³H]-acetylcholine ([³H]ACh) release from phrenic nerve endings of the rat was compared in the absence and in the presence of 1,3-dipropyl-8-cyclopentylxanthine (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 A₂ adenosine receptor.

The experiments were performed at 37°C on rat phrenic nerve-hemidiaphragm preparations gassed with 95% O₂ and 5% CO₂. The bathing solution contained (mM): NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 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-[³H])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. [³H]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 [³H]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 (S₁, S₂ and S₃) was of similar magnitude. NECA was usually applied between S₂ and S₃ and its effect was calculated by comparing the S₃/S₂ ratio with the S₂/S₁ ratio. DPCPX and PD 115,199 were applied 15 min before S₁.

NECA (30–300 nM) decreased in a concentration-dependent manner the evoked release of [³H]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 [³H]ACh by 65±7% (n=3). The excitatory effect of NECA (300 nM) on [³H]ACh release was antagonized in a concentration-dependent manner by PD 115,199 (5–25 nM), i.e. NECA (300 nM) increased [³H]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 [³H]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 β-blocking agents with partial agonist activity occurring through the stimulation of β-2 adrenoceptors (D) or of both β-1 and β-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 weekly 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 (mg/g)
	7-Week	19-Week	7-Week	19-Week	
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

* $p < 0.05$; ** $p < 0.01$ 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 (Götzche, 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 \pm 10 vs 260 \pm 8 g), heart weight (1.53 \pm 0.18 vs 1.22 \pm 0.26 g, p<0.05) and glucose levels (5.8 \pm 1.2 vs 22.4 \pm 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 (EC₅₀ 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 \pm 1.7 vs 6.5 \pm 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 $3.3\text{M}\Omega$ and amplifies each signal to a peak amplitude of 1.25V by means of an autogain circuit (acceptable input range is 20 μ V - 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 repolarisation. 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 1 μ M, 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 (3 μ M) 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 tediamil (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 tediamil 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), although the possibility arose that bradycardia may have contributed, since tediamil 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 tediamil (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%. Tediamil 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 tediamil on reperfusion-induced VF incidence (100% vs 100% in paced controls), but an abolition of SVF (from a 42% incidence in controls). Tediamil's effects on reperfusion-induced VF were not dependent on the duration of preceding ischaemia since in hearts reperfused after 30 min ischaemia tediamil 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 tediamil).

In conclusion, tediamil 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} : $4.5 \pm 1.6 \times 10^{-9}$ M) and chronotropic effect ($1.5 \pm 1.7 \times 10^{-8}$ 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 (V_{max}) produced a parallel shortening of the action potential duration and effective refractory period. Elgodipine also inhibited slow contractions (IC_{50} : $5.8 \pm 2.3 \times 10^{-9}$ M) as well as the amplitude and V_{max} 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} : $1.0 \pm 0.4 \times 10^{-9}$ M), slowed the conduction time through the atrio-ventricular node (lengthened the A-H interval) and increased coronary blood flow (ED_{20} : $5.5 \pm 3.3 \times 10^{-10}$ M), while it had no effect on intraatrial or intraventricular conduction times.

In anaesthetized dogs elgodipine (0.1, 1, 10, 50 and 100 μ g/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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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 (34°C). 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 $1.3 \pm 0.5\%$ of tonic Vmax block, F $1.7 \pm 3.5\%$ and the combination $3.4 \pm 1.2\%$ ($P < 0.05$). At 2 Hz the frequency(use)-dependent Vmax block produced by M was $19.8 \pm 2.4\%$ whereas that produced by F was $18.0 \pm 2.2\%$ and in the presence of the combination increased to $27.3 \pm 1.4\%$ ($P < 0.01$). 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 (τ_{off}) 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 τ_{off} 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 ($9.4 \pm 0.6\%$ vs $7.9 \pm 0.7\%$. $P < 0.05$). 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.

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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 μ g/kg), flecainide (0.5, 1, and 2 mg/kg), prazosin (5, 10, 20 and 40 μ g/kg), phenylephrine (continuous infusion, 50 μ g/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 μ g/kg) had no effect on BP or HR, but reduced ($P < 0.05$) BP at doses of 16 and 32 μ g/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 μ g/kg). Prazosin (5-40 μ g/kg) increased HR, but BP fell only after 20-40 μ g/kg. The HV interval was unaltered, but the AH interval fell after 20 μ g/kg during SR and after 40 μ g/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	UK-52,046	Prazosin	Phenylephrine				
	control	final	8 μ g	16 μ g	10 μ g	20 μ g	23 \pm	33 \pm
		dose	/kg	/kg	/kg	mins	mins	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 50 μ g/ml/min

n=6

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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 -antagonist UK-52,046 at antiarrhythmic doses (2-8 μ g/kg; Uprichard et al, 1988), did not significantly affect AH and HV intervals (during SR), BP or HR.

