163P MICROTUBULE INHIBITORS INTERFERE WITH CALCIUM INFLUX IN HEL MEGAKARYOCYTE-LIKE CELLS

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HEL cells are an early megakaryoblastic cell line sharing some receptor and signalling features in common with platelets. The capacitative theory of Ca^{2+} influx proposes that emptying of intracellular calcium stores promotes the entry of Ca^{2+} across the plasma membrane. This store-depletion Ca^{2+} influx is linked to the appearance of a calcium conductance, known as I_{CRAC}, in the plasma membrane. The underlying mechanism whereby store depletion regulates I_{CRAC} is unknown, although recent literature has suggested a role for the cytoskeletal network and membrane trafficking (Hajnóczy *et al.*, 1994). This prompted us to investigate a role for the microtubule network in HEL cells, using the inhibitors colchicine and nocodazole. In this report we show that colchicine and nocodazole, which depolymerise microtubules, inhibit Ca^{2+} influx in cells that have had their Ca^{2+} stores depleted by the combination of EGTA and the Ca^{2+} ATPase inhibitor thapsigargin.

HEL cells were loaded with Fura-2 to allow intracellular Ca^{2+} measurement by dynamic video imaging. Incubation with microtubule inhibitors was for 2 hours. Ca^{2+} stores were depleted by incubation with 2mM EGTA for 2 hours followed by 1μM thapsigargin for 4 min. Results are shown as mean ± s.e.mean of recordings from 6 - 35 cells from at least two experiments.

After incubation with EGTA addition of thapsigargin (1 μM) produced a small, transient intracellular Ca^{2+} ($[Ca^{2+}]_i$) rise from a basal value of 185 ± 18nM to 228 ± 18nM ($P<0.01$). Subsequent addition of Ca^{2+} (3 mM) to give a free concentration of 1mM raised $[Ca^{2+}]_i$ from 185 ± 18nM to 1135 ± 157nM ($P<0.01$), presumably reflecting Ca^{2+} entry under conditions of impaired uptake into internal stores. Incubation with 20μM colchicine or 30μM nocodazole prior to store depletion had no apparent effect on the response to thapsigargin but reduced the maximum $[Ca^{2+}]_i$ rise on addition of Ca^{2+} (3 mM) to 278 ± 51nM and 322 ± 80nM, respectively. Neither drug had a significant effect on basal Ca^{2+} (190 ± 13nM and 184 ± 9nM, respectively). In contrast, cells incubated with 20μM lumicolchicine, the UV light treated inactive form of colchicine, showed no significant inhibition of $[Ca^{2+}]_i$ rise (185 ± 18nM, resting and 960 ± 139nM after addition, $P<0.05$).

These results suggest that depolymerisation of the microtubule system interferes with calcium influx in HEL cells. This may represent an inhibition of delivery of vesicles, containing the I_{CRAC} channel, to the membrane following store depletion. Further work is required to test this hypothesis.

Hajnóczky, G., Lin, C. & Thomas, A. (1994) *J. Biol. Chem.* **269**, 10280-10287

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164P TYROSINE KINASE DEPENDENT AND INDEPENDENT PATHWAYS OF PHOSPHOLIPASE C REGULATION IN HEL MEGAKARYOCYTE-LIKE CELLS

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HEL cells are an early megakaryoblastic cell line sharing some receptor and signalling features in common with platelets including expression of the fibrinogen receptor, the integrin αIIb-β3, and the low affinity receptor for immune complexes, FcγRIIA. In platelets we have shown that FcγRIIA, in common with collagen and in contrast to the G-protein-coupled receptor agonist thrombin, stimulates phosphoinositide hydrolysis through tyrosine phosphorylation of phospholipase Cγ2 (Blake *et al.*, 1994). In the present report, we show that FcγRIIA cross-linking in HEL cells also induces activation of phospholipase C through a tyrosine kinase dependent pathway and that this is associated with tyrosine phosphorylation of multiple proteins.

HEL cells were pre-labelled with [^{3}H]inositol for 48 h and stimulated with each receptor agonist for 5 min in the presence of 10 mM LiCl to prevent metabolism of inositol phosphates. Total inositol phosphates (i.e. mono-, bis- and tris-phosphates) were measured by Dowex anion exchange chromatography. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) was measured in fura-2 loaded cells by spectrofluorimetry or by dynamic video imaging. Protein tyrosine phosphorylation was measured by western blotting using the monoclonal antibody (mAb) 4G10. FcγRIIA was activated by exposure to mAb IV.3 (1 μg/ml) followed by cross-linking with $F(ab')_2$ (30 μg/ml).

FcγRIIA-crosslinking increases [^{3}H]inositol phosphates to 137 ± 4.6 % of basal values during a 5 min incubation which is accompanied by a transient elevation of $[Ca^{2+}]_i$ in whole cell populations. In single HEL cells, FcγRIIA cross-linking induces a rise in $[Ca^{2+}]_i$ from a basal of 150 ± 39 nM to a peak of 290 ± 73 nM within 10 s, which returns to basal with 2 min. Formation of inositol phosphates is inhibited significantly by the non-selective kinase inhibitor staurosporine (10 μM) and by a selective tyrosine kinase inhibitor, the tyrphostin ST271 (300 μM) to 107.8 ± 8.8 % and 110 ± 7.2 %, respectively ($P < 0.05$). In contrast, collagen (1 - 100 μg / ml) has no significant effect on the level of [^{3}H]inositol phosphates or $[Ca^{2+}]_i$. The G protein receptor agonist thrombin (10 units / ml) increases the level of inositol phosphates to 66.7 ± 8.6 % above basal and this is potentiated significantly by staurosporine (10 μM) and ST271 (300 μM) to 171 ± 17 and 112 ± 12 % above basal, respectively ($p < 0.01$).

Activation of HEL cells by FcγRIIA is associated with marked tyrosine phosphorylation of multiple proteins, most prominently at 38, 45, 67, 72, 120 and 130 kDa. In contrast, thrombin-induces no apparent increase in tyrosine phosphorylation.

FcγRIIA signalling in HEL cells appears to parallel that in platelets, showing characteristics of a tyrosine kinase-dependent pathway. Further work is in progress to elucidate the underlying molecular events.

Acknowledgements: ST271 was kindly donated by the Wellcome Foundation. This work was supported by the Wellcome Trust.

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165P THE INCREASE IN QUANTAL ACETYLCHOLINE RELEASE INDUCED BY A PHORBOL ESTER AT THE *IN VITRO* FROG NEUROMUSCULAR JUNCTION IS SENSITIVE TO NIMODIPINE

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The release of quantal acetylcholine (ACh) from motor nerves is dependent on extracellular Ca^{2+} ions but is insensitive to antagonists of voltage-gated L-type Ca^{2+}-channels (Gotgilf and Magazanik, 1978; Atchison, 1989). Nevertheless in the presence of K^+-channel blockade, waveforms recorded intracellularly from the terminals exhibit components attributable to L-type channels (Morita & Barrett, 1989), suggesting that these Ca^{2+}- channels are not ordinarily activated by nerve impulses. Recently it was reported (Arenson & Gill, 1995) that the protein phosphatase inhibitor, okadaic acid, increased spontaneous quantal release by recruiting otherwise silent L-type channels and by increasing quantal size: observations which suggest that protein phosphorylation and dephosphorylation regulate the activation of L-type channels and the packaging of ACh and which raise questions concerning the identity of the protein kinase(s) responsible for these phenomena. 4-ß-Phorbol esters activate protein kinase C (PKC), increase the release of ACh from motor nerves (Shapira *et al.*, 1987) and in hippocampal cells increase L-type Ca^{2+}-currents (O'Dell & Alger, 1991). The experiments reported here were carried out to ascertain whether activation of L-type Ca^{2+}-channels can account for the phorbol ester-induced increase in ACh release.

Sartorius muscles were equilibrated in Ringer solution containing Ca^{2+} 0.5 and Mg^{2+} 3.5mM. Fibres were impaled with one or two microelectrodes filled with 3M-KCl. Control samples of mepp(c)s (usually > 100) and epp(c)s (140) evoked at 0.33Hz were recorded 15 and 10min respectively before addition of a drug to the chamber. Test samples of spontaneous and evoked signals were obtained 20 and 25 min respectively after the addition and subsequently at further 20min intervals. Changes (mean ± s. e. mean %) in quantal content (M-calculated from the amplitude ratio: mean epp(c)/mean mepp(c)) and in mepp(c) frequency were compared by non-parametric statistics with those that occurred at equivalent times in untreated fibres or in the case of the 4-ß-phorbol ester with those in fibres pretreated with nimodipine (1μM).

4-ß-Phorbol 12 myristate 13 acetate (PMA, 0.2μM) increased M by 82 ± 25% (n = 11) and 129 ± 29% (n = 11) after 25 and 45min incubation respectively. These changes were greater ($P < 0.031$) than those (18 ± 15%, n = 6, 25min and 27 ± 25%, n = 6, 45min) that occurred in the untreated fibres and they persisted after washing with PMA-free Ringer for 1h. In nimodipine pretreated fibres the increases in M induced by PMA were reduced ($P < 0.025$) to 14 ± 7% (n = 13) and 41 ± 14% (n = 10) at 25 and 45min respectively. PMA increased mepp(c) frequency by 70 ± 19% (n =13, $P < 0.035$) and by 162 ± 47% (n = 12, $P < 0.01$) at 20 and 40 min respectively: in untreated fibres the equivalent increases were 4 ± 9% (n = 7) and 18 ± 16% (n = 7). Pretreatment with nimodipine reduced ($P < 0.02$) the PMA- induced increase in frequency at 40min to 42 ± 14% (n = 11). PMA had no effects on the amplitude of the mepc or its decline (τ). Neither 4-α-phorbol 12, 13, didecanoate (0.2μM), which does not activate PKC, nor nimodipine had a significant effect on M or mepp(c) frequency.

Our data are consistent with the view that phorbol esters can increase evoked and spontaneous ACh release by recruiting covert L-type Ca^{2+}-channels but not by increasing quantal size.

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166P EFFECTS OF METHACHOLINE AND ISOPRENALINE ON CYCLIC AMP AND INOSITOL 1,4,5-TRISPHOSPHATE LEVELS IN CHO CELLS STABLY EXPRESSING M_3 MUSCARINIC CHOLINOCEPTORS AND β_2-ADRENOCEPTORS

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Muscarinic receptor subtypes which link to the activation of phosphoinositidase C (PIC), and β-adrenoceptors which activate adenylyl cyclase (AC) are co-expressed in a number of important tissues. In the present study, we have investigated the potential crosstalk between M_3-muscarinic and β_2-adrenoceptor signalling pathways by measuring inositol 1,4,5-trisphosphate ($InsP_3$) and cyclic AMP (cAMP) mass accumulations in a model cell system engineered to express these two receptor subtypes. We have used a previously characterized Chinese hamster ovary (CHO) cell-line expressing M_3-muscarinic receptors (CHO-m3; Tobin *et al.*, 1992) into which we transfected human β_2-adrenoceptor cDNA. Positive clones were selected and characterized by N-[^{3}H]methylscopolamine and [^{3}H]CGP-12177 saturation binding. The clone described here (#27) was found to express M_3 and β_2 receptors at levels of 1456 ± 240 (n=4) and 224 ± 33 fmol/mg protein (n=6) respectively.

Incubations were carried out using confluent cell monolayers maintained at 37°C in a final volume of 500μl Krebs-Henseleit buffer (equilibrated with 95% O_2/5% CO_2). Drug additions were made in 50μl for the times, and at the concentrations, indicated. Reactions were terminated by the addition of ice-cold trichloroacetic acid (0.5 M) and samples were extracted on ice before being neutralized. Measurements of cAMP and $InsP_3$ mass were carried out using standard radioreceptor binding assays.

Methacholine (MCh; 0.1-1000 μM; 20 min) caused a concentration-dependent accumulation of $InsP_3$ (basal, 81 ± 2; +1 mM MCh, 569 ± 16 pmol/mg protein: EC_{50} (geometric mean [range]): 3.2 [2.2-4.5] μM). Incubation of the cells with isoprenaline (ISO; 1μM) had no effect on $InsP_3$ accumulation. ISO (0.1-1000 nM; 10 min) elevated cAMP levels in a concentration-dependent manner (basal, 60 ± 13; +1 μM ISO, 1122 ± 58 pmol/mg protein; EC_{50} 3.5[0.3-50] nM). MCh also elevated cAMP accumulation (to 376 ± 14 pmol/mg protein at 1 mM; EC_{50} 24[7.9-73] μM). The effects of MCh on both $InsP_3$ and cAMP accumulations were antagonized by atropine and were completely unaffected by the presence of the β-adrenoceptor antagonist, timolol (1 μM). The stimulation of cAMP accumulation by ISO was substantially increased in the presence of MCh, thus whilst MCh (1 mM) and ISO (1 μM) caused increases in cAMP of 6-fold and 19-fold-over-basal respectively, MCh + ISO co-addition produced a greater than additive 59-fold increase in cAMP accumulation. The possible role of protein kinase C (PKC) in mediating the enhancement of the ISO response was investigated. However, the PKC activator phorbol myristate acetate (1-3000 nM) did not significantly enhance the cAMP response to ISO.

The results of the present study demonstrate that in CHO-cells co-expressing M_3 and β_2 receptors, activation of M_3 receptors causes accumulation of both $InsP_3$ and cAMP, whilst activation of β_2-adrenoceptors causes only an increase in cAMP. The M_3-mediated $InsP_3$ response is unaffected by co-activation of the β_2-adrenoceptor population; however M_3-receptor activation causes a substantial enhancement of β_2-adrenoceptor-stimulated cAMP accumulation. The mechanism by which M_3-receptor activation causes this effect is unclear, although the involvement of PKC in this crosstalk appears unlikely.

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Tobin, A.B., Lambert, D.G. and Nahorski, S.R. (1992) *Mol. Pharmacol.* **42**, 1042-1048

167P THE EFFECTS OF POTASSIUM CHANNEL BLOCKERS ON THE RAT ISOLATED PERFUSED HEART

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Acidosis inhibits L-type calcium channels in isolated smooth muscle cells and relaxes the mesenteric vascular bed. However in the coronary circulation it causes vasoconstriction(Wilson & Woodward 1995). One mechanism by which voltage sensitive calcium channels could be activated in coronary vessels is by the inhibition of K^+ channels. We have therefore studied the effects of K^+ channel blockers in the perfused rat heart. Experiments were also performed in superior mesenteric beds under basal or pre-constricted states.

Hearts (male Wistar rats 300-350g) were perfused at 10ml.min.$^{-1}$ Developed tension (DT), heart rate (HR) and coronary perfusion pressure (CPP) were measured. Superior mesenteric beds were perfused at 5ml.min.$^{-1}$ with the coronary effluent. Phenylephrine infusion (final conc. 96μM) was used to increase mesenteric perfusion pressure (MPP). Drugs were perfused through the system for 5 min. followed by 10 min washout. Data expressed as mean±s.e.mean, n=4. Statistical analysis performed using t-test except where stated.

The non-selective K^+ channel blockers, tetraethylammonium (TEA), tetrabutylammonium (TBA) and 4-Aminopyridine (4AP)(1μM-10mM) produced concentration-dependent increases in CPP. For 10mM TEA, TBA & 4AP control CPP 80±9, 90±10 & 87±7mmHg increased to 140±17($p<0.01$), 155±8($p<0.02$) and 150±15($p<0.02$) mmHg respectively. Initial increases in CPP were seen at the 10μM concentration. At the 10mM concentration, the increase seen with TBA was not sustained. At concentrations greater than 1mM these agents also decreased DT & HR,. The decrease in HR seen with TEA & TBA did not reverse on washout.

The maxi calcium dependent K^+ channel blocker penitrem A (1-100nM) increased CPP from 75±6 to 100±8mmHg ($p<0.02$) while no effect was seen on DT & HR. Glibenclamide, the ATP-sensitive K^+channel blocker (1n -10μM) had no effect on CPP or HR, but did produce a concentration-dependent decrease in DT. At 10μM, DT was reduced from 5.0±0.7 to 3.25±0.3g ($p<0.02$). In all cases, increases in CPP produced by K^+channel blockers were blocked by 100nM nifedipine ($p<0.05$; Oneway ANOVA).

All compounds had no effect on basal MPP. When pre-constricted, however, different results were obtained. Glibenclamide and penitrem A had no effect at any concentration used. Both TBA and 4AP produced concentration-dependent dilations which occured at concentrations above 1mM. Control MPP for 4AP & TBA of 118±9 & 109±6mmHg became 58±17 & 64±8mmHg with 1mM (both $p<0.05$). In contrast, TEA produced concentration-dependent increases in MPP from 98±10 to 124±13mmHg with 1mM.

These results indicate that perfused coronary and mesenteric vessels respond differently to K^+ channel blockers. CPP increases in the presence of 4-AP, TEA and TBA, while MPP decreases in response to 4-AP and TBA, in contrast TEA increases MPP. These results in perfused vessels could have important implications for interpretation of results obtained in single cell studies and raises the possibility that acidosis induced coronary constriction could be mediated via inhibition of K^+ channel(s), as shown in other cell types (Peers & Green 1991).

D.A.W. is a University Of Bath research student.

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168P CALCIUM-ACTIVATED $^{86}Rb^+$ FLUXES IN THE RAT C6 GLIOMA CELL LINE

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Glial cells are known to play an important role in the regulation of K^+ homeostasis in the brain (Pollen and Trachenberg, 1970). The membrane hyperpolarisation seen in C6 glioma cells after application of neurotransmitters or neuroactive peptides (see, *inter alia*, Reiser et al, 1990) is thought to be due to activation of a K^+ conductance dependent on a rise in cytosolic Ca^{2+}. We have studied the pharmacological characteristics of a putative Ca^{2+}-activated K^+ channel in confluent monolayer cultures of rat glioma C6 cells, using $^{86}Rb^+$ as a radioisotopic tracer for K^+ and the Ca^{2+} ionophore ionomycin to activate Ca^{2+}-dependent fluxes. All experiments were carried out at ambient (21-23°C) temperature.

In the absence of ionomycin, the steady-state basal influx of $^{86}Rb^+$ into C6 cells was 318±20pmol s^{-1} (n=5). Uptake (in the presence or absence of ionomycin) of $^{86}Rb^+$ was linear for the first 60s and under these initial rate conditions, efflux of radioisotope was negligible and the accumulation of isotope within the cells could be considered to be entirely due to an influx component. The threshold for ionomycin activation of $^{86}Rb^+$ influx was approx. 0.1μM and during the initial 30s, the flux rate was proportional to ionomycin concentration. The effect of increasing ionomycin concentration on the total $^{86}Rb^+$ accumulated within the cell was saturable (EC_{50}=0.62±0.03μM, n=5). Only 5% of the total flux was ouabain-sensitive and therefore likely to be energy dependent. Intracellular Ca^{2+} levels were measured directly, using Fura-2. When ionomycin was applied to C6 cells, $[Ca^{2+}]_i$ rose within 30s from a basal level of 42±2nM to 233±17nm (30 cells). Intracellular pH (measured using the the fluoresecent dye, BCECF) fell in the first 30s after ionophore application, from 7.03±0.04 to 6.87±0.03 (30 cells). Membrane voltage was measured during ionophore application, using the perforated patch technique. In all cells studied, ionomycin caused a hyperpolarization from an average resting membrane potential of -34±10mV to a maximum of -76±2mV (n=4), that was accompanied by a fall in membrane resistance.

The effects of potassium channel blockers on ionomycin-activated $^{86}Rb^+$ fluxes were studied with 2μM ionophore during a 30s incubation period. Tetraethylammonium ion (TEA) and quinine completely blocked $^{86}Rb^+$ influx. Although the IC_{50} for TEA block was 10±1.9mM (n=4), complete inhibition was observed at approx 20mM TEA. Both charybdotoxin (IC_{50} = 0.5±0.02nM, n=4) and iberiotoxin (IC_{50} = 800±150nM, n=4) inhibited ionomycin-activated $^{86}Rb^+$ influx in a dose-dependent manner. However apamin, a highly specific blocker of SK channels was ineffective at concentrations up to 100μM. Toxin I (a close homologue of dendrotoxin and a blocker of A-type, voltage-activated K^+ channels) was also unable to block ionomycin-activated $^{86}Rb^+$ influx at concentrations up to 100μM. These results suggest that the Ca^{2+}-activated Rb^+ fluxes that we are observe in C6 cells are either of the BK or IK channel pharmacological subtype.

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169P RUBIDIUM AFFECTS THE ACTIONS OF POTASSIUM CHANNEL MODULATORS IN THE MOUSE INTESTINE

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Yeung *et al.* (1995) have shown quantitative differences between responses to the potassium channel opener, pinacidil (PIN) in the absence or presence of the ATP-sensitive potassium channel blocker glibenclamide (GBC) in normal Krebs' solution (KS) and in KS containing rubidium (Rb). In the present study, the antagonistic effect of GBC on the relaxant response to PIN is examined in mouse ileum exposed to increasing amounts of Rb which were added to replace potassium (K) in KS.

Mucosa-free preparations from the distal ileum of male BKW mice were placed under 0.5g tension (37℃, 95% O_2/5% CO_2) in normal KS containing 5.95mM K (Rb=0) or in isosmotic KS in which K was replaced by increasing concentrations of Rb (1.19-4.75mM). Isometric contractions were elicited by electrical field stimulation (0.2Hz, 30V). When twitch height was constant, a cumulative concentration-response curve to PIN (0.1-200μM) or vehicle was performed. This procedure was repeated following 20min incubation with a single concentration of GBC (0.1-1μM) or vehicle. Relaxant potency was expressed as geometric mean EC_{50} (the concentration required to reduce twitch height by 50%) with 95% CL. The initial twitch response was expressed as tension (g) ± s.e.mean and the effect of GBC was expressed in terms of PIN dose ratio ± s.e.mean. Statistical analysis was performed using Student's unpaired t-test.

PIN-induced relaxation was progressively attenuated by increasing concentrations of Rb (2.38-4.75mM) whereas the initial twitch height was not affected (Table 1). The antagonism of PIN by GBC was concentration-dependent in normal KS and in a solution containing 1.19mM Rb. However, as the concentration of Rb increased (2.38-4.75mM) and the concentration of GBC increased, the ability of GBC to antagonise PIN decreased (Table 1).The vehicle for PIN and GBC produced no effects (n>4). GBC alone produced no effect at the concentrations used (n=4).

These results suggest that Rb may block membrane potassium channels, as suggested by Greenwood & Weston (1993) in the rat isolated aorta. However, a possible intracellular action of rubidium cannot be excluded.

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Yeung, C.K., McCurrie, J.R., & Wood, D. (1995) Br. Pharmacol. Soc. Meeting. (Canterbury).

Table 1 Effect of increasing concentrations of rubidium on responses to pinacidil alone and in the presence of glibenclamide in the mouse ileum.