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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 possess 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 $38 \pm 2^\circ\text{C}$ 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 $13 \pm 2.1\%$ and $15 \pm 1.1\%$ of their respective control values). The control value was 2.4 ± 0.1 g tension (mean \pm 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 $26 \pm 1.5\%$, whereas lignocaine reduced it by $58 \pm 2.7\%$. Atracurium reduced the duration by $20 \pm 1.3\%$, whereas lignocaine reduced it by $40 \pm 5.1\%$. 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 RAPHE 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 [³H]-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 [³H]-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
¶-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 [³H]-8-OH-DPAT, 50mM Tris buffer pH 7.4, 0.1% ascorbate and 10 μM 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 pIC₅₀ values for each compound. pKi values are reported as means \pm 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 1 μM 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 [³H]-PAROXETINE BINDING SITES IN POST-MORTEM SAMPLES FROM DEPRESSED SUICIDES AND CONTROLS

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Two recent reports indicate marked differences in the B_{max} of [³H]imipramine (which labels 5-HT uptake sites) binding sites between left and right hemispheres of post-mortem human frontal cortex. In controls B_{max} 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 [³H]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 [³H]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 [³H]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 B_{max} (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. B_{max} of [³H]paroxetine binding (fmol/mg protein, means ± sem)

Hemisphere	Controls		Suicides	
	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 [³H]imipramine. The asymmetry may be related to sites other than 5-HT uptake sites labelled by [³H]imipramine.

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238P MICROSTRUCTURAL ANALYSIS OF THE EFFECTS OF THE SELECTIVE 5-HT₃ 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-HT₃ 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-HT₃ 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 2H₂O) in doses of 0, 3, 10 and 30 µg·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 10 µg·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.

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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-HT₁ 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-HT₁ agonists on food choice

Diet	8-OH-DPAT(s.c)		RU24969 (i.p.)		mCPP (i.p.)	
	60 ug/kg	500ug/kg	2.5 mg/kg	10 mg/kg	2.5 mg/kg	10 mg/kg
0% protein	233 ± 39 P < 0.01	654 ± 155 P < 0.05	63 ± 7 ns	90 ± 3 ns	55 ± 8 ns	77 ± 8
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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Kennett G. A. & Curzon G. (1988). Psychopharmacology 96, 93-100.

Wurtman J.J. & Wurtman R.J. (1979). Life Sci., 24, 895-904.

241P ID₅₀ VALUES OF ANTAGONISTS versus MCPP-INDUCED HYPOPHAGIA AND 5-HT₂-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-HT_{1C} receptors (Kennett and Curzon, 1988). However 5-HT_{1C} 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 LD₅₀ (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-HT_{1C} sites ($r = 0.84$, $P < 0.01$) but not for 5-HT₂ or 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1D} 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-HT_{1C}/5-HT₂ site affinities ($r = 0.95$ $P < 0.001$). The data thus strengthens evidence that mCPP causes hypophagia by activating 5-HT_{1C} sites.

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Schechter, L.E. and Simansky, K.J. (1988). Psychopharmacology 94, 342-346

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 serotonergic neurones (Sanders-Bush 1982). It has been reported that there exists a circadian variation in behavioural responses to stimulation of 5-HT, but not 5-HT₂ 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 μ M tritiated 5-HT and 10 μ M pargyline. After 3x5 ml washes, 50 μ l aliquots of the tissue suspension were superfused for 30 min with Krebs containing 3.2 μ M paroxetine. The superfusion medium was then changed to iso-osmotic modified Krebs solution containing 25 mM potassium and 3.2 μ M 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 μ M). Apparent pA₂ values were calculated as described by Schlicker and Gothert (1981). Results are expressed as means \pm 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 \pm 0.06(5)	6.648 \pm 0.11(5)	6.724 \pm 0.08(5)	6.763 \pm 0.05(4)

Basal ³H release was unchanged by 5-HT (1 μ M) or methiothepin (1 μ M). 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-HT₁ type and suggest that factors other than terminal autoreceptor sensitivity are responsible for the reported circadian variation in release.