	Pinacidil		Pinacidil + Glibenclamide (Dose Ratio ± s.e.mean; n=4 in all cases)			
Rb (mM)	Twitch Height (g)	EC_{50} (μM) (mean, 95% CL)	n	GBC 0.1μM	GBC 0.3μM	GBC 1.0μM
0	0.58±0.03	1.38 (1.13-1.92)	12	3.42±0.89	5.42±0.85	15.29±2.76
1.19	0.48±0.04	1.47 (1.20-1.97)	12	3.21±0.51	6.40±0.84	14.19±2.13
2.38	0.52±0.04	4.27 (3.50-6.85)**	12	4.45±1.35	5.33±0.93	7.44±2.01
3.56	0.51±0.04	10.33 (8.29-16.29)**	12	2.34±0.18	1.92±0.15*	2.26±0.49*
4.75	0.54±0.04	31.35 (27.38-36.97)**	12	2.35±0.50	1.62±0.18*	1.95±0.30*

*P<0.01, **p<0.001 (0 vs 1.19-4.75mM Rb)

170P ACTIONS OF METHYLENE BLUE ON CONTRACTION AND DELAYED RECTIFIER POTASSIUM CURRENTS IN GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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Methylene blue is often used as an inhibitor of guanylate cyclase; in addition it has been reported to increase cardiac myocyte contraction (Brady et al 1992). The purpose of the present study was to further examine the influence of methylene blue on contraction in single myocytes isolated from guinea pig ventricle and elucidate its underlying mechanisms of action.

Exposure of the cells to 10 μM methylene blue resulted in a progressive increase in contraction amplitude; after 10 minutes contraction (measured from video image of cells viewed microscopically using an edge detection system) was increased by 15±6 % and the rate of relaxation appeared to be reduced (Fig.1). This increase in contraction amplitude was associated with a prolongation of the action potential duration (after 10 minutes APD_{20} was 140±7 % and APD_{90} was 152±7 % of control). Such an observed prolongation of action potential could occur as a result of enhanced L-type calcium current and / or reduced delayed rectifier potassium currents. To further investigate these possibilities L-type calcium currents were activated by step depolarisations from -40mV to 0mV for 200ms at 0.3Hz (switched voltage-clamp). Following exposure to 10 μM and 50 μM methylene blue, the amplitudes. of the calcium currents were not significantly altered (after 10 minutes calcium currents were 99±5% & 96±4 % of control respectively). Delayed rectifier potassium currents were activated by step depolarisations from -40mV to +40mV for 10 to 800 ms and measured as outward tails on repolarisation to -40mV. On exposure to 10 μM methylene blue, a rapid decline in the early component of the delayed rectifier current was observed (within 3 minutes currents at 40ms were reduced to 64±5 % of control, Fig. 2). At 50 μM methylene blue, the early component of the delayed rectifier current at 40 ms was reduced further to 41±4 % of control within 3 minutes.

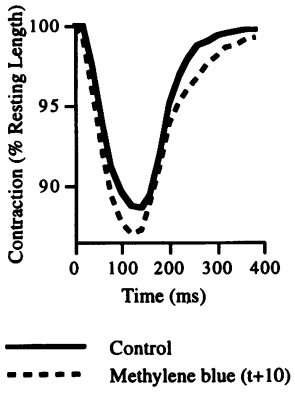

Figure 1

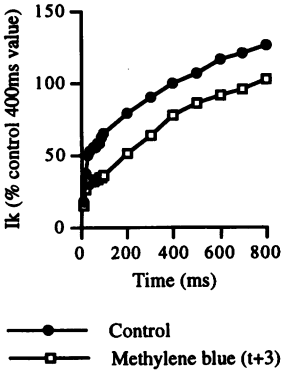

Figure 2

In conclusion the observed increase in contraction amplitude following exposure to methylene blue may be associated with an increased calcium loading of the SR occuring as a consequence of the prolongation of the action potential plateau leading to a reduced calcium extrusion via sodium calcium exchange. The reduction in the early component of the delayed rectifier potassium current by methylene blue would be expected to contribute to such an action potential prolongation. This reduction may result from either a direct channel block by methylene blue or through altered intracellular cGMP levels.

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171P BLOCK OF CARDIAC DELAYED RECTIFIER POTASSIUM CURRENT BY R56865 AND HALOPERIDOL IN ISOLATED GUINEA-PIG VENTRICULAR MYOCYTES

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The aim of this study was to investigate the effects of R56865 (a compound with a variety of actions including block of sodium-activated potassium current, I_{KNa}, Carmeliet & Tytgat, 1991) and haloperidol on cardiac delayed rectifier potassium currents.

Single cells were isolated from guinea-pig ventricular muscle and studied using the whole-cell, patch-clamp technique. The bath solution was a balanced salt solution containing 2.5mM $CaCl_2$ (36°C). Intracellular solutions contained (in mM) 13 KCl, 109 K-glutamate, 10 HEPES, 11 EGTA, 5 $CaCl_2$, 5 NaOH and 14 MgATP, pH 7.2 For reasons unconnected with this study, the intracellular solution in the R56865 experiments contained (in mM), 13 KCl, 109 K-glutamate, 10 HEPES, 1.1 EGTA, 0.1 $CaCl_2$, 5 NaCl and 5 MgCl, 5 K-ATP, pH 7.2. Similar drug effects were observed with either solution. I_{Kr}, the rapidly activating component of I_K was assessed as the deactivating tail current on repolarization to a holding potential of -40mV from a 40ms test pulse to +40mV. Increasing the duration of the test pulse increased the contribution of I_{Ks}, the slowly activating component of I_K, to the total tail current.
Paired t tests were used for comparisons ($p<0.05$ as significant).

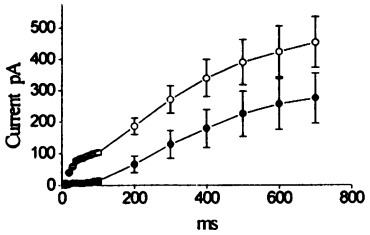

Figure 1. Effect of 500nM R56865 (filled circles) on envelope of tails. Open circles show control values.

R56865 (500 nM), reduced the tail current following a 40ms pulse from 77pA ± 3.5pA to 7pA ± 3.5pA, n=6, and tail currents following 700ms pulses from 454pA ± 105pA to 275pA ± 81pA, n=6, consistent with a predominant effect of R56865 on I_{Kr}, see figure 1. No recovery was observed over a 10 minute wash.

In 3 cells, dofetilide, a potent blocker of I_{Kr} (Carmeliet, 1992) did not block I_{KNa} (measured from ramp depolarisations in the presence of 90mM intracellular sodium), consistent with distinct conductances for I_{Kr} and I_{KNa}. Haloperidol (200 nM), a compound with some structural features similar to R56865, reduced tail currents following a 40ms pulse from 148pA ± 15pA to 60pA ± 7.5pA, n=6, while currents following a 700ms pulse were reduced from 363pA ± 7pA to 144pA ± 18pA, n=6, again consistent with suppression of I_{Kr} with possible additional effects on I_{Ks}. These effects were rapidly reversed on washing. In another series of experiments I_{Kr} and I_{Ks} were separated using an alternative voltage protocol (Carmeliet 1992): I_{Ks} was measured as the deactivating tail current on repolarization to -10mV from a 3s test pulse to +30mV, while I_{Kr} was measured as the tail current on further repolarization to -50 mV (after full deactivation of I_{Ks} by holding at -10mV for 10s). As expected from the earlier experiments, I_{Kr}, measured in this way, was almost completely inhibited (from 115pA ± 12pA to 13pA ± 8pA) while there appeared to be a small reduction of I_{Ks} (from 535pA ± 66pA to 438pA ± 63pA, n=6). Haloperidol (200nM) did not significantly alter I_{Ca} (-1.58nA ± 0.27nA to -1.44nA ± 0.27nA, n=5), measured as the peak inward current on stepping to 0mV, or I_{K1} (-3.4nA ± 0.532nA, to -3.76nA ± 0.35nA), measured as the magnitude of the peak inward currents on stepping to -100mV. Action potential duration (90% repolarization) was prolonged from 201ms ± 20ms to 222ms ± 18ms by 200nM haloperidol.

The observations are consistent with a supressant action of both R56865 and haloperidol on the rapid component of delayed rectifier potassium current in guinea-pig ventricular myocytes with possible small additional effects of haloperidol in I_{Ks}.

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172P INHIBITION OF K_{ATP} CHANNEL CURRENTS BY CICLAZINDOL IN THE CRI-G1 INSULIN-SECRETING CELL LINE

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The anorectic compound ciclazindol has previously been shown to inhibit K_{ATP} channel activity and stimulate insulin secretion in CRI-G1 insulin-secreting cells (Ozanne *et al.*, 1994). In the present study we have examined the structural and pharmacological specificity of this effect. In excised outside-out patches bathed in symmetrical 140mM KCl, the application of 1μM ciclazindol reversibly inhibited single K_{ATP} channel activity in a voltage independent manner. This inhibition of channel activity was concentration dependent, 1μM causing complete inhibition (n=16), 100nM a reduction in channel open-state probability (P_O) by 83.6 ± 8.3% and 10nM by 24.2 ± 11.1% (n=3). The reduction in channel activity was not associated with a change in the single channel conductance (-50mV to +50mV), being 55.4 ± 4.3pS in control and 54.6 ± 5.1pS in the presence of 100nM ciclazindol (n=6). Unlike ciclazindol, mazindol had no effect on K_{ATP} channel activity recorded from outside-out patches (n=4) over the concentration range 100nM to 100μM. Furthermore, three amphetamine-derived anorectic agents, fenfluramine, diethylpropion and phentermine were also ineffective on K_{ATP} channel activity when applied to outside-out membrane patches at concentrations up to 100μM (n=3 for each).

To study the pharmacological specificity of ciclazindol, whole-cell currents were evoked from single CRI-G1 cells bathed in normal physiological saline (which contained (in mM): 135.0 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 10.0 HEPES (pH7.2)). To isolate whole-cell K_{ATP} channel currents the pipette solution contained (in mM): KCl 140.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, K-EGTA 10.0, HEPES 10.0 (pH 7.2) and ±10mV pulses of 200msec duration were applied every 2s from a holding potential of -70mV. Under these conditions, 10μM ciclazindol produced 94.2 ± 2.3% (n=8) inhibition of the whole-cell K_{ATP} channel current. The IC_{50} associated with this effect was 40.01 ± 0.60 nM (n=20) and the Hill coefficient was 1.3 ± 0.3.

The effects of ciclazindol on voltage-sensitive currents were examined. To study the delayed outward currents, 300nM tetrodotoxin (TTX) and 1mM $CdCl_2$ were added to the normal physiological saline and the pipette solution contained (in mM): KCl 140.0, $CaCl_2$ 2.0, $MgCl_2$ 2.5, 2.0 K_2ATP, K-EGTA 10.0, HEPES 10.0 (pH 7.2). To study Ca^{2+} currents 300nM TTX and 10mM $CaCl_2$ were added to the bath solution while the pipette solution containing (in mM): CsCl 140.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, K-EGTA 10.0, HEPES 10.0 (pH 7.2) and Na^+ currents were studied by adding 1mM $CdCl_2$, 20mM tetraethylammonium (TEA) and 4mM 4-aminopyridine (4-AP) to the normal physiological saline and using a pipette solution containing (in mM): CsCl 140.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, K-EGTA 10.0, HEPES 10.0 (pH 7.2). Under these conditions 10 μM ciclazindol inhibited Ca^{2+}, Na^+ and K^+ currents by 3.2 ± 1.4% (n=5), 7.2 ± 4.6% (n=4) and 21.2 ± 5.2% (n=8), respectively.

In conclusion, these findings indicate that ciclazindol is a highly effective and specific inhibitor of K_{ATP} channel activity in the CRI-G1 insulin-secreting cell line.

This work was supported by the Wellcome Trust (Grant Nos 040806 & 038054). K.L. was a Wellcome Prize student (Grant No. 033683).

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173P THE ROLE OF K_{ATP}-CHANNELS AND ADENOSINE IN THE REGULATION OF CORONARY FLOW IN THE ISOLATED BUFFER PERFUSED RAT HEART

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Recent evidence has indicated that ATP-sensitive potassium (K_{ATP}) channels are active under normal physiological conditions and contribute towards the regulation of vascular tone (Jackson, 1993), although this varies between vascular beds (Randall, 1994). Adenosine has similarly been implicated in the regulation of vascular tone. In the present study the participation of both K_{ATP} channels and adenosine in the regulation of coronary vascular tone has been assessed in the isolated rat heart, perfused under constant pressure conditions with physiological buffer. The involvement of K_{ATP}-channels was examined by determining the effects of glibenclamide on coronary flow and mechanical performance, while the role of endogenous adenosine was assessed by the addition of 8-phenyltheophylline (8-PT).

Male Wistar rats (230-380g) were heparinized (1,000U kg^{-1} i.p.) and anaesthetized with sodium pentobarbitone (60mg kg^{-1} i.p.). In each case, following a thoracotomy, the heart was rapidly excised and placed in ice cold Krebs-Henseleit solution to arrest cardiac contraction. The aorta was then cannulated and the heart perfused according to the method of Langendorff at a constant pressure of 60mmHg with oxygenated Krebs-Henseleit buffer containing 2mM pyruvate. A fluid-filled latex balloon catheter, coupled to a pressure transducer, was inserted in to the left ventricle in order to measure developed left ventricular pressure (DLVP) and heart rate was derived from the pressure signal. Mechanical performance was assessed as the mathematical product of DLVP and heart rate (the pressure-rate product, PRP). Coronary flow was measured by means of a transit time ultrasonic flow meter.

In 6 preparations basal coronary flow was 8.78±0.76ml/min/g wet weight (mean±s.e.mean) and the PRP was 21,487±2,577 mmHg/min. Following the addition of glibenclamide (10μM) coronary flow was significantly ($P<0.001$) decreased to 3.89±0.59ml/min/g and the mechanical performance was also significantly ($P<0.01$) depressed with a pressure-rate product of 6,950±1,104 mmHg/min. The reduction in coronary flow was reversed by the addition of sodium nitroprusside (100μM) when it was 9.20±0.80ml/min/g, while the mechanical performance was only partially restored (PRP=14,315±884mmHg/min).

In 6 different preparations basal coronary flow was 10.4±0.6ml/min/g and the PRP was 24,740±1,350mmHg/min. In the presence of 10μM 8-PT these values were significantly ($P<0.001$) reduced to 6.32±0.60ml/min/g and 13,290±1,728 mmHg/min respectively. The subsequent administration of glibenclamide (10μM), in addition to 8-PT, brought about further decreases in these variables to 3.05±0.55ml/min/g and 6,085±1,267mmHg/min. These values were similar to those obtained in the presence of glibenclamide alone.

The results of this study clearly indicate that both K_{ATP}-channels and endogenous adenosine make substantial contributions towards the regulation of coronary flow in the isolated perfused rat heart. The possibility that adenosine is acting through K_{ATP}-channels is currently being investigated.

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174P MODULATION OF VASORELAXATION TO POTASSIUM CHANNEL OPENERS BY BASAL NITRIC OXIDE IN THE ISOLATED PERFUSED RAT SUPERIOR MESENTERIC ARTERIAL BED

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Recent evidence has shown that inhibition of basal nitric oxide production augments vasodilatation to the potassium channel opener (PCO), levcromakalim, in the rabbit ear vascular bed (Randall *et al.*, 1994) and the porcine cardiovascular system (Herity *et al.*, 1994). The present investigation was carried out to examine whether nitric oxide also modulates responses to PCOs in the rat mesenteric arterial bed. To achieve this the vasorelaxant properties of levcromakalim and pinacidil were assessed in the absence and presence of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME).

Male Wistar rats (200-390g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1} i.p.) and the mesenteric arterial bed was cannulated and perfused with oxygenated Krebs-Henseleit solution at 5 ml min^{-1} (Randall & Hiley, 1988). Perfusion pressure was continuously monitored by a pressure transducer placed close to the inflow cannula. Following 30 min equilibration, perfusion pressure was raised (ca. 100mmHg) by addition of methoxamine (1-100μM) and dose-response curves were constructed for the vasodilators in the absence or presence of 100μM L-NAME. In the presence of L-NAME the concentration of methoxamine was reduced, compared to control conditions, to give an equivalent level of tone.

In control preparations basal perfusion pressure was 22.9±4.2mmHg (mean±s.e.mean, n=20) and was increased by 91.3±4.9mmHg following the addition of methoxamine. In preparations treated with L-NAME basal perfusion was 25.3±3.2mmHg and was increased by 107±6mmHg following the addition of methoxamine.

Levcromakalim caused dose-related relaxations of established tone (ED_{50}=4.47±0.70nmol, R_{max}=104±5%, n=10). In 6 preparations treated with L-NAME levcromakalim was significantly ($P<0.01$) more potent (ED_{50}=1.73±0.26nmol), while the maximum relaxation was not significantly altered (R_{max}=101±5%). Pinacidil similarly gave rise to dose-related relaxations of tone (ED_{50}=16.0±4.7nmol, R_{max}=93.2±2.0%, n=6). In the presence of L-NAME, pinacidil was also significantly ($P<0.05$) more potent (ED_{50}=5.43±1.10nmol) and the maximum response was also significantly ($P<0.01$) increased (R_{max}=103±3%, n=6). By contrast the vasorelaxant responses to papaverine were not significantly influenced by L-NAME. In control preparations the dose-response curve for papaverine was described by an ED_{50}=15.6±1.3nmol and R_{max}=96.2±0.9% (n=4) and in the presence of L-NAME the variables were : ED_{50}=19.3±1.2nmol, R_{max}=101±1% (n=4).

The results demonstrate that the vasorelaxant responses to the PCOs, levcromakalim and pinacidil, are augmented following inhibition of basal nitric oxide release. However, the relaxant effects of papaverine were unaffected by the presence of L-NAME, indicating that following inhibition of nitric oxide synthesis the vasorelaxant capacity of the vasculature is not altered. Therefore, the results indicate that basal nitric oxide release modulates the activity of potassium channel openers.

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175P TWO COMPONENTS OF DELAYED RECTIFIER CURRENT, I_K, IN RABBIT SA NODE CELLS

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We have investigated the effects of dofetilide (Gwilt et al., 1990) a selective blocker of the fast component ($i_{K,r}$) of i_K to determine whether i_K in rabbit SA node cells comprises more than one component. Specific fast and slow components, $i_{K,r}$ and $i_{K,s}$, have been found in atrial and ventricular cells (Sanguinetti and Jurkiewicz, 1991, 1992) but in SA nodal cells, only $i_{K,r}$ has been reported (Verheijck et al., 1995).

Rabbits (7-900g.) were killed by cervical dislocation and SA node cells isolated using collagenase (Wako) and elastase (Serva); for details of method see Brown, 1992. Whole cell clamp was used (amphotericin permeabilized patches) with 300 nM Nisoldipine to block $i_{Ca,L}$. i_K activated during depolarizing clamp pulses of different durations from -40mV to +40mV was measured (i_{Kpulse}) and also the peak current on return to -40mV (i_{Ktail}). The ratio i_{Ktail}/i_{Kpulse} will be constant regardless of pulse duration if i_K represents the activation of a single current component ('envelope of tails' test; Sanguinetti and Jurkiewicz, 1990).

In control, however, i_{Ktail}/i_{Kpulse} altered with pulse duration (Fig 1, filled circles), indicating the presence of two subtypes of i_K. In the presence of 200nM dofetilide, (Fig 1, unfilled circles) i_{Ktail}/i_{Kpulse} became constant and independent of pulse duration indicating that a fast component of i_K had been blocked, leaving a slower component.

Fig 2 shows the effect of dofetilide on spontaneous SA node cell activity. Values for control and 200nM dofetilide (mean ± S.E.M., student's paired t-test, n = 5): maximum diastolic potential: -52.2 ± 4.1 mV and -46.8 ± 2.1 mV ($p>0.05$); AP amplitude: 83.8 ± 3.7 mV and 74.4 ± 6.8 mV ($p>0.05$); cycle length: 226.6 ± 18.2 ms and 256.6 ± 18.6 ms ($p<0.05$); APD_{100}: 166.8 ± 18.3 ms and 189.6 ± 12 ms ($p<0.05$).

Thus the i_{Ktail}/i_{Kpulse} data indicate the presence of two components of i_K in rabbit SA node cells. Block of $i_{K,r}$ by dofetilide slows spontaneous SA node cell activity.

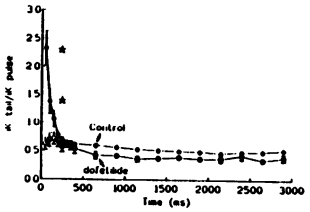

Fig.1. Ratio i_{Ktail}/i_{Kpulse} measured in response to clamp pulses from -40mV to +40 mV. Filled circles: control (n=14). Unfilled circles: with 200nM dofetilide (n=5). 300nM Nisoldipine present. 35°C. Results are means, error bars = SEM. ★indicates $p<0.05$ (student's independent t test).

Fig.2. Spontaneous activity of an SA node cell. Arrowed trace: after 2 minutes exposure to 200nM dofetilide.

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176P POTASSIUM CHANNEL MODULATION BY ZD6169, LEVCROMAKALIM AND NS 1619 IN RAT AND HUMAN BLADDER DETRUSOR MUSCLE

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Cromakalim abolishes the spontaneous mechanical activity of human and rat bladder strips (Foster *et al.*, 1989; Malmgren *et al.*, 1989) and as we have previously shown, levcromakalim induces a glibenclamide-sensitive outward current in rat detrusor cells (Green *et al.*, 1995). In the present study the effects of ZD6169 (Grant *et al.*, 1994), levcromakalim and NS 1619 (Edwards *et al.*, 1994) on K-currents in rat and human detrusor were compared using the whole-cell voltage-clamp technique.

Single smooth muscle cells were isolated from segments of rat and human detrusor by enzymatic treatment. Under Ca-free conditions, stepping to a series of potentials from a holding potential of -90 mV induced the generation of voltage-sensitive, outward K-currents. In both rat and human cells these comprised an A-current ($I_{K(A)}$) and a delayed-rectifier current ($I_{K(V)}$). When cells were held at -10 mV, non-inactivating currents were generated. On clamping rat cells at -10 mV and stepping to a series of test potentials, the resulting I-V relationship was inwardly-rectifying at potentials negative to -60 mV. In contrast, inward rectification was not exhibited by human detrusor cells under these conditions.

In both rat and human detrusor cells held at -10 mV, ZD6169 (10μM) and levcromakalim (10μM) each induced a glibenclamide (10μM)-sensitive, noisy, non-inactivating K-current ($I_{K(ATP)}$). The zero current potential hyperpolarised by 18-25 mV. On stepping from a holding potential of -90 mV, both ZD6169 and levcromakalim simultaneously decreased $I_{K(V)}$.

In both rat and human detrusor cells held at -10 mV, NS 1619 (33μM) increased current noise with relatively little change in the holding current at this potential. This was in marked contrast to the actions of either ZD6169 or levcromakalim. However, on stepping to test potentials positive to 0 mV, the magnitude of the evoked current and associated noise were markedly increased. These effects of NS 1619 were inhibited by iberiotoxin (250nM) indicating an increase in the current ($I_{BK(Ca)}$) flowing through large conductance, Ca-sensitive K-channels ($BK_{(Ca)}$). $I_{BK(Ca)}$ was unaffected by ZD6169 or levcromakalim (each up to 10μM).

These data demonstrate that both ZD6169 and levcromakalim selectively induce $I_{K(ATP)}$ in rat and human detrusor muscle. These effects are not shared by NS 1619 which opens BK_{Ca} channels in these tissues.

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177P PHOSPHOLIPASE A_2 ACTIVITY IN HUMAN COLONIC MUCOSA

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Phospholipase A2 activity in human colonic mucosa has not been fully characterized. Minami *et al.* (1993) using immunoblot analysis suggested a type II PLA_2 was present. PLA_2 activity is elevated in intestinal mucosa in inflammatory bowel disease (Olaison *et al.*, 1989). Type I and Type II (secretory) PLA_2 have no preference for AA-containing phospholipids, are sensitive to dithiothreitol (DTT), are acid resistant and heat resistant at 57°C and require millimolar concentrations of Ca^{++} for optimal activity (Mayer & Marshall, 1993). We have characterized the PLA_2 in macroscopically normal sigmoid colonic mucosa from resections for carcinoma. After homogenizing in 50mM Tris buffer (pH7.5) containing proteinase inhibitors and centrifuging at 10,000xg the supernatant was divided into 100,000xg cytosolic and microsomal fractions. PLA_2 activity was assessed using as substrate E. coli (K12 C600 strain) phospholipids labelled with [^{3}H]oleate ([^{3}H]OA) or [^{14}C] arachidonate ([^{14}C]AA). On TLC the majority of the label was found to be incorporated into phosphatidylethanolamine. The reaction mixture (250µl) contained 50mM Tris-HCl (pH 7.5) plus substrate (10nmol phospholipid/phosphorus) and variable amounts of PLA_2. Incubations were for 15min with 10mM Ca^{++}. In 'micellar' experiments deoxycholate solubilized [^{14}C]dioleoyl phosphatidylcholine ([^{14}C]OAPC) or stearoyl [^{14}C]arachidonyl phosphatidylcholine ([^{14}C]AAPC) labelled in the sn-2 position was utilised as a substrate (2.4nmols phospholipid/ inorganic phosphorus in 150µl). Using [^{3}H]OA E.coli microsome and cytosol PLA_2 activity was related to protein added between 12.5µg and 50µg but with further protein added up to 200µg, PLA_2 activity did not increase. Hydrolysis with 50µg protein was 24.7% ± 2.3 (mean s.e., n=3) in cytosol and 62.7% ± 3.6 (n=3) in microsomes. PLA_2 activity was abolished in the absence of Ca^{++} plus 1mM EGTA. In one tissue PLA_2 activity was assessed in cytosol and microsomes (50µg protein) using EGTA-regulated Ca^{++} buffers. With 10mM Ca^{++} hydrolysis was 68.3% in microsome and 33.72% in cytosol and with 87nM free Ca^{++} was 55.4% and 31.0% respectively. Preincubation at 57°C for 5min of the cytosol or pancreatic PLA_2 (type I; 200ng protein) resulted in 40.3% ± 5.5 (n=4) loss of activity in the cytosol but negligible loss with pancreatic PLA_2 (2.3%, n=2). Cytosol was treated with H_2SO_4 (pH 1.8) for 1h at 4°C and adjusted to pH 7.5. PLA_2 activity was increased to 138.6 % ± 24.5 of control (n=3). Using micellar assay PLA_2 activity in cytosolic fraction (200µg protein) hydrolysed 4.7% ± 0.5 of [^{14}C]AAPC and 2.5% ± 0.4 of [^{14}C]OAPC (n=4 ; $p< 0.014$). But using E.coli no preference for [^{14}C]AA was seen. With micellar [^{14}C]AAPC and cytosol (200µg protein) or pancreatic PLA_2 (100ng protein) dithiothreitol (DTT, 10 mM) reduced PLA_2 activity by 59.8% ± 3.9 (n=4) and 64.1 % ± 4.4, respectively. Our data do not unequivocally confirm the presence of one secretory isoform of PLA_2 in colonic mucosa. There may coexist more than one isoform with different properties or an atypical isoform. Further characterisation is under way.

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178P TETRODOTOXIN DOES NOT INHIBIT THE SHORT-CIRCUIT CURRENT RESPONSE OF HUMAN ILEAL MUCOSA TO CHOLERA TOXIN

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The action of cholera toxin (CT) was originally thought to involve only mucosal epithelial cells, where it was shown to increase intracellular cyclic AMP and cause fluid secretion (Kimberg *et al.*, 1971). More recent evidence suggests the intestinal secretion induced by CT involves local neural mechanisms, and that the myenteric plexus is indispensable for the secretory reflex initiated by CT (Jodal *et al.*, 1993). The aim of the present investigation was to determine whether neural elements were required for cholera toxin to produce a secretory effect in human ileum. Muscle-stripped preparations of human terminal ileal mucosa were mounted in Ussing chambers in which mannitol (11.5 µM) replaced glucose in Krebs fluid bathing the apical side of the tissue. Transmucosal short-circuit current (SCC) was continuously monitored and taken as a measure of electrogenic fluid secretion. After 1h equilibration, CT (10 μgml^{-1}, Swiss Serum & Vaccine Institute, Berne) or control vehicle (H_2O) were added to the solution bathing the apical side and changes in SCC monitored for a further 3h. Finally, a maximally effective concentration of forskolin (25µM) was applied to the basolateral side. Statistical analysis used the Mann-Whitney U test, with $p<0.05$ taken to indicate a significant difference. Data are expressed as mean±s.e.mean.
CT produced a marked but delayed increase in SCC of human ileal mucosa. The increase was not significantly different from controls until 120min had elapsed. Peak effect was observed at 150 min at which point SCC had increased from 57.7±7.6 to 132.4±14.4μAcm^{-2} (n=5). Comparable figures for controls were 62.9±13.6 to 71.4±11.4μAcm^{-2} (n=5). Tetrodotoxin (TTX, 3.1µM both sides of the membrane given 15min before CT) caused a rapid decrease in basal SCC from 71.4±19.7 to 27.8±6.9μAcm^{-2} (n=3). The peak effect of CT was not inhibited in the presence of TTX, SCC having increased from 17.8±4.4 to 152.9±27.9μAcm^{-2}. This concentration of TTX abolished responses to electrical field stimulation (Burleigh & Borman, 1993). Forskolin produced a significantly greater increase in SCC of control tissues compared to CT-treated tissues (64.0±12.2 compared to 23.1±4.5μAcm^{-2}, $p<0.05$). However, the absolute levels of SCC attained in preparations exposed to forskolin plus CT or forskolin alone were not significantly different ($p>0.05$).
In conclusion CT elicited a marked secretory effect in preparations of human terminal ileal mucosa unattached to the myenteric plexus. Although the submucosal plexus was present, neuronal involvement in the response to CT was discounted on the basis of insensitivity of the response to TTX. Furthermore, as the actions of CT and forskolin were not additive, it is probable that both were acting through the same mechanism, namely elevation of cyclic AMP.

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179P L-ALANINE SCAN OF NEUROPEPTIDE Y: EFFECTS OF ALA-ANALOGUES UPON ELECTROGENIC ION TRANSPORT IN RAT JEJUNUM MUCOSA

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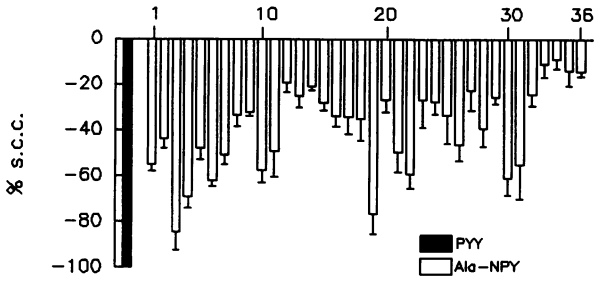
Systematic exchange of single residues along the length of neuropeptide Y (1-36) (NPY) with L-alanine (Ala) provides a means of evaluating the importance of each amino-acid residue (and associated side-chains) in NPY-receptor activation. The four native Ala residues located at positions 12, 14, 18, 23 were substituted by glycine. Previous studies with these Ala-NPY analogues have shown significant differences between structure-activity relationships in tissues expressing either Y_1- or Y_2-receptors (Beck-Sickinger *et al.*, 1994). Here we describe preliminary data with a complete Ala-scan of NPY, in preparations of rat jejunum *in vitro*.

Peptides were synthesised by a combination of automated and solid-phase peptide synthesis, purified by preparative HPLC and analysed by various techniques including electrospray mass spectrometry (Beck-Sickinger *et al.*, 1994). Peptide purity was > 95% and all stocks were stored at -20°C, undergoing no more than one freeze-thaw cycle before use. Preparations of rat (male, Sprague-Dawley, 250-400g) jejunum mucosa were voltage-clamped in Ussing chambers and changes in short-circuit current (s.c.c.) monitored as described previously (Cox *et al.*, 1988). Single additions of Ala-NPY analogues (300 nM) were made to the basolateral reservoir and once the reduced s.c.c. was stable (5-15 min) 100 nM peptide YY (PYY) was added as an internal control. This concentration of PYY produces maximal antisecretory effects in jejunum mucosa (Cox et al., 1988). The cumulative reduction in s.c.c. was recorded as 100% and responses to Ala-analogues calculated as a % of the respective control. Figure 1 shows the mean - 1 s.e.m. (n=4-6) of responses obtained for each Ala-substitution compared with the PYY control (100 nM, 100%). From this single concentration-response data, significant reductions ($P<0.001$, unpaired Student's *t*-test) in biological activity can be seen when residues 2, 5, 8, 9, 11-18, 20, 23-29, and 32-36 inclusive, were replaced. The majority of these residues are conserved between NPY, PYY and pancreatic polypeptide and are either hydrophobic, interdigitating residues found within the core formed by the antiparallel polyproline and α-helices, or are located in the C-terminal region (Schwartz et al., 1990).

Fig. 1 Structure–activity relationship for L–alanine substituted NPY analogues compared with PYY



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180P THE EFFECT OF DEOXYCHOLIC ACID ON THE HYPOTENSIVE RESPONSE TO COLORECTAL DISTENSION IN THE ANAESTHETISED RAT

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In the pentobarbitone-anaesthetised rat, colorectal distension elicits a reproducible fall in arterial blood pressure, which is regarded as the vasomotor response to a noxious stimulus (Ness & Gebhart, 1988). We have investigated the ability of the bile salt, deoxycholic acid (DCA), to sensitise the colorectum of the rat to distension, since in man it has been reported to reduce the rectal distension threshold for balloon perception and urge to defaecate (Edwards *et.al.*, 1989). We have also investigated the possible role played by mast cells in mediating this effect.

Male Wistar rats (220-260g) were lightly anaesthetised with sodium pentobarbitone (60mgkg^{-1}ip) and prepared for measurement of mean arterial blood pressure (MAP). A latex balloon was inserted into the colorectum. Balloon inflation (0.5-2.5ml) at five minute intervals elicited reproducible, volume-dependent falls in MAP, so that distension-effect curves could be constructed. DCA (1.7ml, 60mM) was administered intra-rectally 30min before construction of the final distension-effect curve. All data expressed as mean ± s.e. mean. The paired student t-test was used for statistical comparisons ($p<0.05$ significant). DCA significantly increased the hypotensive responses to colorectal distension [from 23.6±2.8 (non-DCA treated) to 32.1±5.4mmHg (DCA) at 1.5ml distension volume, $p<0.05$, n=6], with no significant effect on resting MAP or intra-balloon pressures. Histological analysis of colorectal tissue showed that repetitive balloon distension produced mild oedema, whilst intraluminal irrigation with DCA resulted in some loss of epithelial cells and infiltration of neutrophils. Mucosal mast cell levels were high when compared with connective tissue mast cells, (CTMC), in both control and DCA-treated animals.

The mast cell stabiliser doxantrazole (10mgkg^{-1}iv), completely inhibited the sensitisation effect when administered 10min prior to DCA [23.6±2.7mmHg (non-DCA treated) compared to 21.0± 2.3mmHg (doxantrazole/ DCA) at 1.5ml distension volume, n=5]. In control animals, doxantrazole had no effect on the hypotensive response to distension. Sodium cromoglycate, a CTMC stabiliser, (10mgkg^{-1}iv) appeared to inhibit the DCA-induced sensitisation [from 24.5±3.1 (non-DCA treated) to 18.0 ±3.8mmHg (DCA/ cromoglycate) at 1.5ml, n=6], however, it also inhibited the hypotensive responses in control animals [from 16.5±4.2 to 2.5±2.1mmHg at 1.5ml, $p<0.05$, n=5], indicating that these effects were not selective for DCA. Compound 48/80 (1mgkg^{-1}, intrarectal administration), a CTMC degranulator, produced a transient fall in MAP (15±4.9mmHg) but did not enhance the hypotensive response to colorectal distension (n=5).

In conclusion, DCA sensitised the rat colorectum to the effects of luminal distension. Selective blockade of the hypotensive response by doxantrazole suggests that this effect may be mediated via mast cell activation, although connective tissue mast cells do not appear to be involved.

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181P EFFECT OF HYPOXIA AND HYPOXIC PRECONDITIONING ON BARRIER FUNCTION OF ISOLATED RAT COLONIC MUCOSA

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Ischaemia may play a role in intestinal mucosal damage in inflammatory bowel disease (Gibson, 1994) and NSAID-induced enteropathy (Taha *et al.*, 1993). The aims of this study were to examine i) if hypoxia (a component of ischaemia) can directly affect the barrier function of isolated colonic mucosa, and ii) if the effect of hypoxia can be attenuated by prior exposure to a short period of hypoxia (a phenomenon described in other organ systems, and known as *preconditioning*, Downey *et al.*, 1993). We have measured electrical resistance (R_t) across the mucosa as an indicator of mucosal barrier function. Statistical significance was tested by t-test.

Rats (AHA, female, 200-250g) were killed and the descending colon removed. The serosal layers of a 2cm section were removed and the mucosa mounted in Ussing chambers (0.64cm^2 exposed mucosa). Tissues were maintained in Krebs-Henseleit solution (37°C, gassed with 5% CO_2 in O_2 for normoxia). R_t was measured using $Ag/AgCl_2$ electrodes connected to an epithelial voltohmeter (WPI) applying an alternating current (±20μA, 12.5Hz). Hypoxia was induced by gassing with 5% CO_2 in N_2.

Initial R_t was 84±3Ω.cm^2 (n=96). All subsequent resistances are quoted as % initial tissue resistance (mean ± standard error). Hypoxia caused a fall in R_t (71±10%, 34±5% & 24±4% at 15, 30 & 60 min after induction of hypoxia, n=5). If normoxic conditions were restored (for 60 min), then R_t recovered, though the extent of the recovery diminished as the duration in hypoxia increased (R_t 102±5%, 74±9% & 59±4% after 15, 30 or 60 min hypoxia, followed by 60 min normoxia, n=5).

The potential for *preconditioning* was investigated by exposing tissues to 15 min hypoxia, followed by 60 min normoxia and then 60 min hypoxia. These tissues were compared to time-matched control tissues not subjected to the initial 15 min hypoxia. In controls, R_t fell during the 60 min hypoxia (84±7% to 42±5%, n=11), whereas in the *preconditioned* group, R_t fell during the initial 15 min hypoxia (to 47±6%, n=11), recovered after 60 min normoxia (86±5%, n=11, $P>0.05$ vs. control), but was unchanged at the end of the 60 min hypoxia (86±5%, n=11, $P<0.001$ vs. control). Hence, an initial short period of hypoxia, followed by recovery in normoxia, protects the tissue against a subsequent longer exposure to hypoxic conditions.

These results demonstrate that the barrier function of isolated rat colonic mucosa is sensitive to hypoxia, and that the effect of prolonged hypoxia may be attenuated by a prior, brief, episode of hypoxia.

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182P INTERACTIONS OF SUPEROXIDE ANION WITH NITRIC OXIDE PRODUCED BY THE INDUCIBLE NITRIC OXIDE SYNTHASE IN RAT ISOLATED AORTA

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Expression of inducible nitric oxide synthase (iNOS) can be stimulated in an isolated artery (Rees *et al.*, 1990) by the presence of contaminating endotoxin in the Krebs solution. If expression of iNOS took place in the vascular smooth muscle cells then nitric oxide would be expected to be produced and promote relaxation within the same cells. Alternatively, if expression of iNOS took place in another cell type in the vessel wall, nitric oxide would need to diffuse from that cell to the smooth muscle in order to promote relaxation. On the basis that superoxide anion destroys nitric oxide, we attempted to establish which of these alternatives was correct by comparing the actions of hypoxanthine/xanthine oxidase, which generates superoxide extracellularly, with those of LY 83583 (Mülsch *et al.*, 1989), which generates superoxide both extracellularly and intracellularly.

Male Wistar rats were killed by stunning and exsanguination. The thoracic aorta was removed and cleared of fat and connective tissue, and the endothelium was removed by gently rubbing the intimal surface with a match stick. Endothelium-denuded aortic rings were suspended in Krebs at 37°C for tension recording. Some rings were incubated overnight (18 h) in Krebs at 37°C prior to tension recording. Expression of iNOS was assessed indirectly by the depression of phenylephrine (PE)-induced contraction. Results are expressed as means ± s.e. mean and were compared using one-way analysis of variance followed by Fisher's test. A probability of less than 0.05 was taken as significant.

Freshly isolated aortic rings contracted in a concentration-dependent manner to PE (1 nM - 10 μM; maximum contraction 1.4 ± 0.1 g, n=16). Following overnight incubation, the maximal contraction to PE was reduced to 0.4 ± 0.1 g (n=8) but this depression was reversed (maximum contraction 1.3 ± 0.1 g, n=4) upon addition of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM), to the tissue bath. Furthermore, the depression of PE-induced contraction was prevented (maximum contraction 0.8 ± 0.1 g; n=6) if the endotoxin scavenger, polymixin B (30 μg ml^{-1}), was present during the overnight incubation. Hypoxanthine (100 μM)/xanthine oxidase (16 mu ml^{-1}), which generates superoxide anion only extracellularly, had no effect on the depression of PE-induced contraction when added to the tissue bath. In contrast, LY 83583 (0.1-1 μM), which generates superoxide anion both extracellularly and intracellularly, produced a concentration dependent reversal of the depression of PE-induced contraction (maximum contractions 0.9 ± 0.1 g, n=7; 1.25 ± 0.1 g, n=5 and 1.4 ± 0.1 g, n=8; in the presence of LY 83583 0.1 μM, 0.3 μM and 1 μM, respectively).

The depression of PE-induced contraction following overnight incubation of rat aortic rings appears to be due to expression of iNOS stimulated by the presence of contaminating endotoxin since it was reversed by L-NAME and polymixin B. The ability of LY 83583 but not hypoxanthine/xanthine oxidase to reverse the depression of PE-induced contraction suggests that nitric oxide is produced and acts within the same smooth muscle cells.

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183P INVOLVEMENT OF TYROSINE KINASE AND PROTEIN KINASE C IN THE INDUCTION OF NITRIC OXIDE SYNTHASE BY LPS AND IFN-γ IN J774 MACROPHAGES

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Macrophages are activated upon exposure to a number of immunological stimuli including bacterial lipopolysaccharide (LPS) and certain cytokines including interferon-gamma (IFN-γ) (Stuehr & Marletta, 1987). These stimuli result in expression of the inducible form of the enzyme nitric oxide synthase (iNOS), leading to production of NO by the cells. Some studies suggest that protein kinase C (PKC)(Severn *et al.*, 1992) is involved in iNOS expression, whereas others suggest involvement of tyrosine kinase (Marczin *et al.*, 1993). The aim of this study was to determine the extent to which activation of PKC and tyrosine kinase contribute to the expression of NO synthase in the J774 macrophage cell line.

J774.7 cells were cultured at 37°C in Techne stirrer bottles. On the day of use, the cells were counted using a haemocytometer, washed with saline and seeded into Costar 24-well plates at a density of 10^6 cells ml^{-1}. Drugs were added and incubation continued for approx. 24 hours. NO production was determined by measuring accumulation of nitrite, its major oxidation product, in the medium bathing the cells by use of the Griess reaction. All results are expressed as the mean ± s.e. mean of n≥6 observations and comparisons were made using one-way analysis of variance followed by Fisher's test. A probability of 0.05 or less is considered significant.

The accumulation of nitrite into the medium bathing unstimulated J774 cells over a 24 hour period was 0.8 ± 0.1 μM. Cells stimulated with 100 ng ml^{-1} LPS and 10 u ml^{-1} IFN-γ produced nitrite levels of 33.5 ± 0.3 μM. Addition of the tyrosine kinase inhibitors, herbimycin A ($5.2x10^{-9}$-$2.7x10^{-7}$ M) and genistein (10^{-7}-10^{-4} M), produced concentration-dependent inhibition of nitrite accumulation stimulated by LPS and IFN-γ: herbimycin A produced a maximum inhibition of 96.1 ± 10.4% at a concentration of $5.2x10^{-7}$ M, and genistein produced a maximum inhibition of 64.8 ± 2.2 % at 10^{-4} M. Two inhibitors of PKC, staurosporine (10^{-9}-$3x10^{-6}$ M) and Ro 31-8220 ($3x10^{-9}$-10^{-5} M) each produced a concentration-dependent inhibition of nitrite accumulation, with maximum inhibition of 96.5 ± 21.3 % at $3x10^{-6}$ M and 73.2 ± 14.2 % at 10^{-5} M, respectively. The highly selective inhibitor of PKC, chelerythrine chloride (10^{-8}-10^{-5} M), had only a small inhibitory effect of 24.0 ± 1.4 % at $3x10^{-5}$ M. Herbimycin A ($1.7x10^{-7}$ M) which inhibited nitrite accumulation by 29.4 ± 1%, and staurosporine ($3x10^{-8}$ M) which produced inhibition of 21.8 ± 1.3 %, together produced additive inhibition (67.1 ± 4.5 %). Combined treatment of herbimycin A with the other inhibitors of PKC, Ro 31-8220 ($3x10^{-6}$ M) and chelerythrine chloride (10^{-5} M), failed to produce additive inhibition.

These results demonstrate that stimulation of tyrosine kinase is an important component in the induction process of NO synthase in J774 cells following stimulation by LPS and IFN-γ. The majority of findings also suggests the involvement of protein kinase C, but future work will be necessary to explain fully the complex interplay between these two second messenger pathways in the regulation of NO synthase expression.

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184P 3-AMINO-1,2,4-TRIAZOLE SELECTIVELY INHIBITS RELAXATION BY HYDROXYLAMINE AND SODIUM AZIDE BUT NOT HYDROGEN PEROXIDE IN RAT AORTA

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Catalase, the enzyme that promotes the breakdown of hydrogen peroxide to water and oxygen, also has peroxidase activity, i.e. it catalyses the oxidation of various substrates in the presence of hydrogen peroxide (Theorell & Ehrenberg, 1952). Two well characterised substrates are the nitrovasodilators, azide and hydroxylamine. In addition to its actions as an oxidant, hydrogen peroxide induces vascular relaxation in endothelium-denuded preparations through activation of soluble guanylate cyclase. It has been proposed that the presence of catalase is vital for hydrogen peroxide-induced stimulation of soluble guanylate cyclase (Burke & Wolin, 1987). We made use of an inhibitor of catalase, 3-amino-1,2,4-triazole (AT, Margoliash & Novogrodsky, 1958), to test the hypothesis that metabolism by catalase was necessary in order to express the relaxant activity of azide, hydroxylamine and hydrogen peroxide.