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Sanders-Bush, E. (1982) in Serotonin in Biological Psychiatry. ed. Ho, B.T. et al. pp 17-34. NY: Raven Press

243P EFFECTS OF THE 5-HT₃ ANTAGONIST GR38032F ON BENZODIAZEPINE WITHDRAWAL IN RATS

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To investigate the ability of the 5-HT₃ 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 ($d = 0.05$, 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 ($d = 0.01$ 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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244P DIFFERENCES IN OPEN-FIELD BEHAVIOUR OF RATS AFTER INJECTION OF 5-HT_{1A} SELECTIVE COMPOUNDS INTO DORSAL AND MEDIAN RAPHE 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) raphe 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 (0.5 μ l/min) 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 ug	DRN				MRN			
		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 5HT₃ 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.

DAY	Repeated Exposure				Acute Exposure			
	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

Treatment	Delay(s)						5-HT depletion (% of controls)
	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-HT₂/5-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-HT_{1A}/5-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 ASSESSMENT 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-HT₃ 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.

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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 - 2 mg 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 α₂-adrenoceptor antagonist properties of 1-PP might play in the effects of buspirone and ipsapirone was studied in more detail as α₂-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 ED₅₀ of 32 µg kg⁻¹. In the presence of idazoxan (1 mg kg⁻¹) the dose-response curve for clonidine was shifted rightwards (ED₅₀ 178 µg kg⁻¹) whereas no such shift occurred in the presence of buspirone (1 mg kg⁻¹; ED₅₀ 25 µg kg⁻¹), diazepam (4 mg kg⁻¹; ED₅₀ 38 µg kg⁻¹) or 1-PP (1 mg kg⁻¹; ED₅₀ 47 µg kg⁻¹).

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 α₂-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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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 pre-treatment were significant ($P < 0.01$, Student's t test).

Compound	Dose (mg kg ⁻¹)	Number of head-twitches
Diazepam	4	4.7 ± 0.4
Buspirone	1	6.8 ± 1.1
Ipsapirone	8	5.5 ± 1.0
MDL 73005EF	2	6.0 ± 0.5
1-PP	2	4.8 ± 0.9

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 mg/kg i.p.	Time in white (s)	White section		Black section	
		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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Kilfoil, T., Michel, A., Montgomery, D. & Whiting, R.L. (1989) *Neuropharmacology* 28, 901-905

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 B₁ (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 underlie 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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Lees, G., Pearson, H.A. & W.-Wray, D. (1988). *Brit. J. Pharmacol.* 95, 744P.

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 $2.9 \pm 0.37 \mu\text{mol g}^{-1}$ wet wt (mean \pm 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 $4.2 \pm 0.44 \mu\text{mol g}^{-1}$ ($P < 0.02$) respectively.

The resting release of GABA from cortical slices prepared from saline injected (control) rats was approximately $6 \mu\text{mol mg}^{-1} 10\text{min}^{-1}$, this was increased by KCl (50mM) to $26 \mu\text{mol mg}^{-1} 10\text{min}^{-1}$ ($P < 0.001$). 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

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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, $0.98 \mu\text{M}$, 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 $1 \mu\text{M}$ and 10nM in 7 of 7 cells tested using separate preparations for each observation, giving a reduction in inhibition of 20-100%. RU 34347, $10 \mu\text{M}$, consistently induced a similar effect to Ro 194603 in 5 of 5 cells tested. However, with $1 \mu\text{M}$ 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 10nM , 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, $1-10 \mu\text{M}$, was prevented or reversed by the benzodiazepine antagonist Ro 15-1788, $10 \mu\text{M}$, in 4 of 7 cells tested. The enhanced inhibition due to RU 34347, $1-10 \mu\text{M}$, was blocked by pre-incubation with RO 15-1788, $10 \mu\text{M}$, 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.

Bagust, J., Gardner, C.R., Hussain, S. & Walker, R.J. (1988) J. Physiol. 400, 61P.
Gardner, C.R. (1989) Drugs of the Future 14, 51-67.

253P THE PHARMACOLOGY OF CHOLINOCEPTORS ON THE SOMATIC MUSCLE CELLS OF THE PARASITIC NEMATODE *Ascaris suum*

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Cholinoreceptors 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 cholinoreceptor was studied. Acetylcholine (10 μ M) elicited a depolarisation of 8.4 ± 0.7 mV ($n=22$, \pm s.e. mean) accompanied by an increase in input conductance of 0.49 ± 0.09 μ S, ($n=22$, \pm 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 IC₅₀ was the concentration of antagonist that reduced the response to 10 μ M ACh by 50%. The pA₂ value was determined from a single dose-ratio for which there was a parallel displacement of the dose-response curve (values are mean \pm s.e. mean).

a)	b)						
AGONIST	R.P.	n	ANTAGONIST	IC ₅₀ μ M	n	pA ₂	n
Dimethylphenylpiperazinium	2	(6)	N-methyllycaconitine	0.23 ± 0.03 (4)	6.98		(1)
Acetylcholine	1		G-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 \pm 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 μ M-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.