Male Wistar rats were killed by stunning and exsanguination. Aortic rings (2.5 mm wide) were suspended in tissue baths containing Krebs solution at 37 °C for isometric tension recording. The endothelium was removed from rings by gently rubbing the intimal surface. The effects of inhibiting endogenous catalase with AT (1-50 mM, 90 min) were studied on relaxations of endothelium-denuded rings induced by hydroxylamine, sodium azide, glyceryl trinitrate and hydrogen peroxide following precontraction with phenylephrine (PE, 10-30 nM). All results are expressed as the mean ± s.e. mean for n ≥5 experiments and comparisons were made using one-way analysis of variance followed by Fisher's test. A probability of 0.05 or less is considered significant.

Hydrogen peroxide (10 μM-1 mM) produced powerful relaxation in endothelium-denuded rings of rat aorta (maximum relaxation 96.0 ± 1.6 %). Methylene blue (30 μM), an inhibitor of soluble guanylate cyclase, powerfully inhibited maximum relaxations induced by hydrogen peroxide in endothelium-denuded rings (to 40 ± 1.2 %). Pretreatment of endothelium-denuded rings with catalase (1000 u ml^{-1}) led to to a 50.4 ± 0.5 % reduction in the maximal relaxation induced by hydrogen peroxide. The presence of AT (50 mM) together with catalase during a 30 min preincubation partially blocked (by 47.0 ± 0.32 %) the ability of catalase to inhibit hydrogen peroxide-induced relaxation. After 90 min preincubation, however, a complete blockade of the ability of catalase to inhibit relaxation was seen. Pretreatment of rings with AT (1-50 mM) to inhibit endogenous catalase shifted the concentration-reponse curves for hydroxylamine and sodium azide to the right in a concentration-dependent manner (maximum shift for hydoxylamine and azide was 189.6- and 1668.1- fold, respectively). In contrast, AT (50 mM, 90 min) had no effect on relaxation induced by glyceryl trinitrate (1-100 nM) or hydrogen peroxide (10 μM-1 mM).

The data suggests that catalase plays a important role in the relaxations induced by hydroxylamine and sodium azide but not hydrogen peroxide or glyceryl trinitrate in isolated rings of rat aorta.

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185P EVIDENCE AGAINST A ROLE FOR SUPEROXIDE ANIONS IN N^G-NITRO-L-ARGININE METHYL ESTER-INDUCED CONTRACTIONS OF THE PORCINE ISOLATED SPLENIC ARTERY

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The ability of N^G-nitro-L-arginine methyl ester (L-NAME) to elicit endothelium-dependent contractions in the porcine isolated splenic artery suggests that basal release of nitric oxide from the endothelium directly, or indirectly, reduces vascular responsiveness (Lot *et al.*, 1993). Another endothelium-derived vasoactive substance, superoxide anions (SO), is thought to constrict blood vessels by a direct effect and also through the inactivation of nitric oxide (Katusic and Vanhoutte, 1989). In the present study, we have examined the evidence for SO contributing to L-NAME-induced contractions in the porcine splenic artery.

Porcine spleens were obtained from a local abattoir and 5mm endothelium-intact (E+) and endothelium-denuded (E-) segments of the splenic artery prepared for isometric tension recording as previously described (Lot *et al.*, 1993). All preparations were stimulated with 60mM KCl until consistent responses were obtained. In some preparations, a concentration-response curve to phenylephrine was constructed, while in others the effect of 10nM Substance P or 150u/ml superoxide dismutase (SOD) against submaximal contractions to phenylephrine was examined. For the SOD experiments, the subsequent effect of 100μM L-NAME was also assessed. The effect of the SO generating system (1,200 u/ml catalase, 10 mu/ml xanthine oxide and 100 μM xanthine; Katusic and Vanhoutte, 1989) was examined in both E+ and E- preparations at rest, prior to the addition of 100μM L-NAME. Responses have been expressed as a percentage of either the induced tone or the maximum response to either phenylephrine or 60mM KCl. These are shown as the mean ± s.e. mean of n observations and considered significantly different if $p < 0.05$ (Student t-test). The negative logarithm of the concentration of phenylephrine required to produce 50% of the maximum response (pD_2) was also determined.

Neither the potency (pD_2 values - 6.39±0.05 (E+) and 6.45±0.05 (E-), n=8) nor the maximum response (18.1±1.8 g wt. (E+) and 18.5±0.9 g wt. (E-), n=8) to phenylephrine were altered by removal of the endothelium. Responses to 0.3-0.5μM phenylephrine in E+ (37.6±6.4%, n=7) and E- (45.6±8.4%, n=5) preparations were inhibited in an endothelium-dependent manner (> 50%) by 10nM substance P. SOD (150u/ml) also produced endothelium-dependent relaxations (45.5±10.4%, n=7) of phenylephrine-induced tone, but with a slow time course (time to equilibrium - 15 min). Subsequent addition of 100μM L-NAME caused a sustained endothelium-dependent contraction equivalent to 261±29.1% (n=7) of the original phenylephrine-induced tone. In E- preparations, phenylephrine-induced tone was not significantly altered by either SOD or L-NAME (n=5). In E+ preparations at rest, exposure to the SO generating system produced a non-sustained contraction equivalent to 8.1±2.7% (n=10) of that produced by 60mM KCl (17.4±1.4 g wt. n=10). The addition of 100μM L-NAME, once the response had returned to baseline, was associated with a large, sustained contraction (14.3±.3.9% of the KCl response, n=10). Neither L-NAME nor the SO generating system affected E- preparations (<2% of the KCl response, n=5).

In summary, as illustrated by the action of SOD in the porcine isolated splenic artery, SO has the potential to limit the effect on vascular tone of basal, endothelium-derived nitric oxide. However, since the exogenous application of SO failed to elicit endothelium-independent contractions, it is unlikely that this anion contributes directly to L-NAME-induced contractions.

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186P EVIDENCE FOR α_2-ADRENOCEPTOR-MEDIATED INHIBITION OF FORSKOLIN-STIMULATED CYCLIC AMP IN THE PORCINE ISOLATED THORACIC AORTA

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Vasoconstrictor α_2-adrenoceptors have been reported to inhibit forskolin-stimulated, but not basal, cyclic AMP accumulation in the porcine isolated palmar lateral vein (Wright *et al.* 1995a). As such, this pathway is thought to have a subsidiary role in contractile events; the importance of which being determined by the prevailing level of cyclic AMP. Vasoconstrictor α_2-adrenoceptors have also been detected on porcine thoracic aorta (PTA) (Wright *et al.* 1995b), and in the present study we have sought to determine (i) whether these receptors are also negatively coupled to cyclic AMP accumulation and (ii) the relationship between the level of cyclic AMP, produced by forskolin, and the inhibition produced by the α_2-adrenoceptor stimulation.

Porcine aortae were obtained fresh, cleaned of adventitia and stored overnight at 4°C in modified Krebs-Henseleit. On the following day, [^{3}H]-cyclic AMP accumulation in 5mm squares of the PTA, in the presence or absence of forskolin, noradrenaline (NA) or UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate), was determined using the [^{3}H]-adenine prelabelling method as previously described (Wright *et al.* 1995a). Where appropriate, agonists were added 10 min before exposure to forskolin and the reaction terminated 5 min later by the addition of 1M HCl. [^{3}H] cyclic-AMP was separated from other [^{3}H] products by sequential Dowex/alumina chromatography (Salomon *et al.* 1974) and measured by liquid scintillation counting. All conditions were carried out in quadruplicate. [^{3}H] cyclic-AMP accumulation produced by forskolin has been expressed as a percentage of the conversion of [^{3}H]-adenine to cyclic-AMP, while the inhibition produced by α_2-adrenoceptor agonists has been expressed as a percentage inhibition of the response to forskolin. The values shown are the mean ± s.e. mean of n observations and differences between mean values are considered significant if $p < 0.05$ (Student t -test).

Forskolin (3-30μM) stimulated [^{3}H] cyclic-AMP accumulation in a concentration-dependent manner; 30μM forskolin causing a ±% conversion (n=5). Noradrenaline, and the α_2-selective agonist UK14304, produced concentration-dependent inhibition of forskolin (30μM)-stimulated [^{3}H] cyclic-AMP accumulation, with pIC_{50} values of 6.90±0.13 (n=3) and 7.55±0.20 (n=3), respectively. The maximum inhibition produced by noradrenaline (63.4±5.7%) and UK-14304 (70.3±1.6%) were not significantly different ($p>0.05$). In contrast, neither agonist (at 10^{-4}M) affected basal levels of [^{3}H] cyclic-AMP accumulation. In a larger series of experiments, the magnitude of the inhibition produced by 1μM UK-14304 (range 7.5% to 59.0%, mean 27.6 ± 3.2% n=16) was significantly ($p < 0.05$) but weakly correlated ($R = 0.26$) to the response elicited by 30μM forskolin (range 1.6-14.4%; mean conversion value 5.9±1.1%, n=16). However, when the concentration of forskolin was varied, no such relationship for the effect of 1μM UK-14304 was apparent; 38.5±6.2% (n=6) inhibition against the response to 3μM forskolin and 25.8±1.6% (n=6) inhibition against that produced 30μM forskolin

In the present study we have shown that α_2-adrenoceptors on the PTA, like those on the porcine isolated palmar lateral vein (Wright *et al.* 1995a) mediate the inhibition of forskolin-stimulated, but not basal, accumulation of cyclic-AMP, and that the magnitude of this effect is relatively independent of the elevation of cyclic AMP.

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187P RELATIONSHIP BETWEEN ELECTRICAL AND MECHANICAL ACTIVITY IN THE GUINEA-PIG ISOLATED TAENIA CAECI

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Field stimulation of the guinea-pig isolated taenia caeci has been shown to elicit inhibitory junction potentials (IJPs) and relaxation (Burnstock *et al.*, 1963). We have shown previously that apamin and ω-conotoxin GVIA (ω-CTX) reveal two different IJPs elicited by a single stimulus (Bridgewater *et al.*, 1995). Here we study the relationship between IJPs and mechanical responses.

Intracellular microelectrodes were used to record changes in membrane potential evoked by single (0.3 ms pw, 30 V) and trains of 10 stimuli at 0.5 - 10 Hz. Isometric tension recording was used to measure mechanical activity (1s trains, 0.2 ms pw, 20 V, 1 - 10 Hz). Data are expressed as mean ± S.E.M.. Statistical analysis was performed using Student's unpaired t test and all data were significant at $P < 0.05$. IJPs elicited by single stimuli had a latency and amplitude of 141 ± 4 ms and 15 ± 0.9 mV respectively (n = 12). In response to a train of stimuli, maximal hyperpolarization was achieved by the first pulse of the train. Facilitation of IJPs was not observed but fade occurred. At frequencies > 2 Hz the membrane potential remained hyperpolarized between stimuli and at frequencies of 4 and 10 Hz summation was seen.

Following cessation of stimulation, a rebound depolarization was observed. In mechanical experiments, basal tone was raised by the application of histamine (10 μM). Under these conditions, field stimulation evoked frequency-dependent relaxations which were maximal at 10 Hz. At frequencies of 8 and 10 Hz, rebound contractions followed the relaxation response. All responses, both mechanical and electrical, were abolished by tetrodotoxin (3 μM).

In the presence of apamin (30 - 100 nM) or ω-CTX (100 nM) the control IJP was abolished to reveal an underlying IJP of smaller amplitude (5.4 ± 0.5 mV, n = 8) and longer latency (231 ± 9 ms, n = 8). The characteristic features of the response to trains of stimuli also differed from control IJPs in that the membrane hyperpolarization was not seen to fade during stimulation and maximal hyperpolarization was often only achieved following the 7th pulse of the train. Interestingly, it was noted that following ω-CTX the rebound depolarization seen at higher frequencies of stimulation in controls and in the presence of apamin was abolished. Apamin and ω-CTX partially inhibited the mechanical relaxation response. Apamin was more potent, inhibiting relaxation at 10 Hz by 71 ± 8 % (15 strips from 4 animals) compared to 37 ± 7% for ω-CTX (17 strips from 4 animals). Apamin enhanced and ω-CTX abolished the rebound contraction.

Inhibitors of nitric oxide synthase reduce nerve mediated relaxations of the guinea-pig taenia (Poitrowski *et al.*, 1994). The effects of Nω-nitro-L-arginine (NOArg; 10 μM) were therefore studied. IJPs in the presence of apamin and ω-CTX were unaffected by NOArg. However, on average, nerve mediated relaxations were reduced by 27% (n = 32).

Thus, there are at least four mechanisms mediating relaxation in the guinea-pig taenia caeci, one of which is independent of any detectable change in membrane potential under the conditions of the present experiments.

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188P N^G-NITRO-L-ARGININE METHYL ESTER CAN ELICIT ENDOTHELIUM-INDEPENDENT CONTRACTIONS IN THE PORCINE ISOLATED CORONARY ARTERY

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Inhibitors of nitric oxide synthase produce endothelium-dependent contractions of the porcine isolated coronary artery (Pacicca *et al.*, 1992) and splenic artery (Lot and Wilson, 1994). In preliminary studies, however, we noted that N^G-nitro-L-arginine methyl ester (L-NAME) produced contractions in segments of the porcine coronary artery in which the endothelium had been removed. In the present study we have examined the pharmacological characteristics of these responses, and addressed the basis of the discrepancy between our results and those of Pacicca *et al.*, (1992).

Porcine hearts were obtained from a local abattoir and coronary arteries were dissected and stored overnight at 4°C in Krebs-Henseleit (K-H) saline (with 2% ficoll). The following day, endothelium-intact (E+) and endothelium-denuded (E-) segments (5mm in length) were prepared for isometric tension recording (see Lot and Wilson, 1994) and placed under a initial resting tension of 10 g wt. After establishing the maximal response to 60mM KCl, preparations (at rest) were exposed to putative inhibitors of nitric oxide synthase. For some vessels, 1μM dexamethasone was included in both the medium used for overnight storage and that employed for the experiment. Responses have been expressed as a percentage of the KCl (60mM, 14.4±1.1 g wt., n=28) response and are shown as the mean ± s.e. mean. Differences between mean values were considered significant if $p < 0.05$ (Student t-test).

Substance P (10nM) relaxed submaximal contractions to the thromboxane-mimetic U-46619 in E+ (> 80%) but not in E- segments (<5%). L-NAME (100μM) produced slowly developing contractions (time to equilibrium - 40min) in 20 out of 28 segments of the coronary artery (11.6±2.0%, n=20). The size of these contractions were approximately 50% of those observed in E+ segments (24.3±3.6%, n=28). L-arginine (1mM) relaxed L-NAME-induced responses (> 70%) in both E+ and E- preparations. Qualitatively similar contractions were elicited by 100μM L-nitro-arginine monomethyl arginine in E+ (17.9±2.7%, n=7) and E- (10.0±3.6%, n=7) segments, but not by 100μM aminoguanidine (<2% in E+ and E- segments, n=6), a putative, selective inhibitor of inducible nitric oxide synthase (Corbett *et al.*, 1992).

Contractions to 100μM L-NAME were significantly smaller in magnitude for both E+ (12.8±1.5%, n=8) and E- (2.9±1.1%, n=7) preparations examined on the day of collection. Furthermore, the presence of 1μM dexamethasone (during storage and the experiment) was associated with a significant reduction in the responses to 100μM L-NAME in E- (from 7.6±2.0% to 1.9±1.1%, n=8, $p<0.01$) preparations.

In summary, endothelium-independent contractions of the porcine isolated coronary artery to L-NAME are enhanced by overnight storage of the vessels, which may explain the discrepancy between our findings and those of Pacicca *et al.*, (1992). However, while these responses were suppressed by incubation with dexamethasone and by L-arginine, the pharmacological evidence does not support a role for the inducible form of nitric oxide synthase.

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189P INFLUENCE OF INDOMETHACIN AND N^{ω}-NITRO-L-ARGININE METHYL ESTER (L-NAME) ON THE RENAL EFFECTS OF ANGIOTENSIN II

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The renal haemodynamic effects of angiotensin II (AII) may be influenced by prostaglandins (Baylis & Brenner, 1978) or nitric oxide (NO; Sigmon et al., 1992), although changes in blood pressure or renin release may complicate results. We have examined the effects of intrarenal artery (i.r.a.) infusion of AII in captopril-pretreated, pentobarbitone-anaesthetised rats (male, AH/A, mean body weight 304±9g), in the presence of saline, indomethacin (INDO) or a non-pressor dose of L-NAME.

Mean blood pressure (MBP) was measured from the carotid artery. A catheter (tip diameter ≈0.2mm) was guided into the left renal artery via a femoral artery. The ureter was cannulated for determination of urine output (Uv), and renal blood flow (RBF) was measured from an ultrasonic flow probe around the left renal artery. Glomerular filtration rate (GFR, inulin clearance) and absolute sodium excretion (UNaV) were derived. Two groups of rats received INDO (10mgkg^{-1} i.v.) or L-NAME (10μgkg^{-1}min^{-1} i.v.) 30min after surgery; controls received saline only. Captopril (50μgkg^{-1}min^{-1} i.v.) was then administered to all rats. Forty-five minutes later, renal function was measured before (basal) and during infusion of AII (30ngkg^{-1}min^{-1} i.r.a.). Data are shown as arithmetic mean ± s.e.mean.

In control rats, AII decreased RBF, but did not affect GFR, Uv, or UNaV (Table 1). MBP was unchanged (from 114±9 to 113± 8mmHg). After INDO pretreatment, AII decreased RBF *and* GFR, with a small decrease in MBP (110±4 to 102±4mmHg; $P<0.05$), but Uv and UNaV were not significantly changed. In the presence of L-NAME, AII reduced RBF, GFR and Uv (Table 1). UNaV decreased, although not significantly ($P=0.07$). Although MBP fell (111±6 to 93±7mmHg; $P<0.05$), Uv of the right (non-infused) kidney was maintained at the basal level.

These data suggest that the glomerular effects of AII are opposed by cyclooxygenase-derived prostaglandins and NO. However, NO synthase inhibition also reveals AII-induced reductions in urine excretion.

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Table 1 : Renal function before (basal) and after AII 30ngkg^{-1}min^{-1} (+AII), in the absence and presence of INDO or L-NAME. * and # denote $P<0.05$ compared with Basal (Student's t-test for paired data) or Control (Student's t-test for unpaired data), respectively.

		RBF ml min^{-1}		GFR ml min^{-1}		Uv μl min^{-1}		UNaV μmol min^{-1}	
Group	n	Basal	+AII	Basal	+AII	Basal	+AII	Basal	+AII
Control	6	13.0±1.2	8.2±1.0*	1.3±0.1	1.2±0.1	34.5±6.7	35.5±9.4	6.8±1.6	6.9±1.9
+ INDO	5	12.0±2.5	8.3±2.1*	1.3±0.1	0.8±0.1*	37.7±5.5	28.3±3.5	6.8±1.6	5.0±0.9
+ L-NAME	7	7.9±1.9#	3.7±0.7*	1.4±0.2	0.7±0.1*	45.5±5.8	25.6±6.2*	7.7±1.0	5.1±1.3

190P ENDOTHELIUM-DEPENDENT RELAXATION, NITRIC OXIDE AND VASCULAR TONE IN CONTROL AND CHRONIC HYPOXIC RAT PULMONARY ARTERIES

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Pulmonary hypertension has been associated with disruption of the vascular endothelium. Impaired endothelium-dependent relaxations have been observed in human pulmonary artery rings from patients with chronic obstructive lung disease (Dinh-Xuan *et al.*, 1993), and in isolated lungs from rats with pulmonary hypertension due to exposure to chronic hypoxia (Adnot *et al.*, 1991). Alternatively it has been suggested that nitric oxide synthase (NOS) activity may be increased due to exposure to chronic hypoxia to compensate for the increase in vascular tone (Xue *et al.*, 1994). In this study, we examined the effects of chronic hypoxia on endothelium dependent relaxation, nitric oxide inhibition and vascular tone in rat extrapulmonary artery branches and pulmonary resistance arteries. Experimental male Wistar rats (age 30 days) were exposed to hypobaric hypoxia (10% O_2) for two weeks, with aged matched controls being maintained in normal room air. Rats were killed with sodium pentobarbitone and the heart and lungs removed. Right and left pulmonary artery branches (3-5mm i.d.)were dissected free and mounted in 5ml organ baths under 1.5g tension. Intralobal pulmonary resistance arteries (150μm i.d.) were dissected out and mounted on a wire myograph under appropriate tension. All vessels were bubbled with 16%O_2, 6% CO_2 balance N_2. Following 1 hour equilibration vessels were subjected to 1 of the following protocols a) preconstriction with 1μM noradrenaline (NA) followed by 1μM acetylcholine (ACh) to assess endothelium-dependent relaxation, b) inhibition of NOS with 100μM L-NAME c) addition of 1μM sodium nitroprusside (SNP) to assess the presence of endogenous tone (in endothelium denuded preparations). The results are shown in Table 1. From these results we can see that endothelium-dependent relaxation is decreased in the pulmonary artery branches of rats exposed to chronic hypoxia, however, in pulmonary resistance arteries the endothelium dependent relaxation is unaffected. L-NAME was shown to increase vascular tone to a greater extent in chronic hypoxic vessels compared to controls, and in a similar fashion relaxations to SNP were greater in chronic hypoxic vessels than in controls. The effect of L-NAME and SNP was greater in the large capacitance arteries. In conclusion our results suggest that pulmonary hypertension induced by chronic hypoxia causes an increased in endogenous tone. Agonist induced NO release is decreased only in the larger pulmonary artery studied. In both large and small pulmonary arteries from chronic hypoxic rats, basal NO release attenuates inherent tone present in these vessels.

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Table 1 a. Relaxation response to 1μM ACh in NA-precontracted PA rings as % of NA contraction. b. Contraction response to 100μM L-NAME as % 50mM KCl response. c. Relaxation to SNP as a % 50mM KCl response. PAB = pulmonary artery branch, PRA = pulmonary resistance artery. n/n = number of ring preparations/number of animals

Preparation	a. % relaxation	n/n	b. L-NAME response	n/n	c. SNP response	n/n
Control PAB	38 ± 3	28/14	26 ± 5	8/4	34 ± 8	12/6
Chronic hypoxic PAB	28 ± 3*	28/14	111 ± 19**	8/4	97 ± 16**	12/6
Control PRA	50 ± 2	30/15	3 ± 1	17/15	2 ± 1	14/14
Chronic hypoxic PRA	50 ± 2	30/15	13 ± 3**	17/15	7 ± 2**	14/14

Statistical difference using Students t-test for unpaired data. *$p< 0.05$, **$p<0.01$ chronic hypoxic vs control.

191P CEREBROVASCULAR RESPONSIVENESS TO NITRIC OXIDE SYNTHASE INHIBITION IN SPONTANEOUSLY DIABETIC RATS

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Impaired cerebrovascular responsiveness is associated with diabetes mellitus (*Mayhan et al., 1991*) and may reflect pathophysiological changes in the structure and function of cerebral blood vessels. The purpose of this investigation was to measure local cerebral blood flow (LCBF) in insulin-dependent, spontaneously diabetic (B/B) rats in order to assess firstly, whether the disease processes result in altered basal LCBF and secondly, by treating these rats with a nitric oxide (NO) synthase inhibitor, whether NO process might be involved in any dysfunction of cerebrovascular control.

Experiments were performed on conscious, lightly restrained, age-matched diabetic (DP, n=8) and diabetes-resistant (DR, n=8) B/B rats, 8-20 weeks after the onset of diabetes (median age at onset, 90 days). Diabetic animals received daily injections of insulin (2.4-4.0 IU, s.c.). The last injection was given 4hr before the measurement of LCBF was performed using the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique (*Sakurada et al., 1978*). Half of the animals from each group received N^G-nitro-L-arginine methyl ester (L-NAME) (30 mg/kg i.v.) 20 minutes prior to the measurement of LCBF. The other half received saline. Data (presented as mean ± SD) were analysed using the Bonferroni t-test correction for multiple comparisons. Acceptable significance was set at $P<0.05$.

No significant difference in plasma glucose was found between saline-treated groups, although in both DP and DR rats (226 ± 156 vs 204 ± 120 mg/dl) the coefficient of variation was high (>50%). Heart rate was significantly lower in the DP group compared to the DR group (315 ± 30 vs 405 ± 30 beats/min) whilst the haematocrit was higher (52.5 ± 2% vs 47.8 ± 1%). No differences in blood gas tensions or pH were found, but a base excess of 3.23 ± 1.8 in DP rats confirmed the metabolic disturbances of diabetes. In diabetic rats injected acutely with saline, LCBF was significantly lower in some regions (eg hippocampus 72 ± 8 vs 92 ± 8 ml.100g^{-1}.min^{-1}; globus pallidus 54 ± 6 vs 73 ± 2; lateral geniculate 109 ± 12 vs 142 ± 14) compared to DR controls. There was no correlation between plasma glucose and basal LCBF in individual rats. Injections of L-NAME reduced LCBF in most brain areas in DR rats, but had no significant effect in the DP group. This lack of response in diabetic rats was most pronounced in the striatum where LCBF following L-NAME treatment (84 ± 9ml.100g^{-1}.min^{-1}) was not significantly different from saline controls (87 ± 3ml.100g^{-1}.min^{-1}). There were no significant differences in mean arterial blood pressure between the L-NAME-treated groups (147 ± 8 vs 142 ± 4mmHg), suggesting that in the systemic circulation as a whole, L-NAME-induced vasoconstriction is not affected by the diabetic condition.

This study shows that basal LCBF is reduced in insulin-dependent diabetes. However the effects are regionally heterogeneous, possibly reflecting differential susceptibility to the disease in a sub-population of cerebral blood vessels. The attenuated response to L-NAME in diabetic rats is consistent with an involvement of the NO pathway in the observed perturbation of basal LCBF.

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192P EFFECT OF NITRO-L-ARGININE (L-NOARG) ON ENDOTHELIN-1 (ET-1) AND SARAFOTOXIN 6C (SX6C) RESPONSES IN RAT ISOLATED PERFUSED LUNGS

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Endothelins (ETs) cause vasodilation in many vascular beds indirectly via the production of nitric oxide (NO), this is associated with an activation of ET_B receptors (Warner et al., 1993; Karaki et al., 1993). However we have not seen ET-induced vasodilation in an isolated perfused lung model, probably because of low basal tone (Lal et al., 1995). In the present experiments the effects of ETs and SX6C were studied in perfused lungs with an elevated perfusion pressure.

Lungs from male Wistar rats (300-350 g) were isolated and set up as described previously to allow simultaneous measurements of pulmonary perfusion pressure (PPP), lung weight (LW) and pulmonary inflation pressure (PIP) (Lal et al. 1994). The actions of ET-1 or SX6C were studied under basal conditions (PPP 5 ± 0.5 mmHg ; mean ± SEM) or after elevation of PPP (9 ± 0.5 mmHg, n= 16) with an infusion of the thromboxane mimetic U46619 (40-80 nM). Data were analysed using the Student's t-test. Acceptable significance was set at $p< 0.05$.

When PPP was elevated with U46619, low doses of ET-1, ET-3 and SX6C (dose range 1.25-40 pmol) produced dose-dependent falls in PPP. ET-1 (ED_{50} 3.6 ± 0.6 pmol, n=5) was equipotent with ET-3 (ED_{50} 3.9 ± 1.05 pmol, n=4), whereas SX6C was significantly more potent (ED_{50} 2.2 ± 0.14 pmol, n=7; $p< 0.05$). In the presence of L-NOARG (100μM) these vasodilator responses were converted to vasoconstrictor responses, 20 pmol of ET-1 or SX6C now produced increases in PPP of 6 ± 2 mmHg and 5 ± 2.5 mmHg (n=4) respectively. This represents an approximate 5 fold increase in sensitivity, as under basal perfusion conditions 100 pmol of both these agents are needed to elicit similar increases in PPP (Lal et al., 1995). Perfusion of nitro-D-arginine (100 μM) had no effect on the ET-1 and SX6C mediated falls in PPP.

Under basal perfusion conditions the effects of ET-1 or SX6C (50-400 pmol) on PIP and LW were potentiated in the presence of L-NOARG (100 μM). In control lungs, 400 pmol of ET-1 increased LW by 2 ± 0.8 g (n=7) which was potentiated in the presence of L-NOARG to 6.3 ± 1.35 g, n=4 ($p< 0.001$). Similarly LW increases in response to SX6C (200 pmol) were potentiated to 8 ± 1.35 g, n=5 ($P< 0.001$) vs. 1.84 ± 0.5 g, (n=13) in control lungs. Increases in PIP in response to ET-1 (50- 800 pmol) were significantly augumented by L-NOARG (control ED_{50} 242 ± 33 pmol, n=7 vs.132 ± 5 pmol, n=4 ($p< 0.05$). SX6C-induced increases in PIP were also potentiated (ED_{50} in control 85 ± 6 pmol, n=13 vs. 48 ± 5 pmol, n=5 ($p< 0.01$).

In summary, the pulmonary vasodilator potencies of ETs and SX6C suggest an action mediated via ET_B receptors linked to NO production (as evidenced by the inhibitory effects of L-NOARG). The finding that L-NOARG also potentiated the rises in PPP , the increases in LW and the rises in PIP caused by ET-1 or SX6C suggests that endogenous nitric oxide modulates the vasoconstrictor and bronchoconstrictor activities of these agents in this preparation.

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193P CHARACTERISATION OF [^{125}I]ENDOTHELIN-1 AND [^{125}I]BQ3020 BINDING TO RAT CEREBELLAR ET_B RECEPTORS

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The potent vasoactive endothelins (ET-1, ET-2 and ET-3), which produce long lasting vasoconstriction are known to interact at two endothelin receptor subtypes called ET_A and ET_B receptors which have also been cloned from mammalian tissues. All the endothelins and the structurally similar sarafotoxins (STX-6b and STX-6c) display equipotency at ET_B receptors whilst ET-1, ET-2 and STX-6b exhibit between 50 and 100-fold high potency at ET_A receptors as compared to ET-3 and STX-6c. There has recently been suggestions of more than two endothelin receptor subtypes, based on pharmacological evidence obtained using selective ET_A and ET_B ligands. Endothelin receptors are found in the brain with highest densities being located in cerebellum. Using quantitative receptor autoradiography with 20 pM [^{125}I]ET-1, endothelin receptors were confined mostly to the granule cell layer (4.43 ± 0.30 fmol/mg tissue) with lower binding to molecular layer (0.67 ± 0.14 fmol/mg tissue). We have attempted to further characterise these endothelin receptors in rat cerebellum to discover whether the current classification into ET_B receptors is justified in relation to the speculation of subtypes of ET_B receptors which display subtle differences in their pharmacology (Bax and Saxena, 1994). Receptor binding studies were performed on rat cerebellar homogenates obtained from male and female AP rats (Wistar strain). The binding of [^{125}I]ET-1 and the putative selective ET_B agonist [^{125}I]BQ3020 were examined using 100 nM ET-1 to define non-specific binding. Both radioligands bound to single population of sites with high affinity ([^{125}I]ET-1 K_D= 0.31 ± 0.05nM, Bmax =110 ± 19 fmol/mg protein, [^{125}I]BQ3020, K_D = 1.22 ± 0.52nM, Bmax = 47.1 ± 3.3 fmol/mg protein, mean ± S.E.M.). Competition experiments in rat cerebellar homogenates using 20 pM of each radioligand demonstrated a pharmacological profile typical of that described for ET_B receptors (Table 1) with high affinites for ET-1, ET-3, STX-6c but lower affinities for selective ET_A antagonists such as JKC-301(Ristea et al., 1994), BQ123 and BMS-182,874. The putative ET_B agonists, BQ3020, IRL1620 and [$Ala^{1,3,11,15}$]ET-1 displayed a moderate affinity at cerebellar endothelin receptors. The affinities of the endothelin ligands at [^{125}I]ET-1 and [^{125}I]BQ3020 binding sites was highly correlated (0.97; $p<0.0001$, two-tailed) suggesting that both ligands recognised the same site. The non-selective endothelin antagonists, Ro-46,2005 and Ro-47,0203 (bosentan) demonstrated moderate affinities at the endothelin site in rat cerebellum.

Table 1. Affinity of endothelin ligands at cerebellar endothelin receptor. (pKi, mean ± S.E.M., n = 3-4). N/D = not determined.

	[^{125}I]ET-1	[^{125}I]BQ3020
ET-1	8.36 ± 0.09	8.39 ± 0.09
ET-3	8.71 ± 0.08	9.08 ± 0.33
STX-6c	7.74 ± 0.07	8.34 ± 0.05
BQ3020	7.67 ± 0.01	7.46 ± 0.06
IRL1620	6.39 ± 0.16	6.29 ± 0.24
IRL1038	6.67 ± 0.35	N/D
Ro-46,2005	6.07 ± 0.03	6.82 ± 0.21
Ro-47,0203	6.21 ± 0.11	
[$Ala^{1,3,11,15}$]ET-1	6.70 ± 0.16	7.01 ± 0.13
BQ123	5.03 ± 0.00	5.01 ± 0.00
BMS-182,874	<3	<3
JKC-301	<3	<3

In conclusion, the pharmacological profile of [^{125}I]ET-1 and [^{125}I]BQ3020 binding to rat brain homogenates is similar to that of ET_B-type receptors.

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194P EFFECT OF DEVELOPMENTAL AGE ON ENDOTHELIN RECEPTOR-MEDIATED VASOCONSTRICTION IN RABBIT PULMONARY RESISTANCE ARTERIES

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Persistent pulmonary hypertension of the newborn (PPHN) is responsible for a significant number of neonatal deaths, normally occurring from 0-3 days old (Gersony, 1973). Elevated plasma endothelin-1 [ET-1] levels are associated with pulmonary hypertension (Stewart *et al.*, 1991). Hence it of interest to identify which functional ET receptors are evident in foetal and newborn rabbit pulmonary arteries and how the sensitivity of these to agonists varies with development. In this study, we used ET-1 and the selective ET_B receptor agonist sarafotoxin S6c (SxS6c) to determine the receptor subtype(s) involved in the ET-induced vasoconstriction of rabbit pulmonary resistance arteries (PRAs). Foetal, neonatal and adult NZW rabbits were killed with sodium pentobarbitone. Small PRAs were dissected out and mounted as ring preparations (2mm length) on a wire myograph (under ~125mg tension) in Krebs (at 37°C) bubbled with 3% O_2/6%CO_2 balance N_2 for the foetal vessels and 16% O_2 for all others. Cumulative concentration-response curves (CCRCs) were constructed for ET-1 and SxS6c (1pM-0.3μM). The results are shown in Table 1. They show that adult PRAs are extremely sensitive to ET-1 and SxS6c which are equipotent indicating the presence of ET_B receptors. ET-1 is least potent at 4 and 7 days whilst SxS6c is most potent at 0-24 hours and at this age, more potent than ET-1 itself. The results indicate that ET_B receptors are prevalent in rabbit PRAs as has been shown in larger pulmonary arteries (Fukoroda *et al.*, 1994). They also demonstrate that the sensitivity of rabbit PRAs to ET agonists can vary with developmental age. There is a high sensitivity of the PRAs to ET_B receptor stimulation at 0-24 hrs. If this hypersensitivity occurs in man, it could only contribute to the high pulmonary resistance prevalent in PPHN.

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Table 1. pEC_{50} values for ET-1 and SxS6c and maximum responses to SxS6c as a % of the the response to 0.1M ET-1 in the same preparation. n/n = number of ring preparations/number of animals.

Developmental age	Agonist	n/n	pEC_{50}	SxS6c maximum response
Foetal	ET-1	6/6	8.7 ± 0.2	
	SxS6c	6/6	9.0 ± 0.2	67.8 ± 13.4
0-24 hrs	ET-1	6/6	8.8 ± 0.2	
	SxS6c	6/6	10.1 ± 0.1†^{aaa}	58.5 ± 11.1
4 days	ET-1	6/5	8.6 ± 0.1*	
7 days	ET-1	6/6	8.2 ± 0.1**	
Adult	ET-1	4/4	9.2 ± 0.4	
	SxS6c	14/7	9.2 ± 0.2	73.6 ± 4.7

Statistical difference from adult agonist pEC50, Student's unpaired t-test 1) ET-1,*P<0.05; **P<0.01: 2) SxS6c, †P<0.05. Statistical difference from ET-1 at same age point ^{aaa}P<0.001.

195P INVOLVEMENT OF PROTEIN KINASE C AND PROTEIN TYROSINE KINASE IN ENDOTHELIN-3-INDUCED PROLIFERATION OF WOUNDED ENDOTHELIAL CELLS

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Endothelin-1 (ET-1; Yanagisawa *et al.*, 1988) is one of three isopeptides, designated ET-1, ET-2 and ET-3, encoded in the human genome (Inoue *et al.*, 1989). It is mitogenic in vascular smooth muscle cells (Komuro *et al.*, 1988) and brain endothelial cells (Vigne *et al.*, 1990) which suggests that endothelins may act as vascular growth regulators *in vivo*. Using a novel *in vitro* multichannel wounding device, repair of endothelial cell injury in the presence of endothelins was studied using human umbilical vein endothelial cells (HUVEC).

HUVEC (pooled cells from Clonetics; TCS Biologicals, Buckingham) were grown to confluence on 13 mm diameter Thermanox™ coverslips. A wounder was used to produce 11 parallel wounds (each of 200 μm width) across the monolayer of cells (Fan & Frost, 1990). The coverslips were rinsed and placed in a new well containing 450 μl complete medium and 50 μl drug or vehicle (medium E199). After 18 h incubation, the regeneration of HUVEC into the denuded area was quantified using a Seescan (Cambridge) semi-automated computerised image analysis system and the percentage recovery after wounding was calculated. Statistical comparison was by analysis of variance followed by Fisher's LSD test using $P < 0.05$ as the level of significance.

In the presence of 1-1000 nM ET-1 there was no significant change in wound repair of HUVEC (control, 26.8±1.9% recovery; 1000 nM ET-1, 29.5±1.2%, $n = 3$). However, ET-3 facilitated the recovery of HUVEC over 18 h in a concentration-dependent fashion with an $EC_{50} \approx 2$ nM but did not accelerate mitogenesis in sub-confluent cells. Thus, it appears that recovery from injury is accelerated by ET-3 by means of a receptor which is insensitive to ET-1 at concentrations up to 1 μM.

The involvement of protein kinase C (PKC) and protein tyrosine kinase (PTK) in the repair response of HUVEC to ET-3 was then investigated. Neither calphostin C (21.9±1.1% recovery), the PKC inhibitor, nor lavendustin A (20.5±1.1% recovery) or genistein (23.2±1.3% recovery), the PTK inhibitors, affected the basal rate of recovery from wounding in the absence of ET-3, although ET-3 (100 nM) increased recovery to 37.6±1.1% from a control of 20.7±1.0% ($n = 3$ for all, $P < 0.001$ ET-3 versus control). In the presence of calphostin C (50 nM), the response to ET-3 was suppressed (recovery = 21.7±0.9%, $n = 3$ for all, $P < 0.001$ relative to ET-3 alone). Lavendustin A (11 nM), but not its negative control lavendustin B (11 nM), also suppressed the ET-3 response (recovery = 22.3±1.2%, $n = 3$, $P < 0.001$ relative to ET-3 alone) while the response to ET-3 in the presence of lavendustin B was 37.7±1.4% ($n = 3$). In the presence of genistein (50 μM, recovery = 36.9±1.9%, $n = 3$) no difference was detected compared to ET-3 alone.

These results suggest that PKC and PTK are involved in the repair response of wounded endothelial cell monolayers to ET-3 although the results with genistein and lavendustin A suggest that only some type or types of PTK are activated.

HL is an MRC Research Student.

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196P SUPPRESSION OF VASOCONTRACTILITY IN RAT AORTA AND PORTAL VEIN CAUSED BY PROGESTERONE AND PREGNANEDIOL

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Progesterone has been implicated in the suppression of postural vasoconstriction observed by Hassan *et al.*, (1990), and as a vasoactive agent in the aetiology of menstrually-related migraine (Leathard & Eccles 1991). Other progestational agents, including pregnanediol, have also been shown to have a suppressive effect on excitable cells (Phillis, 1986). We have now attempted to identify possible mechanisms of the vasodilator action of progesterone.

Male Wistar rats (~350 g), killed by cervical dislocation, provided aorta and portal vein preparations. These tissues were kept in aerated mammalian Tyrode saline (composition (mM): NaCl 137.0, KCl 5.4, $CaCl_2$ 1.79, $MgSO_4$ 1.04, NaH_2PO_4 0.34, $NaHCO_3$ 11.9, Glucose 5.6) maintained at 37 °C. The tissues were set up in organ baths either as 'whole' preparations (portal vein) or as helically-cut strips (aorta) to give measurable changes in force recorded via isometric tension transducers.

Both aorta and portal vein were observed to contract in response to KCl (20-120 mM) and to Ca^{2+} levels (0.25-2.5 mM) in a concentration-dependent manner. The effect of 30 min incubation with 10^{-6} M progesterone or 10^{-6} M pregnanediol on the above responses was compared with that of the vehicle (ethanol 1:1500 or 1:750 respectively). Neither progesterone nor pregnanediol caused any suppression of tonic contractions evoked by KCl in aorta or portal vein. Progesterone did, however, suppress 0.5-2.5 mM Ca^{2+}-evoked tonic contractions of portal vein (30.5 ± 3.6 % inhibition (mean ± s.e.mean) at 2.0 mM Ca^{2+}; n=8, P<0.05) and also caused significant suppression of phasic contractions of the portal vein at low levels of KCl depolarisation (25.0 ± 3.6 % inhibition at 20 mM KCl; n=8, P<0.05); pregnanediol had an even greater suppressive effect upon these phasic contractions (62.2 ± 5.9% inhibition at 20 mM KCl; n=8, P<0.01). Statistical analyses utilised Student's t-tests for unpaired data.

This suppression of contractility may involve inhibition of voltage-dependent Ca^{2+} entry by either direct Ca^{2+} channel blockade or by K^+ channel activation, hyperpolarising the membrane and thus reducing calcium channel opening. Preferential effect of hormones on contractions elicited by low K^+ concentrations in the portal vein suggest that these may be acting via a K^+ channel opening mechanism (Weir and Weston 1986; Leathard *et al.*, 1990).

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197P EFFECTS OF UNIVERSITY OF WISCONSIN PRESERVATION SOLUTION ON RABBIT AORTIC ENDOTHELIUM AND SMOOTH MUSCLE

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The University of Wisconsin (UW) preservation solution has been widely used for preservation of organs during transplantation (Belzer & Southard, 1988). However, the effects of the high potassium concentration in UW on the preservation of vascular endothelium and smooth muscle function for longer durations (up to 72h) are not well known (Pearl *et al*, 1994). In this study we have used rings (3-5mm width) of male New Zealand White rabbit thoracic aorta to assess the effects of hypothermic storage (4°C) in UW on endothelial and smooth muscle function. Following prolonged, hypothermic storage in UW (24, 48 and 72h), the tissues were equilibrated in modified Kreb's-Ringer ($CaCl_2$ 1.8 mmol/L) solution for 2h, maintained under 2 g tension, and cumulative concentration-response curves to various agents were determined. Isometric tension developed by the tissues was recorded with a Grass Force Transducer (FT03) on a Grass Polygraph 7P. ANOVA with Fisher's protected multicomparison test was utilized to compare the differences among the groups. No significant difference in contractile response to potassium chloride (KCl) and noradrenaline (NA) was observed between endothelium-denuded aortic rings stored in UW for 24, 48 and 72h and control rings (CR, 0 h). In contrast, the magnitude of maximum contraction of endothelium-intact circular rings to KCl (but not NA) was increased after 72 h of storage when compared to CR (8.0 ± 0.5 vs 5.8 ± 0.4 g tension respectively, n = 16, P<0.05). The magnitude of maximum relaxations elicited by acetylcholine (ACh) in endothelium-intact aortic rings, precontracted with KCl, were reduced after 48 and 72 h of storage in UW, when compared to CR and rings stored in UW solution for 24 h (19.7 ± 1.8 and 20.2 ± 2.9% vs 27.8 ± 2.8 and 31.8 ± 5.8% cumulative relaxation respectively, from 50% maximal contraction, n = 16, P<0.05). Tissues stored for 24 h, precontracted with NA, showed the greatest relaxation response to ACh. The maximum relaxatory response to sodium nitroprusside (SNP) in endothelium-denuded vessels, precontracted with KCl or NA, was not changed by long-term storage in UW (up to 72 h). Sensitivity to KCl was unchanged while sensitivity to NA increased in endothelium-denuded rings after 72 h of storage when compared to CR (-log EC_{50} 6.82 ± 0.05 vs 6.72 ± 0.03 respectively, n = 16, P<0.05). Sensitivity of ACh was unchanged while sensitivity to SNP was reduced in both KCl and NA-precontracted rings after 72 h of storage, compared to CR (-log IC_{50} 4.47 ± 0.06 vs 4.85 ± 0.06 and 5.56 ± 0.07 vs 5.87 ± 0.03 respectively, n = 16, P<0.05). The study demonstrates that 72 h of storage in UW, at 4°C, significantly impairs the functional ability of the vascular endothelium and the relaxation response of the underlying smooth muscle in rabbit thoracic aorta and that these changes may contribute, in part, to the observed increase in the contractile response of the smooth muscle. These alterations in the functions of vascular endothelium and smooth muscle were not significant at 24 h and were slight at 48 h of storage.

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198P THE AT_1 RECEPTOR ANTAGONIST, LOSARTAN, INHIBITS CONTRACTION AND ENHANCES PGI_2 RELEASE IN HUMAN SAPHENOUS VEIN STIMULATED BY ANGIOTENSIN II

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Contractions of rings of human saphenous vein (SV) in response to angiotensin II (AII) are attenuated by endogenous endothelial derived vasodilators such as prostacyclin (PGI_2; Barker *et al.*, 1994a, b). As well as antagonism at the AT_1 receptor, losartan exhibits other actions such as the release of PGI_2 from cells in culture (Jaiswal et al, 1991). We have now measured both contractions and PGI_2 release from SV in response to AII in the presence of losartan.

Human SV isolated during coronary bypass surgery were cut into rings (2-4mm long) and mounted in Krebs buffer (pH 7.4, 37°C, 95% O_2; 5% CO_2) to measure isometric tension. Functional integrity of each ring was assessed by contraction to KCl (bath concentration 90mM) and relaxation, following precontraction, to the endothelium-dependent vasodilator, bradykinin (BK). Only those rings which contracted with a force greater than 10mN to KCl and relaxed (at least 10% of the precontraction) in response to BK were used. The KCl contraction of each ring was used as a standard response for that ring and subsequent contractions to AII were calculated as a percentage of this response. For the contractile responses, AII was given cumulatively (1, 10 and 100nM) over 20 min. Losartan (100 or 300nM) was incubated with the rings for 20 min prior to and during exposure to AII. For the measurement of PGI_2 production, rings were incubated with losartan for 20 min before exposure to AII (100nM) for 5 min. The whole of the organ bath buffer was collected and 6-oxo-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) content was measured by radioimmunoassay. Basal release from the same ring was measured for 20 min with no treatment and release on stimulation corrected for basal release.