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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 speed 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. 832, 840

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 (BZ₁) 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-pentylenetetrazole	Anti-Electroshock	Exploratory Activity	Rota-rod			
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 (BZ₁) 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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Seighart, W., (1983) Neurosci. Lett. 38, 73-78

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 (Driessens 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 μ mol/l to 310 μ mol/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 μ mol/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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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 \pm 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 \pm 0.5	0.8 \pm 0.5
DW + Vehicle	12	6.1 \pm 1.4*	2.4 \pm 0.4*
DW + Flumazenil (10 mg/kg)	8	2.3 \pm 0.8+	0.4 \pm 0.2+
DW + CGS 8216 (5 mg/kg)	12	15.6 \pm 2.8++	10.7 \pm 1.3++
DW + CGS 8216 + Flumazenil	8	2.9 \pm 0.6	1.6 \pm 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 μ m 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 μ m 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 μ m from the recording electrode were used to apply the electrical stimulus. Up to 2 μ M 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 μ M DA; n=4) and was also dependent on stimulus duration (50 to 500 ms; 0.60 ± 0.17 to 2.10 ± 0.43 μ M 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 μ m, overflow was $62 \pm 22\%$; 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.

Single square wave pulses (0.1 ms, 10 V) typically evoked 0.15 ± 0.05 μ M DA (n=20); a train of square wave pulses (50 Hz, 10 V, 0.1 ms for 100 ms) evoked 0.61 ± 0.12 μ M DA (n=4), which did not differ ($P>0.05$) from sine wave stimulation (10 V, 100 ms, 50 Hz; 0.41 ± 0.07 μ M 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.

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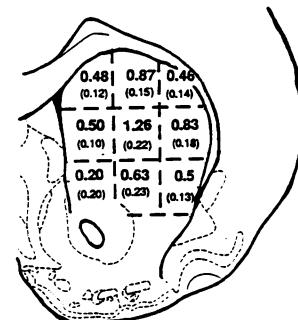
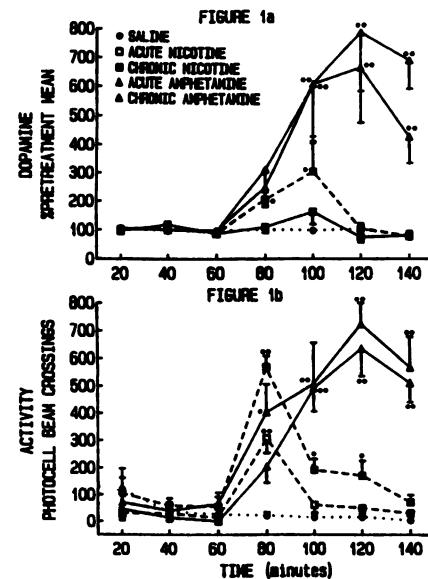


Figure 1 shows the average amounts of DA overflow (μ M) in different areas of striatum (\pm s.e.mean; n=9 to 16).

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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.



The results are means±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*.

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261P OPPOSING EFFECTS OF DOPAMINE D₁ AND D₂ 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 stereotactically 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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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 [³H]-sulpiride to label the low affinity state, and the agonist [³H]-NO437 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 [³H]-sulpiride for 10 min at room temperature in the presence of 100µM GppNHp. A similar concentration of untreated striatal membranes were incubated with 1nM [³H]-NO437 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 [³H]-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 [³H]sulpiride binding.

Compound	[³ H]-Sulpiride Kapp (µM)	[³ H]-NO437 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: IC₅₀ 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 [³H]-sulpiride)/(Kapp in [³H]-NO437). *: 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 PVC 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 [³H]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 [³H]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 ≤ 3 mg/kg ip, whereas doses > 5 mg/kg ip suppressed responding. Bupropion (10^{-7} - 10^{-5} M) also had no effect on [³H]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 ≤ 10 mg/kg ip, but generalised to amphetamine at 30mg/kg ip. Methamphetamine (10^{-8} - 10^{-4} M) markedly and dose-dependently enhanced [³H]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 ≥ 0.3 mg/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 μ M). 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 μ M 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 μ M) 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.

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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 CaCl₂ and 5 μ M 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 \pm 21%, P<0.001, n=7-8), DOPAC (+400 \pm 38%, P<0.001, n=7-8) and HVA (+197 \pm 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.