AII induced concentration-related contractions of the SV; a maximum of 53% (34, 67; median and 95% confidence limits) of the KCl response was attained at 100nM AII (n=12). Pre-treatment with losartan decreased contractions to AII; at 100nM losartan, 100nM AII now gave 11% (7, 31; n=11) of the KCl response and at 300 nM losartan, responses were further reduced to 4% (0, 6; n=5: P<0.05, Wilcoxon). Release of 6-oxo-$PGF_{1\alpha}$ in the organ bath buffer was not increased after AII (100nM) alone (0.26 ± 0.22ng, mean±s.e.m.; n=4). After treatment with losartan (100nM), this concentration of AII now produced a higher release of 6-oxo-$PGF_{1\alpha}$ (0.8 ± 0.1ng; n=4: *P*<0.05, paired t-test).

We conclude from these results that the contractile responses of the human SV to AII were mediated by the AT_1 receptors and that, in this tissue, losartan increased the output of PGI_2 during stimulation by AII. The latter effect would contribute to the decreased contractions to AII in the presence of losartan.

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199P MATURATIONAL CHANGES IN ENDOTHELIUM-DEPENDENT VASODILATATION IN RABBIT PULMONARY ARTERIES

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Acetylcholine(ACh)-induced vasorelaxation in arteries has been shown to be due to the release of the endothelium-derived relaxing factor nitric oxide (NO) (Furchgott & Zawadzki, 1980). Recent studies have shown that NO is an important mediator of vasomotor tone in ovine developing pulmonary circulation (Shaul *et al*, 1993; Abman *et al*, 1991). Here, we studied the vasorelaxant responses to ACh in noradrenaline(NA)-precontracted pulmonary artery rings from NZW foetal and neonatal rabbits. The rabbits were killed with sodium pentobarbitone. The main pulmonary artery (PA) & left branch (extra-lobe region) were dissected out. The rings were mounted under tension in Krebs-filled (5ml) organ baths (37^0C) and bubbled with 16% O_2/ 6% CO_2 balance N_2 (3% O_2/ 6% CO_2/ balance N_2 for foetal tissue). The PA rings were pre-constricted with 1μM NA and after a stable contraction had been reached, cumulative responses to ACh (0.1, 0.3, and 1.0 μM) were obtained. The response to 100μM L-NAME in vessels under basal tone was also studied. The results are shown in Table 1.The results show that relaxation to ACh in precontracted rabbit PAs varies significantly with developmental age and, in the case of the foetus, vessel location. Responses were significant in the foetal vessels and were most pronounced at 4 days. They were, however, absent at 0-24 hours. Inhibition of nitric oxide synthase with L-NAME increased vessel tone, the magnitude of which varied with developmental age and in the foetus, vessel location. These responses, however, were absent in 0-24 hour neonates. These results suggest both basal and ACh-induced NO is absent in 0-24 hour neonates and may explain the changing sensitivity of the neonatal pulmonary vasculature to circulating vasoconstrictors (Morecroft & MacLean 1995). A high mortality rate is seen in neonates with persistent pulmonary hypertension (PPHN) at 0-3 days of age. The absence of protective NO through 'endothelial dysfunction' in these babies may partly explain this as well as the observation that inhaled NO therapy has shown some success in the treatment of PPHN (Kinsella et al, 1993). This work was supported by the Wellcome Trust & The Royal Society (London). The laboratory is a member of the EUC Biomed project 'EureCa' BMHI-CT94-1375.

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Table 1 a. Relaxation response to 0.3 μM ACh in NA-precontracted PA rings as % of NA contraction. b. Contraction response to 100μM L-NAME as % 50mM KCl response. n/n = number of ring preparations/number of animals

Preparation		*A. % Relaxation*	*n/n*	*B. L-NAME response*	*n/n*
Foetal	-main	49.8 ± 3.9	15/15	1.1 ± 1.1	6/6
	-extra-lobe	74.6 ± 6.0†††	17/17	36.6 ± 5.6†††	4/4
0-24h	-main	0.0 ± 0.0***	10/10	2.4 ± 1.8	14/14
	-extra-lobe	-2.4 ± 2.4***	10/10	0.0 ± 0.0***	6/6
4 day	-main	94.8 ± 5.2***	6/6	30.5 ± 5.6***	5/5
	-extra-lobe	90.8 ± 2.5	6/6	24.3 ± 5.7	6/6

Statistical difference using one-way ANOVA, 1) from foetal response ***P<0.001 ; 2) in vessel location: †††P<0.001

200P A COMPARISON OF RITODRINE- AND PINACIDIL-INDUCED RELAXATION OF THE HUMAN MYOMETRIUM

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The β_2-adrenoreceptor agonist ritodrine is presently the tocolytic agent of choice in the treatment of preterm labour although evidence indicates that it has no significant benefit for neonatal outcome (Cashore & Stern, 1984). We have investigated the relationship between ritodrine, the potassium channel opener, pinacidil and their antagonists, propranolol and charybdotoxin respectively.

Myometrial tissue was obtained with consent from pregnant, non-labour donors undergoing elective caesarean section at term gestation. Tissue strips were mounted under 2g tension at 37^0C and continually gassed with 95%O_2/5%CO_2 for isometric recording and stimulated with 0.5nM oxytocin. Cumulative concentration-response curves to either pinacidil (5nM-5mM) or ritodrine (2nM-2mM) alone or in the presence of charybdotoxin (400nM) or propranolol (10μM) for a 30 minute incubation period were evaluated.

Ritodrine produced a concentration-dependent inhibition of oxytocin-induced myometrial contractions, best fitted to a 2-site model, with EC_{50} values of 12.9±6.5nM (mean±SEM) and 197.4±102.9μM (n=5). In the presence of charybdotoxin and propranolol, the EC_{50} values were 9.1±6.1nM and 284.7±98.9μM (n=5) and 12.8±6.6nM and 178.6±67.2μM (n=3) respectively. Using a two-tailed unpaired t-test, none of these values were significantly different from ritodrine alone (P>0.1).

Pinacidil is a potent uterine relaxant *in vitro* (Morrison *et al.*, 1993) and it activates uterine BK_{Ca} channels (Ashford *et al.*, 1993). In the presence of pinacidil, the concentration-effect curve, best fit to a one-site model, had an EC_{50} of 0.20±0.04μM and a Hill slope of 0.9±0.2 (n=4). In the presence of charybdotoxin, the EC_{50} was 1.8±0.2μM with a Hill slope of 0.70±0.06 (n=3) and for propranolol, an EC_{50} of 1.5±0.2μM and a Hill slope of 0.60±0.04 (n=4). The EC_{50} and Hill values for both charybdotoxin and propranolol were significantly different (P<0.005) to the values obtained for pinacidil alone.

As propranolol interfered with the pinacidil-induced relaxation, its effect on the uterine BK_{Ca} channel in freshly dispersed myocytes was studied. In the outside-out patch configuration with normal physiological saline in the bath (in mM) 135 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES (pH 7.2) and an electrode solution consisting of (in mM) 140 KCl, 50nM free Ca^{2+}, 1 $MgCl_2$, 1 KEGTA, 10 HEPES (pH 7.2), propranolol (10-20μM) caused a flickery block of the BK_{Ca} channel accompanied by a reduction in channel open state probability (n=4). In inside-out patches, under symmetrical K^+ conditions, propranolol (10-20μM), applied to the intracellular membrane surface, had no significant effect on the BK_{Ca} channel.

These results indicate that ritodrine relaxes the human myometrium by a mechanism unrelated to BK_{Ca} channel activation. Propranolol, on the other hand, is a potent blocker of uterine BK_{Ca} channels and can antagonize the effects of pinacidil thereby indicating an action distinct from its β-adrenoreceptor.

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201P A PRELIMINARY STUDY ON THE EFFECTS OF BRADYKININ AND RELATED COMPOUNDS ON THE HUMAN ISOLATED UMBILICAL ARTERY

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Bradykinin (BK) receptors (B) were originally divided into B_1 and B_2 subtypes (Regoli & Barabé, 1980), and more recently this has been extended to B_3 subtypes (Farmer *et al.*, 1989). On human umbilical artery (HUA) BK has been shown to induce a contractile response (Altura, 1972). This study is a preliminary characterisation of B receptors on HUA, using selective B_1, des-Arg^9-BK, and B_2, BK and Lys-BK, agonists and antagonists.

Samples of human umbilical cord were obtained from full term pregnancies (all patients gave written consent) and placed immediately into Krebs' solution at room temperature. The rings of umbilical artery were suspended in Krebs' solution at 37°C in a 10ml organ bath and oxygenated with 2.5% O_2/8% CO_2/balance N_2 as described by Amin *et al.* (1995). Agonists were added directly into the organ bath in a cumulative fashion. Concentration effect curves were constructed to BK, Lys-BK and des-Arg^9-BK. Where an antagonist was used, it was allowed to equilibrate for at least 30mins before the agonist concentration effect curve was repeated. Schild analysis was used to calculate pA_2 values. In all cases n=5.

Cumulative addition of BK (1-1000nM), Lys-BK (1-1000nM) and des-Arg^9-BK (5-50000nM) resulted in concentration-dependent constriction with desensitisation at the highest concentration used. The EC_{50} values (95% C.L.) for BK, Lys-BK and des-Arg^9-BK were 38nM (31-46), 11nM (5.8-17.8) and 320nM (151-605), respectively. The presence of indomethacin (2.79μM) had no significant effect on the response to the kinins. In the presence of indomethacin the EC_{50} values for BK, Lys-BK and des-Arg^9-BK being 43nM (32-54), 19nM (7-38) and 418nM (112-1000), respectively.

The presence of HOE-140, a B_2 receptor antagonist, (Wirth *et al.*, 1991) resulted in competitive antagonism of the response to BK and Lys-BK.

Agonist	pA_2 of HOE-140	slope
BK	7.54	1.03
Lys-BK	7.43	1.02

HOE-140 (1μM) had no effect on the contractile response to des-Arg^9-BK (EC_{50}=440nM (115-1004)). The presence of 1μM des-Arg^9(leu^8)BK, a B_1 receptor antagonist, caused a progressive rightward shift of the dose response curve to des-Arg^9-BK but did not significantly alter the response to BK (EC_{50}=49nM (21-87)) and Lys-BK (EC_{50}=20nM (3-46)). The pA_2 for des-Arg^9(leu^8)BK against des-Arg^9-BK=5.9, (slope, 1.11) (pA_2=6.5 on rabbit mesenteric artery (Churchill & Ward, 1986)).
The results of this study suggest that BK and Lys-BK are acting mainly through B_2 receptors and des-Arg^9-BK through B_1 receptors. None of these constrictor responses were mediated through cyclooxygenase products. The B_3 subtype remains to be investigated.

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202P A COMPARATIVE STUDY OF THE THROMBOXANE MIMETICS, U46619 AND I-BOP ON THE HUMAN UMBILICAL ARTERY

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It has been known for some time that prostaglandins cause constriction of human umbilical artery (Hillier *et al.*, 1968) but as yet the receptor population has not been defined. Previous studies in this laboratory have suggested the presence of a TP-receptor on human umbilical artery (Amin *et al.*, 1995). In the present study an attempt has been made to characterise further the TP-receptor population on human umbilical artery using the TP-receptor selective agonists U46619 (11, 9-epoxymethano PGH_2) and, I-BOP ([1S-(1α,2β(5Z), 3α (1E,3S*), 4α)]-7-[3-(3-hydroxy-4-(4'-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1] heptan-2-yl]-5-heptenoic acid), in conjunction with the TP-receptor antagonists, Bay u3405 and SQ 29548 (Coleman *et al.*, 1994).

Samples of human umbilical cord were obtained from full term pregnancies (all women gave written consent) and placed immediately into Krebs solution at room temperature. The cords were then transported to the laboratory within 60 minutes. The rings of umbilical artery (with intact endothelium) were suspended in Krebs solution containing indomethacin (2.79 μM) at 37°C in a 10ml organ bath and oxygenated with 2.5 % O_2/ 8 % CO_2/balance N_2 as described previously (Amin *et al.*, 1995). Cumulative concentration-effect curves were constructed to U46619 and I-BOP. Where antagonists were used they were allowed to equilibrate for at least 30 minutes before the agonist concentration-effect curve was repeated. In all cases n=5.

The thromboxane mimetics, U46619 and I-BOP both evoked purely contractile responses but the maximal response to I-BOP was approximately 50% of that seen with U46619.

Table 1:- EC_{50} values for the TX-agonists U46619 and I-BOP

TX-agonist	Dose Range (M)	EC_{50}
U46619	$1x10^{-9}$-$3x10^{-5}$	4.0×10^{-7} M
I-BOP	$1x10^{-9}$-$3x10^{-6}$	2.3×10^{-8} M

We have previously shown that the pA_2 values on this tissue for Bay u3405 and SQ 29548 against U46619 are 8.3 and 8.6 respectively (Amin *et al.*, 1995). Both antagonists (10^{-6}M) caused a significant shift to the right of the concentration-effect curve to U46619 (P<0.05 for Bay u3405; P<0.01 for SQ 29548). The responses to I-BOP remained unaffected by the presence of either antagonist at 10^{-6} M or less.

These results suggest that the TX-mimetics U46619 and I-BOP maybe acting through different TP receptors rather than on a single receptor. This situation could be clarified as sub-type selective TP-receptor agonists and antagonists become available. The results of this study suggest the possibility that two TP-receptor subtypes may be present on human umbilical artery. This work is currently being extended to resolve the components of the TP response and to determine the other prostaglandin receptors that may be present on the human umbilical artery.

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203P COMPARISON OF THE EFFECT OF ACUTE HYPOXIA ON ILIAC ARTERY RINGS FROM NORMAL AND CHRONICALLY HYPOXIC RATS

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We recently showed that, in rings of rat iliac artery, moderate hypoxia (a decrease in bath pO_2 from 100 to 70mmHg) reduced the maximum contraction evoked by noradrenaline (NA_{max}) by a mechanism that was not dependent on the presence of the endothelium or on the synthesis of nitric oxide (NO); Bartlett & Marshall, 1994. NA_{max} was also found to be reduced in the aorta *in vitro* and in mesenteric and skeletal muscle arterioles *in vivo*, of chronically hypoxic (CH) rats (Doyle & Walker, 1991; Mian & Marshall, 1994), the mechanisms are not known.

In the present study, on 2mm rings of rat iliac artery taken from normal (N) rats and CH rats (kept in a hypoxic chamber at 12% O_2 for 3-5 weeks), we have compared responses evoked in normoxia (bath pO_2 102±1mmHg; mean±s.e.m., n=5) and in hypoxia (54±1mmHg, comparable to the PaO_2 of CH rats breathing 12% O_2; Mian & Marshall, 1994). Rings were mounted with (E+) or without (E-) endothelium under 0.5g tension at 37°C and cumulative concentration response curves to NA (10^{-9}-10^{-4}M) were constructed.

In normoxia, the NA_{max} of E+ rings from CH rats was lower than that of N rats, but acute hypoxia substantially reduced the NA_{max} in both cases (Table 1). The NA_{max} of E- rings from CH and N rats were similar in normoxia and they were similarly reduced by acute hypoxia. Hypoxia had no effect on EC_{50} values (-Log NA(M)) of E+ or E- rings from either CH or N rats.

Table 1. Max. responses to NA (mg) in rat iliac arteries. Values are mean±s.e.mean, n=5. *, **, ***; p<0.05, 0.01, 0.001 hypoxia vs normoxic control; §, p<0.05 E+ normoxia vs E- normoxia, ANOVA with Scheffe test. †, P<0.05 chronic vs normal rings, unpaired Students' *t* test

	N	CH
E+ NORMOXIA	1650±169	770±105†
E+ HYPOXIA	575±126***	460±42*
E- NORMOXIA	1225±210	1450±105§
E- HYPOXIA	635±59**	670±123**

Further, in normoxia, E+ rings from CH rats, precontracted with phenylephrine (EC_{80}), gave a significantly greater relaxation to 10μM acetylcholine (Ach) than E+ rings from N rats (82±2% vs 71±4% PE contraction; P<0.05 Students' unpaired *t* test). No E- rings relaxed to Ach.

These results suggest that, in normoxia, NA_{max} of iliac arteries from CH rats is reduced by a mechanism that is dependent on the endothelium. However in acute hypoxia, both the NA_{max} of iliac arteries of both CH and N rats is reduced regardless of the presence or absence of the endothelium.

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204P THE NOVEL β_3-ADRENOCEPTOR AGONISTS SR 59119A AND SR 59104A ARE STEREOSPECIFICALLY ANTAGONISED BY SR 59230A

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We assessed the *in vitro* agonist potency and selectivity of the novel compounds SR 59104A (N-[(6-hydroxy-1,2,3,4-tetrahydronaphthalen-(2*R*)-2yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) and SR 59119A (N-[(7-methoxy-1,2,3,4-tetrahydronaphthalen-(2*R*)-2-yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) in several preparations suitable for monitoring subtype-specific ß-adrenergic responses (Landi et al., 1993). We also tested these responses' susceptibility to the first selective β_3-adrenoceptor antagonist, SR 59230A (1-(2-ethylphenoxy)-3-(1,2,3,4-tetrahydronaphthalen-(1*S*)-1-ylamino)-propan-(2*S*)-2-ol oxalate) (Manara et al, 1995); see Landi et al., 1993 for methodology. Male, Crl:(CD)BR rats (200-250g) or Crl:(HA)SPF guinea-pigs (400-600g) were used. SR 59104A and SR 59119A selectively inhibited rat proximal colon motility (RC, β_3) with virtually no effect on tissues containing either β_1- (guinea-pig right atrium, GPA) or β_2-adrenoceptors (guinea-pig trachea, GPT; rat uterus, RU). (±)Alprenolol and SR 59230A (30 min contact time) competitively antagonized RC responses to SR 59104A (mean apparent pA_2 ± sem, slope ± sem, 6.7±0.2, 1±0.2 and 8.6±0.2, 0.85±0.1 respectively) and SR 59119A (6.7±0.1, 1.04±0.1 and 8.4±0.2, ~1). The stereoselective action of SR 59230A (*SS*-isomer) was confirmed by its *RR*-enantiomer's (SR 59483A) inability to antagonize the β_3-responses up to 1 μM. Thus, SR 59119A and SR 59104A were as potent as SR 58611A (Bianchetti and Manara, 1990) as β_3-adrenoceptor agonists, with apparently even greater selectivity. SR 59104A, and SR 59119A showed low affinities at 5-HT_{1A} (Ki, respectively 25 and 162 nM, in rat hyppocampus) and 5-HT_{1B} receptors (respectively 1.6 μM and 5 μM, in rat striatum) and neither compound, up to 1 μM, displaced specific radiolabelled ligands from several binding sites including 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_{1C}, 5-HT_{1D}, 5-HT-uptake, α_1, α_2, histamine H_1, muscarinic, GABAa and b, opioid, dopamine D_1 and D_2 (Mennini et al., 1993; Manara et al., 1995). This further attests to the selectivity of SR 59104A and SR 59119A in a more general context and confirms their value as better tools for characterization of the β_3-adrenoceptor.

Table 1 - Effect (EC_{50}, nM) of SR 59104A and SR 59119A in several isolated tissue preparations

	RC motility inhibition	GPA chronotropic action	GPT relaxation	RU motility inhibition
SR 59104A (RR)	3.3 (2-5)	>30,000	>30,000	925 (463-1847)
SR 59119A (RR)	2.6 (2-3.2)	>30,000	>30,000	944 (463-1542)

RC, rat colon; GPA, guinea-pig atrium; GPT, guinea-pig trachea; RU, rat uterus. In parentheses 95% confidence limits (n = 7-10)

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205P THE ACTIONS OF CLOZAPINE, CHLORPROMAZINE AND THIORIDAZINE AT THE A_1-ADRENOCEPTORS OF THE RAT VAS DEFERENS AND SPLEEN

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While classical antipsychotic drugs are considered to bring about their therapeutic effects via dopamine D_2 receptors, the "atypical" antipsychotic clozapine has a lower affinity for this site and is likely to act via other receptor mechanisms (Reynolds & Czudek, 1995). Most of these drugs have high affinity for the α_1-adrenoceptor which can result in postural hypotension and may also contribute to their therapeutic effects (Prinssen et al., 1994). The present study investigates the action of clozapine, and two classical antipsychotics, chlorpromazine and thioridazine at the functional α_{1A}-adrenoceptors of the rat vas deferens and the α_{1B}-adrenoceptors of the rat spleen.

Epididymal vas deferens and hemispleen of the rat were set up in gassed Krebs-bicarbonate solution at 37°C. Concentration-response curves to phenylephrine were constructed in the absence and presence of clozapine, chlorpromazine and thioridazine after a 30 min antagonist equilibration period. All experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM). Single cumulative concentration-response curves were constructed on spleen. Non-cumulative concentration-response curves were obtained on vas deferens.

At the α_{1A}-adrenoceptors of the rat vas deferens all three drugs had high affinities, the pK_B values (mean±sem, n≥12) being 8.2 ± 0.1, 9.2 ± 0.1 and 8.5 ± 0.1 for clozapine, chlorpromazine and thioridazine respectively. Schild plots for all three drugs had slopes not significantly different from unity (1.06±0.21, 1.24±0.54 & 0.91±0.15 respectively) and maximum responses were not altered.

The drugs also acted as competitive antagonists at the α_{1B}-adrenoceptors of the rat spleen causing dextral shifts of concentration-response curves without altering maximum responses and producing Schild plots with slopes not significanly different from unity. The affinities however were significantly lower than those obtained on the vas deferens. The pK_B values (with Schild slopes) were 7.0 ± 0.1 (0.82±0.13) for clozapine, 7.1 ± 0.1 (0.87±0.09) for chlorpromazine and 6.2 ± 0.1 (0.81±0.15) for thioridazine.

Thus, all three antagonists were selective for the α_{1A}-adrenoceptors of the rat vas deferens over the α_{1B}-adrenoceptors of the rat spleen, thioridazine having the greatest selectivity.

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206P CHARACTERISATION OF THE α_1-ADRENOCEPTOR SUBTYPE IN THE RAT PINEAL GLAND

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The nocturnal synthesis of melatonin in the rat pineal gland is controlled by noradrenaline (NA) released from the sympathetic neurons which innervate the gland. NA acts on ß- and α_1-adrenoceptors (α_1-AR) to regulate the induction of the enzymes responsible for melatonin synthesis. The aim of the present experiments was to characterize the subtype(s) of α_1-AR present in the rat pineal gland.