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267P SODIUM DEPENDENT BINDING OF [³H]-RACLOPRIDE TO RAT STRIATAL MEMBRANES

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The substituted benzamide, [³H]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 [³H]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 CaCl₂, 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 [³H]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 (K_d) and maximum number of binding sites (B_{max}) 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 [³H]raclopride was less than 20% of total binding, thus K_d and B_{max} 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 K_d, Table 1). This was accompanied by an increase in B_{max} (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 [³H]raclopride binding

[Na ⁺] mM	30	60	90	120
K _d (nM)	10.5 ± 0.7	5.8 ± 0.1	3.6 ± 0.1	1.3 ± 0.1
B _{max} (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 β₁-ADRENOCEPTOR NUMBER IN RAT CEREBRAL CORTEX

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β-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 β-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 β₁-adrenoceptor. In this study we set out to examine the effects of DSP-4 on β₁- and β₂-adrenoceptor sub-types in rat cerebral cortex by selective labelling of total and β₁-adrenoceptors using [¹²⁵I]-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. β-Adrenoceptor number and affinity were characterised using [¹²⁵I]-pindolol essentially as described by Beer et al (1987). Specific binding of [¹²⁵I]-pindolol to total and β₁-adrenoceptors was defined by 100 nM isoproterenol and 100 nM CGP 20712A respectively. Binding data was analysed by non-linear regression analysis.

	TOTAL β-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 β₁-adrenoceptors labelled by [¹²⁵I]-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 β₁-adrenoceptor number when assayed 3 days later. However when tested 14 days after treatment both total and β₁-adrenoceptor number were increased by 23% and 28% respectively. Subtraction of the β₁-from the total β-adrenoceptor capacity in each case indicated that β₂-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 β-adrenoceptor number in the rat cerebral cortex is restricted to an increase in β₁-adrenoceptors. This confirms the suggestion by Dooley et al (1983) and reinforces the proposal that in the rat brain β₁-adrenoceptors are located close to noradrenergic nerve terminals whereas β₂-adrenoceptors are located some distance from such nerve terminals.

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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 50 μ g of membrane extract

	(Age) (years)	(Plaques) (/mm ²)	optical density value, arbitrary units)				
			G_o	$G_s(H)$	$G_s(L)$	G_i	G_2
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_{ss} polypeptides with relative masses of 45 and 42 kDa respectively. Note: Comparisons can be made between G_i and G_2 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.

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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	*P<0.05
CA ₄	87.9 ± 24.1	116.7 ± 24.1	97.8 ± 30.2	89.0 ± 8.8	**P<0.001
CA ₃	97.5 ± 16.4	116.8 ± 34.2	70.9 ± 51.0	74.3 ± 5.8	Pmoles/g
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	

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 \pm sem, 42 \pm 3 years; post-mortem delay, 35 \pm 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 \pm 3 years; 40 \pm 3 h). β -Adrenoceptor binding sites were measured by saturation binding of [³H]CGP 12177 in well-washed membranes (De Paermentier et al., 1989b).

There were no significant differences in K_d or B_{max} values for total β -adrenoceptor binding sites, or β_1 -subtypes, between controls and depressed suicides in any of the brain regions (Table 1), although B_{max} 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 \pm 5, controls 86 \pm 5 fmol/mg protein, $p=0.03$, Wilcoxon rank sum test).

Table 1. B_{max} of β -adrenoceptor binding sites (fmol/mg protein, means \pm sem)

	Total β -adrenoceptors		β_1 -adrenoceptors		β_2 -adrenoceptors	
	Control	Suicide	Control	Suicide	Control	Suicide
Caudate	103 \pm 9	92 \pm 4	89 \pm 5	79 \pm 3		
Putamen	134 \pm 9	123 \pm 7	120 \pm 8	109 \pm 6		
Hippocampus	61 \pm 3	57 \pm 3	28 \pm 1	25 \pm 1	36 \pm 2	34 \pm 2
Amygdala	44 \pm 2	42 \pm 3	22 \pm 1	22 \pm 2	22 \pm 1	21 \pm 2
Thalamus	44 \pm 3	45 \pm 2	22 \pm 1	24 \pm 2	23 \pm 1	22 \pm 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.

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272P EFFECTS OF RS15385-197 IN *in vivo* PREPARATIONS

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RS-15385-197 is the most potent alpha₂-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 μ g/kg clonidine, s.c.) in pentobarbitone-anaesthetised rats with an ID₅₀ of 7 μ g/kg, i.v. and 95 μ g/kg p.o. ($n = >6$). In conscious rats RS-15385-197 (1 and 10 mg/kg, p.o.) antagonized clonidine (100 μ g/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 μ g/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-methoxyphenylglycol (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 $K_d = 0.41 \pm 0.03$ nM, $B_{max} 70.7 \pm 4.4$ fmol/mg protein, $n=9$; RS-15385-197, $K_d 0.34 \pm 0.03$, $B_{max} 51.2 \pm 4.4$, $n=10$). However, this dosing schedule of RS-15385-197 did not change the number (control B_{max} , 103 \pm 8 fmol/mg protein, and RS-15385-197, 97 \pm 4, $n=8$) or affinity (control $K_d 0.32 \pm 0.02$ nM ; RS-15385-197, 0.35 \pm 0.01), of frontal cortical 5-HT₂ receptors. In conclusion, RS-15385-197 is a potent and selective alpha₂-adrenoceptor antagonist which selectively increases noradrenergic activity in rat cortex and down regulates beta-adrenoceptors without a change in the number of 5-HT₂ 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 ³H-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 100 μ M) 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 100 μ M) 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.

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274P NEUROCHEMICAL EFFECTS OF IDAZOXAN GIVEN CONTINUOUSLY BY OSMOTIC MINIPUMP IN THE RAT

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The α_2 -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 β -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 μ l 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 β -binding (saturation analysis using [¹²⁵I]-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.		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 n=6	1	168±11	98±7	294±27	982±33	445±28
	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 n=6	1	144±14	69±14	270±19	845±27	383±26
	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 [¹²⁵I]-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 α_2 -receptors. Such increased release may similarly explain the observed reduction of brain β -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-H₂ ANTAGONISTS PROCONVULSANT?

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Initial studies of histamine-H₂ 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-H₂ 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-H₂ antagonists 15 or 30 mins before induction of seizures using either pentylenetetrazole (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****	* p <0.02
Metiamide	100	180**	3.8****	** p <0.05
Zolantidine	10	88*	2.7***	*** p <0.005
	3	88*	3.2****	**** 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-H₂ antagonism *in vivo* and *in vitro*. They are thus consistent with possible H₂ involvement in the demonstrated histaminergic control of seizures (Fairbairn & Sturman, 1989).

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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 responses; 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 2 μM (+)-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 2 μM (+)-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.

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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 stereotactically 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 Mg²⁺-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 IC₅₀s 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.

Lambert, J.J., Peters, J.A. & Cottrell, G.A. (1987) Trends Pharmacol. Sci. 8, 224-227.

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 Mg²⁺ (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 EC₅₀ value for NMDA (5 μM and 20 μM) in the absence and presence of Mg²⁺ (1 mM) ethanol had an apparent EC₅₀ 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 Mg²⁺ and 3 mM Ca²⁺. The non-NMDA antagonist, 6,7-dinitro-quinoxaline-2,3-dione (DNQX, 10 μM), and the GABA_A channel antagonist, picrotoxin (10 μM) were then added to the medium. This treatment allows the measurement of a greater than 90% pure NMDA-mediated synaptic potential in the presence of Mg²⁺. 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 particular 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 IC₅₀ 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-aminocyclopropane carboxylic acid, which in radioligand binding assays acts as an agonist at the glycine recognition site of the NMDA receptor complex (Marivon 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.

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281P THE EFFECTS OF POLYAMINES ON THE BINDING OF [³H]-MK-801 TO RAT CORTICAL MEMBRANES

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The polyamine, spermidine, stimulates the binding of [³H]-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.

P₂ 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, [³H]-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 [³H]-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 [³H]-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 [³H]-MK-801 binding (Table 1). Acrolein was inactive. In agreement with Carter et al (1989) ifenprodil reversed the spermidine (100μM) enhancement of [³H]-MK-801 binding (IC₅₀, 15 ± 7μM, mean ± s.e. mean, n=3). Putrescine also reversed the effects of spermidine (100μM)(IC₅₀, 81 ± 16μM, mean ± s.e. mean, n=3).

Table 1 Effects of glutamate, glycine and polyamines on [³H]-MK-801 binding (% Basal)

Drug concentration	Glutamate	Glycine	Spermidine	Spermine	N-acetyl Spermidine	N-acetyl Spermine	Putrescine	Ornithine	S-adenosyl-methionine	putrescine
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 PROPOFOLOL 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.

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.