In radioligand binding studies, [^{125}I]iodo-2-[ß-(4-hydroxyphenyl) ethylaminomethyl] tetralone (^{125}I-HEAT), a selective α_1-AR ligand with no subtype specificity, was incubated (25°C, 20 min.) with rat pineal gland membranes (3-5 μg). Saturation studies used 2-800 pM ^{125}I-HEAT and competition experiments ~100 pM. Non-specific binding was defined using phentolamine (10 μM). For detection of α_1-AR mRNA, total RNA was extracted from cultured rat pinealocytes using guanidinium isothiocyanate. Reverse transcription combined with polymerase chain reaction (RT-PCR) analysis was carried out for 35-40 cycles (1 min. at 94°C, 1 min. at 55°C and 2 min. at 72°C, with a final extension of 10 min. at 72°C) using specific primer pairs designed from rat α_{1B}- and α_{1C}-AR cDNA sequences (Voigt *et al.*, 1990; Stewart *et al.*, 1994). Products were analysed by agarose gel electrophoresis.

Specific ^{125}I-HEAT binding was saturable with a K_d 81.7 ± 13.4 pM and B_{max} 273 ± 19 fmol/mg protein (mean ± SEM, n=4). Chloroethylclonidine (CEC) pretreatment of membranes (10 μM, 10 min. in 10 mM HEPES buffer) significantly reduced B_{max} to 99.6 ± 19.2 fmol/mg protein (n=4, $p<0.005$). Higher concentrations of CEC (upto 100 μM) or longer pretreatment (upto 40 min.) did not produce any additional reduction in specific ^{125}I-HEAT binding. Competition experiments with subtype selective antagonists gave monophasic inhibition curves with Hill coefficients close to unity, and mean log K_i values (n=4-5) for phentolamine, WB 4101, 5-methylurapidil, benoxathian, (-) and (+)-niguldipine of -7.25±0.06, -7.74±0.03, -6.94±0.06, -8.12±0.09, -6.88±0.04, -6.92±0.05 respectively. Competition experiments with α_1-AR agonists, done in the presence of GTP (100 μM), had Hill coefficients close to unity and gave mean log K_i values (n=4) for (-)-adrenaline, (-)-NA, phenylephrine, methoxamine, cirazoline, oxymetazoline and SDZ NVI-085 of -5.96±0.05, -5.68±0.08, -5.54±0.11, -3.74±0.06, -6.77±0.08, -7.11±0.10 and -5.41±0.13 respectively. K_i values for (-)- and (+)-niguldipine were not altered in membranes which had been pretreated with CEC. RT-PCR revealed single bands corresponding to the expected size of the α_{1B}- and α_{1C}-AR DNA fragments. The identity of the α_{1B}- and α_{1C}-AR fragments was confirmed by restriction digestion with Sau3AI, HinfI, SmaI.

These data suggest that the majority of α_1-AR present in rat pineal membranes are the α_{1B}-AR subtype. The small proportion of α_1-AR which remain after pretreatment with the irreversible inhibitor CEC have a relatively low and equal affinity for (-) and (+)-niguldipine indicating that these sites are not α_{1C}-AR, but likely represent remaining α_{1B}-AR sites which escape inactivation by CEC perhaps because they are inaccessible to this hydrophilic compound. Despite the evidence that the majority of α_1-AR expressed in rat pineal are the α_{1B}-subtype, RT-PCR analysis revealed the presence of both α_{1B}- and α_{1C}-AR mRNA. Further studies are needed to determine which cells express these mRNA and their relative abundance.

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207P α_1-ADRENOCEPTOR SUBTYPE AFFINITIES OF TAMSULOSIN METABOLITES

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Tamsulosin is an α_1-adrenoceptor antagonist targeted for patients with benign prostatic hyperplasia. Since tamsulosin is selective for α_{1A}- relative to α_{1B}-adrenoceptors in rat tissues (Michel et al. 1993) and among cloned subtypes (Michel & Insel 1994), we have now investigated whether metabolites of tamsulosin also discriminate α_1-adrenoceptor subtypes. The metabolites M1, M2, M3, M4 and AM1 and the synthesis byproduct R4 were tested. M1, M2, M3, and R4 are tamsulosin derivatives in which the phenoxyring has been substituted with a 2-hydroxy, 4-hydroxy, 5-hydroxy or 2-methoxy group, respectively, in M4 the benzenesulfonamide group has been 2-hydroxy substituted, and AM1 is o-ethoxyphenoxyacetic acid. All compounds were eveluated in radioligand binding studies using [^{3}H]prazosin as the ligand in rat liver (homogeneous α_{1B}-adrenoceptors) and kidney (mixed α_1-adrenoceptor subtypes) and with cloned rat α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors transiently expressed in COS-1 cells as previously described (Michel et al. 1993; Michel & Insel 1994). Data are mean ± s.e.mean of 3-5 experiments.

Generally the rank order of potency was tamsulosin ≈ M4 ≈ R4 > M1 > M2 ≈ M3 >> AM1 in each model system, with AM1 having only neglible affinity (-log K_i < 4) and not being discussed further. All other compounds had steep competition curves in rat liver but shallow and biphasic competition curves in rat kidney with 35-50% high affinity sites. Among cloned subtypes the order of potency for all compounds except for M4 was $\alpha_{1A} \approx \alpha_{1D} > \alpha_{1B}$. Affinities (-log K_i) for tamsulosin, M4 and R4 are given as examples in Table 1.

Table 1: Drug affinities (-log K_i) of tamsulosin derivatives in model systems of rat α_1-adrenoceptor subtypes

	tamsulosin	M4	R4
liver	9.38±0.07	9.45±0.03	9.62±0.04
kidney high affinity site	10.53±0.12	10.62±0.08	10.71±0.33
kidney low affinity site	8.64±0.17	8.84±0.02	8.76±0.20
cloned α_{1A}	9.75±0.09	9.46±0.02	9.47±0.04
cloned α_{1B}	8.86±0.05	8.58±0.05	8.85±0.01
cloned α_{1D}	9.41±0.23	10.23±0.02	9.57±0.04

We conclude that the tamsulosin metabolites M1, M2, M3 and M4 and the synthesis byproduct R4 retain the selectivity of the parental compound for α_{1A}- relative to α_{1B}-adrenoceptors. Some of them have similarly high affinity as tamsulosin.

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208P COMPARISON OF Rec 15/2739 AND TERAZOSIN α_1-ADRENOCEPTOR SUBTYPE AFFINITIES

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The α_1-adrenoceptor antagonists Rec 15/2739 (N-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide; also known as SB 216469) and terazosin are under clinical investigation for the symptomatic treatment of benign prostatic hyperplasia. Therefore, we have compared the selectivity of Rec 15/2739 and terazosin for α_1-adrenoceptor subtypes in rat tissues and with cloned subtypes transiently expressed in COS-1 cells using [^{3}H]prazosin as the radioligand as previously described (Michel et al. 1993; Michel & Insel 1994). The α_{1A}-adrenoceptor-selective antagonists (+)-niguldipine and WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl) were studied as reference compounds. Data are mean ± s.e.mean of 3-6 experiments.

All compounds competed for [^{3}H]prazosin binding in rat liver and spleen (homogeneous α_{1B}-adrenoceptors) with steep and monophasic competition curves; the calculated affinities were similar (less than 0.5 log units difference) to those at the cloned α_{1B}-adrenoceptor (see below). In rat cerebral cortex and kidney (mixed α_1-adrenoceptor subtypes) Rec 15/2739, (+)-niguldipine and WB 4101 had shallow and biphasic competition curves; in both tissues all three compounds recognized approximately 50% high affinity sites which is consistent with previous estimates of α_{1A}-adrenoceptors in these tissues (Michel et al. 1993). In contrast terazosin had steep and monophasic competition curves in rat cerebral cortex and kidney. Drug affinities at the cloned rat α_1-adrenoceptor subtypes are given in Table 1. Affinities at the cloned bovine α_{1A}-adrenoceptor were slightly (0.5-0.8 log units) higher than those at the rat α_{1A}-adrenoceptor.

Table 1: Drug affinities (-log K_i) at cloned α_1-adrenoceptor subtypes transiently expressed in COS-1 cells (bovine α_{1A}-adrenoceptor for terazosin, rat clones in all other cases)

	α_{1A}	α_{1B}	α_{1D}
Rec 15/2739	9.63±0.08	8.00±0.02	8.70±0.14
terazosin	8.35±0.07	8.75±0.08	8.88±0.05
(+)-niguldipine	9.79±0.10	7.78±0.02	7.39±0.25
WB 4101	9.92±0.17	8.21±0.05	9.47±0.06

Thus, Rec 15/2739 and WB 4101 are approximately 40-fold selective for α_{1A}- relative to α_{1B}-adrenoceptors and have intermediate affinity for α_{1D}-adrenoceptors. (+)-Niguldipine is approximately 100-fold selective for α_{1A}- relative to α_{1B}-adrenoceptors with even lower affinity at α_{1D}-adrenoceptors. In contrast terazosin lacks selectivity for α_1-adrenoceptor subtypes. The relevance of the difference between Rec 15/2739 and terazosin remains to be determined in clinical studies.

Michel, M.C., Büscher, R., Kerker, J. et al. (1993) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 348: 385-395.
Michel, M.C. & Insel, P.A. (1994) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 350: 136-142.

209P EFFECTS OF THE α_2 ADRENOCEPTOR AGONIST UK 14304 ON ADIPOSE TISSUE LIPASE AND cAMP IN OBESE MICE AFTER CHRONIC ETHANOL TREATMENT

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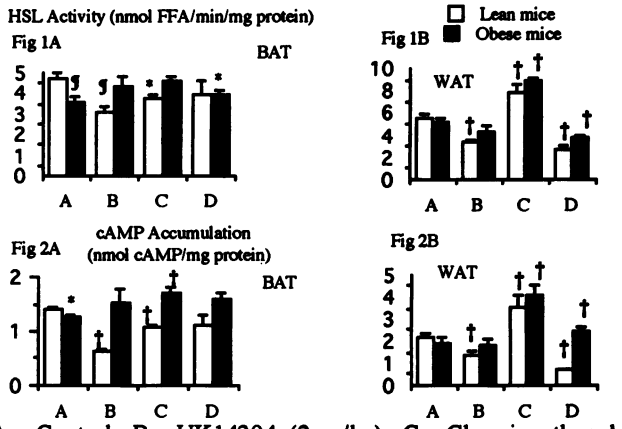
Obese CBA/Ca mice have lower Giα protein expression in white adipose tissue than lean mice but normal expression of Gsα protein (Palmer *et al*, 1992). Activation of α_2-adrenoceptors inhibits lipolysis via Gi coupling protein, which prevents cAMP accumulation thus inhibiting the activation of hormone-sensitive lipase (HSL), the key enzyme of lipolysis. Chronic ethanol treatment has already been shown to ameliorate the diabetes and obesity in male CBA mice (Connelly & Taberner, 1986). Here, we have used the same treatment to compare the effect of ethanol on α_2-adrenoceptor action in terms of brown (BAT) and white (WAT) adipose tissue HSL activity and cAMP accumulation in both lean and obese mice.

HSL was assayed using [^{3}H] triolein as substrate and cAMP accumulation was measured by a saturation binding assay (Shih & Taberner, 1994). Groups of 8 male lean and obese CBA mice were given pelleted food and water or 20% (w/v) ethanol solution for 4 weeks then injected i.p. with UK 14304 or saline as control 1 hr prior to the assays. Differences between groups (means ± s.e.mean) were analysed by Students t-test.

Obese mice had lower HSL activity and cAMP production in BAT ($p<0.01$ and $p<0.05$) but not in WAT. CET in obese mice increased HSL activity and cAMP formation in both BAT and WAT ($p<0.05$, $p<0.005$). In contrast, in lean mice HSL activity and cAMP level were decreased ($p<0.05$, $p<0.005$). UK 14304 2 mg/kg was sufficient to reduce HSL activity ($p<0.01$) and cAMP formation ($p<0.005$) in both BAT and WAT of lean mice but had no effect on either measure in obese mice. However, 1 mg/kg of UK 14304 was able to affect both WAT HSL activity ($p<0.005$) and cAMP level ($p<0.005$) in CET lean and obese mice.

Untreated obese mice were less sensitive to UK 14304 than their lean littermates. This may be due to the low Giα protein expression in WAT. The changes in adipose tissue HSL activity after acute UK 14304 in both control and CET lean and obese mice paralleled changes in cAMP formation. Since chronic exposure to ethanol increases Giα protein expression (Wand *et al*, 1993), this may explain why ethanol-treated mice were more sensitive to the α2-agonist than controls. Chronic ethanol drinking may thus have an important role in regulating inhibitory G-protein expression which, in turn, affects cAMP formation and HSL activity.



A= Control; B= UK14304 (2mg/kg); C= Chronic ethanol treatment (CET); D= CET+ UK14304 (1 mg/kg)
* $p < 0.05$; ¶ $p < 0.01$; † $p < 0.005$

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210P β_3-ADRENOCEPTOR AGONIST ACTIVITY IN RAT ISOLATED ADIPOCYTES IS INFLUENCED BY ADENOSINE DEAMINASE

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Measurements of the lipolytic effects of β-adrenoceptor agonists in isolated adipocytes *in vitro* could be affected by accumulation of endogenous adenosine, which is antilipolytic (Vannucci *et al.*, 1989.) The purpose of the present study was to examine the effect of removal of endogenous adenosine by adenosine deaminase (ADA) on the lipolytic response of rat adipocytes using β_3-adrenoceptor agonists of differing intrinsic efficacies, including isoprenaline, CL316,243 (Bloom *et al.*, 1992) and ZD7114 (Growcott *et al.*, 1993).

Rat epididymal adipocytes were isolated by collagenase digestion and concentration-response curves to the lipolytic effects of β-adrenoceptor agonists were constructed according to Quayle *et al.* (1993). Glycerol concentration in the extracellular medium was used as an index of lipolytic activity.

In the presence of ADA (1 unit.ml^{-1}), both basal and maximal isoprenaline-stimulated lipolysis were significantly elevated compared to control (Table 1). ADA treatment reduced the EC_{50} values of both isoprenaline and CL316,243 by about 7-fold, but did not significantly affect the intrinsic activity of CL316,243 compared to isoprenaline. In contrast, the EC_{50} of the low efficacy agonist ZD7114 was not significantly affected, but its intrinsic activity compared to isoprenaline was substantially increased following ADA treatment.

In conclusion, it appears that in rat adipocytes, endogenous adenosine has an inhibitory influence on basal and β_3-adrenoceptor agonist-stimulated lipolysis.

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Table 1. Lipolytic potencies of β_3-adrenoceptor agonists.

	-ADA		+ADA	
	EC_{50} (nM)[1]	I.A.[2]	EC_{50} (nM)[1]	I.A.[2]
Isoprenaline	45 (23-91)	1.0	6.1 (1.0-36)**	1.0
CL 316,243	5.4 (1.1-26)	1.02 ±0.04	0.77 (0.11-5.4)*	0.96±0.09
ZD7114	81 (6.6-1000)	0.36±0.06	73 (8.3-641)	0.99±0.10**

Basal lipolysis (μM glycerol) = 18±4 (-ADA) & 59±8** (+ADA). Maximum isoprenaline-induced lipolysis = 80±7 (-ADA) & 121±12** (+ADA). 1. geometric mean (95% C.L.), 2. arithmetic mean (±s.e.m.) of 4-11 determinations. Significantly different from corresponding value (-ADA), *$p<0.05$, **$p<0.01$ (Student's t-test.)

211P INHIBITION BY D609 OF HISTAMINE RECEPTOR COUPLING TO Ca CHANNELS IN SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA-PIG ILEUM

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D609 (tricyclodecan-9-yl-xanthogenate) is a phospholipase C inhibitor (Müller-Decker, 1989). The effect of this agent on transient and sustained phases of histamine-induced Ca^{2+}-current (I_{Ca}) suppression in ileal smooth muscle cells (Beech, 1993) was investigated in the present study. Suppression of I_{Ca} was determined in whole-cell recordings from freshly isolated cells using a CsCl pipette solution (in mM: CsCl 130, $MgCl_2$ 2, $CaCl_2$ 0.05, HEPES 10, EGTA 0.2, ATP 3 and GTP 0.1; pH 7.4), and a NaCl bath solution (in mM: NaCl 135, $MgCl_2$ 1.2, $CaCl_2$ 1.5, glucose 8 and HEPES 10; pH 7.4). The I_{Ca} was elicited by stepping to 0 mV from a holding potential of -60 mV for 50 ms every 5 s.

The effect of bath-applied 10 μM histamine was determined alternately in cells recorded from with and without 1.8 mM D609 in the pipette solution. When D609 was absent, histamine induced transient and sustained inhibitions of I_{Ca} of 40±9% and 35±6% respectively (means±s.e.m., n=5). When D609 was present, the transient effect of histamine was absent and the sustained inhibition was only 3±2% (n=6). The effect of histamine was also significantly (Student's *t*-test, $P>0.05$) but not completely inhibited by 180 μM D609. In the control recordings, transient and sustained inhibitions of I_{Ca} were 45±7% and 29±5% (n=11), whereas with 180 μM D609 in the pipette, they were 23±5% and 17±3% (n=15). D609 (1.8 mM) did not have significant effects on the amplitude of I_{Ca}, muscarinic receptor-coupling to cation channels, or IP_3-induced release of Ca^{2+} from stores. The peak amplitude of I_{Ca} at 0 mV prior to the application of histamine was -250±36 pA with D609 present (n=6), and -342±59 pA in the absence of D609 (n=5). Cationic current induced by 10 μM carbachol at the holding potential (-60 mV) was -102±50 pA with D609 present and -184±33 pA in the absence of D609 (n=5 for each). The amplitude of the Ca^{2+}-activated K^+-current induced by flash photolysis of intracellular caged-IP_3 (D-*myo*-inositol 1,4,5-triphosphate, $P^{4(5)}$-1-{2-nitrophenyl}ethyl ester) was 1.2±0.3 nA with D609 present and 0.75±0.3 nA in the absence of D609 (n=4 and n=8 respectively); in these experiments, KCl replaced CsCl in the pipette and the holding potential was 0 mV.

These data suggest that D609 blocks transient and sustained phases of histamine-induced suppression of voltage-gated Ca channel activity in ileal smooth muscle cells, and that both effects may require the action of a phospholipase C.

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212P EFFECT OF SOME SUBSTITUTED AZOLES ON THE *IN VITRO* METABOLISM OF RETINOIC ACID

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Retinoic acid (RA) is one of the principal endogenous compounds that control growth and differentiation of epithelial tissues in mammals (Lotan, 1980). As RA possesses a short $t_{0.5}$, inhibition of its (oxidative) metabolism is a strategy for enhancing its action. In animal models of prostate cancer, liarozole, a 17,20 steroid lyase inhibitor owes part of its effectivness to the inhibition of P450-mediated RA oxidation (Van Wauwe et al., 1992). Ketoconazole also inhibits RA metabolism (Van Wauwe et al., 1988). In a series of substituted azoles (Back & Tjia, 1985), it was found that inhibition of tolbutamide oxidation in the rat was dependent on substitution in the azole ring. The present study examines whether a structure-activity relationship occurs for substituted azoles in respect of inhibition of RA metabolism.

(11,12 ^{3}H)-RA (3 μM) was incubated with male albino rat hepatic microsomes (10 mg protein) and NADPH (2 m mol) in PBS pH 7.4, in the absence and presence of one of several azoles (100 μM in DMSO) for 15 min at 37°C. Following extraction into ethyl acetate, ^{3}H-RA and its oxidative metabolites were separated by reverse phase HPLC (Ahmad et al., 1994). Metabolism was determined from the % conversion of RA into its metabolites based on AUC values.

The results presented in Table 1 indicate that azoles with substituents in positions other than the 1-position on the ring are very weak inhibitors of RA metabolism. The most effective inhibitors (ketoconazole, itraconazole, bifonazole and clotrimazole) are 1-substituted and possess relatively large aromatic groups in the molecule. Further study of these structure-activity relationships may provide useful lead compounds that could offer a novel approach to cancer treatment.

Table 1 **Inhibition of in vitro hepatic microsomal metabolism of ^{3}H-RA by various substituted azoles (100 μM)**

Compound (position of azole substituents)		% Inhibition (mean ± sd, n=3)
Ketoconazole	(1)	87.5 ± 0.4
Itraconazole	(1)	68.7 ± 0.4
Bifonazole	(1)	47.5 ± 2.9
Clotrimazole	(1)	34.4 ± 3.7
Econazole	(1)	14.0 ± 3.7
Miconazole	(1)	13.5 ± 0.9
Nafimidone	(1)	12.0 ± 1.7
Sulconazole	(1)	8.7 ± 1.1
1-Benzylimidazole	(1)	7.6 ± 1.1
Cimetidine	(4,5)	1.0 ± 0.1
2-Methyl-5-phenyl benzoxazole	(2,5)	18.0 ± 1.4
4(4-Bromophenyl)-1, 2 3-thiadiazole	(4)	11.6 ± 1.4
5(4-Methylphenyl)-1,2 4-thiadiazole	(5)	4.8 ± 1.6
5(3-Chlorophenyl) oxazole	(5)	8.6 ± 1.3
2 (Thien-2 yl)-1, 3, 4-oxadiazole	(2)	0

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213P THE EFFECT OF CISPLATIN AND/OR PROBENECID ON THE G401, A HUMAN RENAL CELL LINE

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Introduced by Dr. A. Markham.

We have previously reported (Charlton & Simmons, 1993; Kumar et al, 1995) results from an evaluation of a human renal cell line, G401, in response to known nephrotoxic aminoglycoside antibiotics. We have now determined mitochondrial enzyme activity in the G401 using the MTT assay (Mossman, 1983) in response to the anti-cancer agent, cisplatin, another known nephrotoxic agent and probenecid, an inhibitor of the renal anion transporter which is believed to carry cisplatin.

Three experiments were performed (n=18 per experiment):
i) a 24h incubation with varying concentrations of cisplatin
ii) a 24h incubation with varying concentrations of probenecid
iii) incubation with 10μM probenecid for 24h either before, at the same time as or 24h after a 24h incubation with 1μM cisplatin. Total incubation time being 48h.

G401 cells (passage 92-95) were seeded (10^4 cells/well) in 96 well plates and grown to confluence in McCoy's 5α medium supplemented with 10% Fetal Clone. Once confluent, medium was replaced with fresh media containing cisplatin and/or probenecid and incubated for a further 24h or 48h after which time the MTT assay was performed. Results are shown as absorbance readings at 595nm.

Results of experiments i and ii are shown in Table 1.

Results from experiment iii)

There was no significant decrease in mitochondrial activity in cells that had been incubated for 24h with probenecid prior to incubating with cisplatin for 24h (0.910±0.055) compared to cells with neither cisplatin and probenecid (1.031±0.023), suggesting that probenecid had prevented the effect of cisplatin. Adding probenecid at the same time as (0.824±0.033) or 24h after cisplatin (0.893±0.018) did not prevent cisplatin's' significant reduction ($p<0.001$) of MTT activity compared to medium alone (1.031±0.023).

Table 1
The effect of a 24h incubation with varying concentrations of cisplatin (0-5mM) or probenecid (0-5mM) on MTT activity in G401 cell line (n=18). Results are expressed as mean ±SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to media only, two-tailed paired t-test.

Conc.	Cisplatin (i)	Probenecid (ii)
0	1.042±0.079	0.763±0.021
0.5μM	0.899±0.139**	0.696±0.017**
50μM	0.637±0.128***	0.718±0.017*
100μM	0.470±0.051***	0.681±0.019***
500μM	0.175±0.122***	0.668±0.024***
5mM	0.100±0.018***	0.682±0.030***

Despite probenecid having a significant effect on MTT activity when applied alone, when applied before cisplatin it appeared to reverse cisplatin-induced reduction in mitochondrial activity.