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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 400 μ m 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, CaCl₂ 2, NaHCO₃ 26, MgSO₄ 1.19, KH₂PO₄ 1.18, D-Glucose 11; equilibrated at 37°C with 95% O₂/5% CO₂. In some experiments MgSO₄ or CaCl₂ was omitted. Hypoglycaemia was produced by reducing the glucose concentration to 2 mM and anoxia was produced by equilibrating solutions with 95% N₂/ 5% CO₂. 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 5 μ m wax sections cut and stained with haematoxylin/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 \pm s.e. mean): control 72 \pm 3 (n=21), NMDA 10 μ M 63 \pm 8 (n=3), NMDA 30 μ M 18 \pm 4 (n=10), NMDA 100 μ M 9 \pm 2 (n=11). Pre-equilibration with MK801 (10 μ M) or Ca⁺⁺-free medium completely prevented NMDA-induced neurotoxicity; number of intact cells: NMDA + MK801 65 \pm 18 (n=4), NMDA + Ca⁺⁺-free 64 \pm 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 \pm 7 (n=7), 20-min anoxia + low-glucose 58 \pm 16 (n=4), 30-min anoxia + low-glucose 65 \pm 15 (n=6), 60-min anoxia + low-glucose 54 \pm 29 (n=3). In Mg⁺⁺-free Krebs, hypoglycaemia or anoxia alone was also ineffective; no. cells: low-glucose 67 \pm 7 (n=7), 60-min anoxia 63 \pm 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 \pm 8 (n=3), low-glucose + 30-min anoxia 35 \pm 4 (n=4), low-glucose + 60-min anoxia 10 \pm 2 (n=9). The damage produced by 60-min anoxia/hypoglycaemia was prevented by MK801 (10 μ M); no. cells 58 \pm 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 [³H]glycine from rat striatal longitudinal slices (400 μ m thick).

Striatal slices accumulated [³H]glycine by a Na⁺-dependent, high-affinity (K_m =35 μ M) process, with maximal uptake by 15 min. Interestingly, inclusion of NMDLA in the medium (1 or 10mM) significantly increased [³H]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 [³H]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 1 μ M [³H]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⁺⁺-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 [³H]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, NaHCO₃ 25, CaCl₂ 1.3, KH₂PO₄ 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 iodoacetate (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 ± 0.5 mV) upon which was superimposed a rapid spike-like depolarisation (magnitude 7.4 ± 0.5 mV). In other tissues (n=20) only a single peak was observed (magnitude 6.8 ± 0.5 mV). 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 ± 0.7 mV, 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 (NMDA, 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 ED₅₀ 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 NMDA with an ED₅₀ (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 aspergillin 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 principal 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 μ g/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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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 ($ED_{50}=2\text{pg}$) 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 10-100 μg) injected locally into the paw. It was also blocked by local injection ($ED_{50}=5\mu\text{g}$) 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 (50 μg) 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 κ -agonists in mice (Portoghesi *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 $37.7 \pm 0.3^\circ\text{C}$ 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 (0.39 ± 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

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)*

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).

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291P D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ 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-NH₂ (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 [³H]-[D-Ala², MePhe⁴, Gly-ol⁵] enkephalin (1nM), the δ -sites with [³H]-[D-Pen², D-Pen⁵] enkephalin (1.5nM) and the κ -sites with [³H]-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 1 μ M CTOP caused less than 20% inhibition of δ - or κ -binding. [³H]-CTOP labelled a single class of binding site with a K_d of 0.12±0.01nM (n=3). The binding of [³H]-CTOP was readily displaced by [D-Ala², MePhe⁴, Gly-ol⁵] enkephalin and naloxone whereas selective δ - and κ -ligands displayed low potencies.

In the myenteric plexus, the antagonist dissociation constant (K_e) for CTOP against the μ -ligand [D-Ala², MePhe⁴, Gly-ol⁵] enkephalin was 16.1±1.9nM (n=5) and against the κ -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*.

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292P INCREASED FORMATION OF DIACYLGLYCEROL METABOLITES FOLLOWING MUSCARINIC CHOLINERGIC STIMULATION OF SYNAPTOSONES

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Synaptosomes were isolated from cerebral cortices of 2-4 rats by centrifugation on discontinuous percoll gradients (Dunkley *et al.*, 1986) and labelled by incubation with [²-³HH]-glycerol (6 μ Ci/mg protein) for 20 min. at 37°C. The labelled synaptosomes were then incubated with carbachol (10 μ M-1mM) in the presence or absence of atropine (10 μ M) for 10 min. at 37°C before termination of reactions by addition of trichloroacetic 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 v/v 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 10 μ M (p 0.05), dose-dependent (EC₅₀ values approx 20 μ M for both MAG and phospholipids), and were antagonised by atropine (no significant increases in the presence of 10 μ M atropine).

We conclude that occupancy of synaptosomal muscarinic receptors causes a rapid, dose-dependent increase in the flux of glycerol through synaptosomal glycerophospholipids. This probably results from very rapid turnover of 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 [³H]acetylcholine (Ach) release from fresh human temporal cortex tissue, having previously demonstrated that ATII is potent to inhibit the release of K⁺-stimulated [³H]Ach from rat entorhinal cortex. 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 [³H]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 [¹²⁵I]ATII (1988 Ci nmol⁻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-leucine³ 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 (1.0μmol⁻¹) significantly inhibited K⁺ stimulated [³H]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 [³H]Ach release. ACE activity in human temporal cortex was 1.03nmoles min⁻¹ mg⁻¹ protein (mean of a triplicate determination). Scatchard analysis of [¹²⁵I]ATII saturation data identified a homogenous population of high affinity [¹²⁵I]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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Nicotinic 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 interfering 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.

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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 synthesized 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.

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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+} ($[\text{Ca}^{2+}]_o$), intrasynaptosomal total calcium(Ca_r), intrasynaptosomal free Ca^{2+} ($[\text{Ca}^{2+}]_i$) and calcium accumulation, in rat cortical synaptosomes. Ca_r , assayed by atomic absorption spectrometry(Gitelman,1967), was estimated to be a maximum of 10.7mM(n=8) at 1mM $[\text{Ca}^{2+}]_o$. $[\text{Ca}^{2+}]_i$ was measured by fluorescence spectrophotometry(Adamson et al,1987) using Fura-2. Basal $[\text{Ca}^{2+}]_i$ was 118nM(n=10) in 1mM $[\text{Ca}^{2+}]_o$. This agrees with earlier reports that $[\text{Ca}^{2+}]_i$ is a small fraction (1/10,000th) of the Ca_r (Gibson and Peterson,1987). However, at various $[\text{Ca}^{2+}]_o$ (0-5mM), there are approximately linear relationships between $[\text{Ca}^{2+}]_i$, Ca_r and $[\text{Ca}^{2+}]_o$.

^{45}Ca accumulation into synaptosomes was linearly dependent on $[\text{Ca}^{2+}]_o$. Under physiological conditions(1mM $[\text{Ca}^{2+}]_o$ and 5mM $[\text{K}^+]_o$), ^{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_r . ^{45}Ca accumulation into synaptosomes during a 2min incubation was dependent on $[\text{Ca}^{2+}]_o$ (2.8, 4.1, 10 and 13 nmol/mg protein at 0.1, 1, 10 and 60mM $[\text{K}^+]_o$, respectively), i.e., as would be expected, the process is depolarisation dependent. However, at physiological $[\text{K}^+]_o$, 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_r is approximately 10,000 times higher than $[\text{Ca}^{2+}]_i$; c) only 10% of Ca_r is freely exchangeable at 1mM $[\text{Ca}^{2+}]_o$; d) the freely exchangeable pool is higher(x1,000) than the free pool; e) varying external Ca^{2+} alters Ca_r , $[\text{Ca}^{2+}]_i$ and accumulation in a linear fashion, suggesting that the $[\text{Ca}^{2+}]_i$ 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,Y). 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% CO₂. Protein content was used as a measure of cell growth. Protein content, intracellular polyamines and reduced glutathione were determined as described previously (Brunton et al., 1989).

Spermine produced a dose-dependent inhibition of cell growth in both cell lines, with the P,Y 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 approximately 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,Y cells the intracellular concentration was only twice that of the medium.

Table 1

Exposure Time (h)	Spm Added (mM)	Protein Content (mg)	Intracellular		Spm Added (mM)	Protein Content (mg)	Intracellular	
			Spermine (nmoles/mg protein)	Glutathione (nmoles/mg protein)			Spermine (nmoles/mg protein)	Glutathione (nmoles/mg protein)
BHK Cells	-	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,Y 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,Y 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,Y cells may also protect these cells from spermine induced damage.

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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) Oxypheral (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 ($1.75 \pm 0.22 \mu\text{mol}/\text{min}/\text{mg}$ protein; n = 4). LAE activity in male rats was unaffected by injection of either F-DA or FC-43 with overall mean values of $2.13 \pm 0.15 \mu\text{mol}/\text{min}/\text{mg}$ protein (n = 12) and $2.09 \pm 0.14 \mu\text{mol}/\text{min}/\text{mg}$ 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 ($2.08 \pm 0.19 \mu\text{mol}/\text{min}/\text{mg}$ 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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