We conclude that the G401 is sensitive to cisplatin and probenecid and may thus be of some future value in predictive nephrotoxicity.

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214P SURFACTANT POTENTIATION OF THE ANTI-BACTERIAL ACTION OF AMPICILLIN AND CHLORHEXIDINE

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Patch-clamp analysis of model lipid bilayers indicates that the non-ionic surfactant, Pluronic F-68 (PF-68), may cause the formation of trans-membrane pores (King *et al.*, 1991). Such pores would help to explain the increased uptake of fluorescein diacetate into yeast (King *et al.*, 1991) and 2-deoxyglucose uptake and amino acid incorporation into animal cells (Cawrse *et al.*, 1991) in the presence of this compound. PF-68 and other surfactants may be useful for modifying bacterial responses to antibiotics, by altering cell permeability. The effects have been studied of PF-68 or Triton X-100 (TRIT) on the sensitivity of *Escherichia coli* to ampicillin (AMP) or chlorhexidine (CHLOR). *E. coli* (RP437) was grown overnight in LB medium (5.0 ml) at 180 cycles min^{-1} and 37°C. Experimental cultures (0.12 ml of overnight culture in 12 ml medium) were then grown for up to 6 h in either LB medium alone or medium containing 1.0% (w/v) of (i) PF-68 (ICI, U.K.) or (ii) TRIT (Sigma, U.K.). In test cultures, the medium was supplemented with AMP (5.0 μg ml^{-1}; Sigma, U.K.) or CHLOR (10.0 μg ml^{-1}; Sigma, U.K.) after 180 min; culture growth was assessed by optical density (O.D.) at 600 nm.

Growth of *E. coli* with 1.0% (w/v) PF-68 was similar to control whereas 1.0% (v/v) TRIT inhibited culture growth such that after 180 min, the mean O.D. was reduced by 31%. Beyond 250 min, growth rates of treated and control cultures were identical. Addition of AMP to untreated cultures after 180 min reduced the mean (± s.e.mean; n = 3) O.D. after a further 70 min to 0.85 ± 0.03 compared to 1.06 ± 0.02 in control ($P < 0.01$). Cultures grown with PF-68 had a mean O.D. of 0.70 ± 0.02 at 70 min after addition of AMP. Addition of AMP to cultures grown for 180 min with 1.0% (v/v) TRIT reduced the mean O.D. after a further 70 min to 0.60 ± 0.02 compared to 1.06 ± 0.03 ($P < 0.01$) for cells grown with TRIT alone. Addition of CHLOR to cultures after 180 min reduced the mean O.D. after a further 70 min to 0.59 ± 0.02 compared to 1.07 ± 0.06 ($P < 0.01$) in control. Cultures grown with 1.0% (w/v) PF-68 had a mean O.D. of 0.50 ± 0.02 at 70 min after addition of CHLOR. Addition of CHLOR to cultures grown for 180 min with 1.0% (v/v) TRIT reduced the mean O.D. after a further 70 min to 0.42 ± 0.02 compared to 1.07 ± 0.06 ($P < 0.01$) for cells grown with TRIT alone. These results show that the growth of *E. coli* is partially inhibited by AMP or CHLOR and that PF-68 or TRIT can potentiate such growth inhibition. AMP inhibits bacterial cell wall formation by blocking transpeptidation of peptidoglycans (Stanier *et al.*, 1987), while CHLOR alters membrane permeability causing ion leakage (Franklin & Snow, 1989). If PF-68 induces the formation of transmembrane pores (King *et al.*, 1989), this may alter the membrane-bound cell wall biosynthetic apparatus, thus enhancing sensitivity to AMP, or exacerbating ion loss due to CHLOR.

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215P ABSENCE OF AN ANXIOLYTIC EFFECT OF THE 5-HT$_{2C/2B}$ ANTAGONIST SB200646A IN THE CONDITIONED EMOTIONAL RESPONSE MODEL OF ANXIETY IN THE RAT

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The 5-HT$_{2C/2B}$ receptor antagonist SB 200646A (N-1-methyl-5-indolyl)-N-(3-pyridyl), has been reported to induce anxiolytic-like effects in rats (Kennett *et al.*, 1994, 1995a). In this study, the effects of SB 200646A (SB) were compared to those of the 5-HT$_{1A}$ receptor partial agonist, ipsapirone (Ips) in the rat conditioned emotional response (CER) model of anxiety. Male Lister Hooded rats (approximately 250 g) were trained to lever press for food reward and given sessions in which two light presentations signalled mild electric footshock. Suppression in lever pressing during the light was used to index conditioned fear. Rats were trained until a stable level of conditioned fear was observed and occasional drug test sessions were then introduced. Prior to testing, rats were allocated to either drug or vehicle groups (n=9,11) such that the groups were matched on the behavioural measures. Pre and during-light (Dur) response rates (per min) and suppression ratios, (SR)=Dur/(Dur+Pre), were analysed using 1- and 2-way ANOVA and post-hoc Student Newman Keuls tests. When a significant Trial X Group interaction occurred, each light presentation was considered separately. SB and Ips, dissolved in 0.3% hydroxypropylmethylcellulose wt/volume in distilled water, were given p.o. 30 (Experiment 1) or 60 min (Experiment 2) prior to testing. Before Experiment 3, rats were food deprived for 4, 8, 16 or 23 h but not injected with drugs. Ips (50 mg/kg), increased SRs (eg., reduced conditioned fear) without enhancing pre-light response rates but SB (15-60 mg/kg) had no effect on conditioned fear in either experiment. SB tended to increase pre-light response rates, although this effect only reached statistical significance on Trial 1 of Experiment 2. Food deprivation also increased pre-light response rates without affecting conditioned suppression of responding. The absence of an anxiolytic effect of SB in the CER model contrasts with the report of an anxiolytic effect of this compound in the Geller-Seifter test (Kennett *et al.*, 1995a). The effects of SB in the CER test resemble the effects of increasing food deprivation, and it is noteworthy that SB has been shown to enhance feeding (Kennett *et al.*, 1995b). Therefore, it is possible that the anxiolytic-like effects of SB observed in the Geller-Seifter test may represent a false positive due to enhanced motivation to respond for food.

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Table 1 The effect of SB, Ips and Food Deprivation on CER performance. Data are expressed as mean ± SEM *=P<0.05 as compared to vehicle or FD 4 h

Experiment	Group	mg/kg	Pre (per min)	Dur (per min)	SR	Experiment	Group	mg/kg	Pre (per min)	Dur (per min)	SR
1	Vehicle		16.8±2.3	0.3±0.15	0.02±0.01	2	Vehicle		7.7±1.4	0.6±0.25	0.07±0.03
	SB	15	21.4±3.5	1.2±0.66	0.06±0.03		SB	15	8.9±1.9	1.2±0.79	0.09±0.03
	SB	30	25.0±3.8	2.7±1.65	0.09±0.04	Trial 2	SB	30	10.0±1.7	1.3±0.73	0.15±0.07
	SB	60	18.1±2.8	0.9±0.41	0.04±0.02		SB	60	8.2±1.1	1.2±0.72	0.11±0.07
	Ips	50	14.0±3.8	3.8±1.61	0.22±0.06*		Ips	50	11.6±2.0	4.4±1.52	0.25±0.07
2	Vehicle		6.8±1.8	0.1±0.07	0.03±0.02	3	FD 4 h		8.6±1.3	0.2±0.09	0.03±0.01
	SB	15	15.8±2.7*	0.5±0.25	0.03±0.01		FD 8 h		10.2±1.1	0.1±0.04	0.01±0.00
Trial 1	SB	30	12.6±0.4	0.8±0.51	0.05±0.03		FD 16 h		16.2±1.5*	0.3±0.17	0.01±0.01
	SB	60	10.9±1.1	0.3±0.15	0.03±0.01		FD 23 h		19.0±2.0*	1.3±0.69	0.05±0.02
	Ips	50	13.3±2.7	2.7±0.93*	0.21±0.08*						

216P REPEATED ADMINISTRATION OF A SELECTIVE 5-HT UPTAKE INHIBITOR DOWNREGULATES 5-HT$_{2C}$ BUT NOT 5-HT$_{1A}$ RECEPTOR-MEDIATED BEHAVIOUR IN THE RAT

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Decreases in 5-HT$_{2C}$ receptor function have been reported following chronic treatment with selective 5-HT reuptake inhibitors (Kennett et al., 1994). We have studied the effects of repeated administration of the selective 5-HT uptake inhibitor, Wy 27587 (Fletcher et al., 1988), on behavioural responses to a selective 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 5-HT syndrome and hypothermia) and to a 5-HT$_2$ receptor agonist 6-chloro-2(1-piperazinyl)pyrazine (MK-212; hypolocomotion) which were used as indices of 5-HT$_{1A}$ and 5-HT$_{2C/2B}$ receptor function in vivo (Hjorth et al., 1982; Knight & Fletcher, 1989).

Wy 27587 (10 mg/kg p.o. b.i.d.) or vehicle was administered to male Sprague Dawley rats (250-350g) for 14 days and behavioural testing performed 24h after the last dose. The 5-HT syndrome was assessed immediately following i.v. administration of 8-OH-DPAT using a sequential up/down procedure (Fletcher & Forster, 1989) or 15 min after s.c. administration (0.03 - 0.3 mg/kg; n≥8) using an arbitrary rating scale of 0-10 with increasing severity. Oesophageal temperature was measured prior to, and 15 and 30 min after, 8-OH-DPAT administration (0.03-0.3 mg/kg s.c.; n=8). The hypolocomotor response to MK-212 (0.75 and 2.0 mg/kg i.p. 30 min predose; n=6) was measured for a 2min period in automated open fields.

Chronic treatment with Wy 27587 had no significant effect on the 5-HT syndrome assessed using the sequential up/down method [ED$_{50}$ values and confidence limits for 8-OH-DPAT (mg/kg i.v.), vehicle 0.048 (0.040-0.058); Wy 27587 0.043 (0.038-0.049)], or on syndrome and hypothermic responses (maximum change in body temperature) to s.c. administration of 8-OH-DPAT (Table 1). However, the hypolocomotor response to MK-212 (2 mg/kg i.p.) was significantly attenuated by chronic pretreatment with Wy 27587 (Table 2). These data indicate that functional downregulation of the 5-HT$_{2C/2B}$ receptor may occur more readily than downregulation of the 5-HT$_{1A}$ receptor.

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Table 1: Effect of Wy 27587 on 5-HT$_{1A}$ receptor-mediated behaviours

8-OH-DPAT (mg/kg s.c.)	5-HT syndrome group totals (max = 80)		Hypothermia (temp dec.°C) mean ± s.e.mean	
	Vehicle	Wy 27587	Vehicle	Wy 27587
0	0	1	-0.1±0.1	0.1±0.1
0.03	7*	7	0.4±0.1*	0.4±0.1
0.1	28**	25**	1.0±0.1**	1.1±0.1**
0.3	61**	65**	2.6±0.2**	2.5±0.2**

Table 2: Effect of Wy 27587 on MK-212-induced hypolocomotion

MK-212 (mg/kg i.p.)	Locomotor activity (beam breaks/2min) mean ± s.e.mean	
	Vehicle	Wy 27587
0	120.5±5.3	127.8±7.1
0.75	92.3±7.3*	110.7±2.9*
2	28.3±7.1**	66.8±4.1** §

*P<0.05; **P<0.001 versus respective vehicle-controls; §P<0.05 versus respective chronic -vehicle group using Mann-Whitney U-test (5-HT syndrome) or two-way ANOVA/Fisher's protected LSD (hypothermia and locomotor activity)

217P FUNCTIONAL ANTAGONISTIC AND ANXIOLYTIC EFFECTS OF THE 5-HT$_{2C/2B}$ RECEPTOR ANTAGONIST, SB-200646A, IN RODENTS

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Non-selective 5-HT$_2$ receptor antagonists are active in some rodent models of anxiety (Kennett, 1993). Recently a selective 5-HT$_{2C/2B}$ antagonist, SB-200646 (SB), was reported to exhibit anxiolytic activity in the rat social interaction test (Kennett *et al.*, 1994). We have examined the activity of SB in two further rodent models of anxiety (the rat elevated zero-maze and the mouse light/dark box) and in a putative *in vivo* model of 5-HT$_{2C}$ receptor function in the rat, MK-212-induced hypolocomotion (Knight and Fletcher, 1989).

Male SD rats (250-350g; n=6) received SB or vehicle p.o. 30 min before the i.p. injection of MK-212 (1.5 mg/kg) or saline. Locomotor activity (LMA; infra-red counts) was measured 30-34 min later in automated open fields. In the rat elevated zero-maze (Shepherd *et al.*, 1994), rats (n=12-24) were injected p.o. with SB, chlordiazepoxide (CDP; 5 mg/kg) or vehicle and 60 min later were placed onto a closed quadrant of the maze for a 5 min test session. The following behaviours were scored: (a) open quadrant entries (OE), (b) head dips (HDIPS) and (c) stretched attend postures (SAP). The mouse light/dark box apparatus was as described previously (Bill *et al.*, 1992). Female Tuck T/O mice (28-35g; n=10) were dosed p.o. with SB, diazepam (DZP; 0.5 mg/kg) or vehicle and 60 min later were placed into the light compartment. The following behavioural measures were recorded in a 5 min test session: (a) rearing in the light (LCR) and dark compartments and (b) time spent in the light (%LC TIME).

The results are presented in Table 1. SB dose-dependently attenuated (but did not completely reverse) MK-212-induced hypolocomotion with a minimum effective dose of 20 mg/kg p.o. The compound was also active in the rat zero-maze, significantly increasing OE and HDIPS and decreasing SAP, and in the mouse light/dark box, significantly increasing LCR and %LC TIME. In both models the minimum effective dose was 10 mg/kg p.o. These data confirm the activity of SB-200646A in rodent models of anxiety and also demonstrate a correlation between anxiolytic efficacy and *in vivo* functional antagonism at 5-HT$_{2C}$ receptors.

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Table 1.

MK212-induced hypolocomotion		Rat elevated zero-maze				Mouse Light/Dark box		
Treatment (p.o./i.p.)	LMA (counts) / 4 min	Treatment (mg/kg p.o.)	HDIPS	SAP	OE	Treatment (mg/kg p.o.)	LCR	%LC TIME
Veh/Veh	224.2 ± 23.3	Veh	3.2 ± 0.6	2.7 ± 0.6	7.0 ± 0.9	Veh	12.8 ± 1.2	44.7 ± 2.0
Veh/MK (1.5)	'84.7 ± 11.4	SB(5)	3.8 ± 0.9	1.6 ± 0.5	8.5 ± 1.8	SB(3)	13.4 ± 2.1	50.5 ± 2.2
SB(10)/MK	91.0 ± 16.9	SB(10)	8.8 ± 2.2*	0.8 ± 0.4*	11.6 ± 1.8*	SB(10)	24.2 ± 1.7*	54.5 ± 2.8*
SB(20)/MK	139.7 ± 12.5*	SB(20)	7.8 ± 0.9*	0.6 ± 0.2*	12.0 ± 0.9*	SB(30)	22.5 ± 1.3*	52.8 ± 2.0
SB(40)/MK	164.8 ± 20.0*	SB(40)	8.3 ± 1.4*	1.2 ± 0.4*	12.6 ± 1.0*	SB(100)	26.6 ± 1.6*	62.8 ± 3.0*
SB(80)/MK	173.5 ± 22.2*	CDP(5)	14.3 ± 1.3*	0.6 ± 0.2*	14.0 ± 1.1*	DZP(0.5)	27.2 ± 1.9*	60.5 ± 3.5*

Data presented are mean ± s.e.mean. *P<0.05 versus Veh/MK-212 or vehicle- control groups (one-way ANOVA/Dunnett's test).

218P DESENSITISATION OF THE TRANSFECTED HUMAN 5-HT$_{2C}$ RECEPTOR IS ACCOMPANIED BY A DECREASED AFFINITY OF THE RECEPTOR FOR [^{3}H]5-HT

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Desensitisation of G-protein coupled receptors is thought to occur by receptor/G-protein uncoupling (Hausdorff et al., 1990). Using a human neuroblastoma cell line, SH-SY5Y, transfected with the cDNA for the human 5-HT$_{2C}$ receptor, we have previously shown that receptor mediated phosphoinositide (PI) hydrolysis desensitises rapidly upon continued stimulation by 5-HT (Briddon et al., 1995). In order to investigate the role of uncoupling in desensitisation of this receptor, we have compared changes in the binding of the agonist [^{3}H]5-HT and antagonist [^{3}H]mesulergine after exposure to 5-HT.

For ligand binding studies, SH-SY5Y/5-HT$_{2C}$ cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 5% dialysed foetal calf serum. This was replaced with DMEM without serum for 16 hours prior to assay, after which time cells were exposed to 5-HT. Cells were then rinsed with phosphate buffered saline, scraped and collected by centrifugation. Membranes were prepared, then washed 3 times by centrifugation (36,000g/4°C) before final resuspension in incubation buffer (50mM Tris , 5mM $MgCl_2$, 1mM EGTA, pH7.4). [^{3}H]Mesulergine (0-8nM) or [^{3}H]5-HT (0-40nM) was incubated with membranes for 60 min at 37°C and the reaction terminated by rapid filtration over Whatman GF/C filters. In both cases non-specific binding was determined by co-incubation with 10μM mianserin.

Binding of [^{3}H]mesulergine to SH-SY5Y/5-HT$_{2C}$ membranes was saturable and of high affinity (B_{max}=644±140fmol/mg protein, pK_d = 8.98±0.05M, mean±s.e.mean, n=5). [^{3}H]5-HT also bound to a single site (B_{max}= 122±23 fmol/mg protein; pK_d=8.30±0.06M, n=6). Though there was some variability in the B_{max} between assays, [^{3}H]5-HT consistently labelled a sub-population of receptors representing 19±3% (n=4) of those labelled by [^{3}H]mesulergine. Binding of [^{3}H]5-HT (5nM) was inhibited by mesulergine > 5-HT >mianserin >> ketanserin consistent with binding to 5-HT$_{2C}$ receptors. Incubation of cells with 1μM 5-HT for 15 min caused no significant change in the B_{max} or affinity for mesulergine binding (B_{max} = 538±129 and 489±73 fmol/mg protein; pK_d= 8.96±0.06M and 9.05±0.03M for control and treated respectively, n=4). Desensitisation caused a significant decrease in the affinity of [^{3}H]5-HT for the receptor (pK_d= 8.37±0.05M and 7.61±0.04M control and treated respectively, n=4, p<0.001, unpaired t-test), without a significant change in the number of sites labelled (B_{max} = 108±19 and 176±34 fmol/mg protein, n=4, p=0.18, unpaired t-test).

In summary, incubation of SH-SY5Y/5-HT$_{2C}$ cells with 5-HT for a period which causes substantial desensitisation of the PI response (Briddon et al., 1995), produces no change in the number of binding sites for either [^{3}H]mesulergine or [^{3}H]5-HT. However, the affinity of the agonist ligand is decreased, consistent with the process of uncoupling of the receptor from its G-protein. Desensitisation of the 5-HT$_{2C}$ receptor in this cell line therefore appears to be accompanied by a shift in the agonist affinity state of the receptor from a high to a low affinity form.

S.Briddon is a Wellcome Prize Student.

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219P 5-HT_{2A} BUT NOT 5-HT_{2C} OR 5-HT_{2B} RECEPTORS MAY MODULATE BLINKING INDUCED BY THE TRH ANALOGUE MK771

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We have previously demonstrated that low doses of 5-HT1A agonists modulate blinking induced by the TRH analogue MK771(pyro-2-aminoadipyl-histidyl-thiazolidine-4-carboxamide) (McCreary and Handley, 1992). The 5-HT2A/2C antagonists ritanserin and ICI169,369 attenuate blinking in TRH treated mice (Dursun and Handley, 1991; Dursun, 1992) but it is not known whether this modulation is through 5-HT2A or 5-HT2C receptor mechanisms. We have therefore tested 5-HT antagonists with varying selectivity for 5-HT2A, 5-HT2C and 5-HT2B receptors (and prazosin, given the alpha-1 adrenoceptor affinity of ketanserin (Leysen *et al.*, 1981) in a paradigm designed to investigate MK771-induced blinking (McCreary and Handley, 1992).

Aston-bred male MF-1 mice, 20-30g in weight, were administered vehicle (10.0ml/kg), ritanserin (Rit) (n≥6/group), ketanserin tartrate (Ket) (n≥5/group), prazosin (Praz) (n≥7/group) or SB 200646A (5-HT2B/2C antagonist, Forbes *et al.*, 1993) (n=10/group) 22.5 min. (or 52.5 min. SB 200646A experiment) prior to MK771 (2.5mg/kg i.p.) or vehicle. Blinking was counted 7.5 min. later for 5 min. All drugs were dissolved in 0.9% saline vehicle except SB 200646A which was suspended in 1% methyl cellulose (in 0.9% saline).

Ritanserin (1.0mg/kg i.p.) and ketanserin (1.0 and 5.0mg/kg s.c.) attenuated MK771-induced blinking whereas SB 200646A (20.0 and 40.0mg/kg p.o.), prazosin (2.0mg/kg i.p.) and ritanserin (3.0 and 5.0mg/kg i.p.) were without effect (*Table 1*). These results suggest that 5-HT2A receptors but not 5-HT2C, 5-HT2B, or alpha-1 adrenergic receptors may modulate TRH receptor related blinking.

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Table 1. Effects of antagonists on MK771-induced blinking (mean blinks in 5min. ± S.E.M.), NS=not significant; *, $p<0.025$ and **, $p<0.0005$ *cf.* control (analysed with unpaired t-test or 2-Way ANOVA (SB 200646A experiment)).

Antagonist	dose (mg/kg)	Vehicle +MK771	Drug +MK771
SB200646	20.0	178.8±35.90	184.80±12.9 NS
(n=10)	40.0	178.8±35.90	178.73±7.42 NS
Rit (n≤6)	1.0	104.05±14.37	70.611±7.42 *
	3.0	179.60±20.51	156.91±15.96 NS
	5.0	128.54±21.81	120.58±15.96 NS
Ket (n≤5)	1.0	148.87±29.38	60.454±19.57 *
	5.0	158.27±13.95	76.615±12.85 **
Praz (n≤7)	2.0	118.46±11.36	101.66±19.72 NS

220P *IN VIVO* EVIDENCE THAT D-FENFLURAMINE RELEASES 5-HT FROM NERVE TERMINALS OF BOTH THE DORSAL AND MEDIAN RAPHE NUCLEI

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Recent interest has focussed on possible differences in the pharmacology of 5-hydroxytryptamine (5-HT) neurones derived from the dorsal and median raphe nuclei (DRN and MRN). For example, there is evidence (albeit indirect) that the anorectic agent, d-fenfluramine (d-FEN), selectively releases 5-HT from nerve terminals of the DRN. Thus, the acute 5-HT releasing effect of d-FEN is attenuated in rats pretreated with p-chloroamphetamine (Series et al., 1995) which some studies (Mamounas et al., 1992), but not all (McQuade et al., 1994), suggest causes a selective loss of DRN 5-HT neurones. Furthermore, repeated treatment with high doses of d-FEN itself is claimed to induce DRN-selective 5-HT lesions (Mamounas et al., 1992). Investigation of the source of nerve terminals releasing 5-HT in response to d-FEN is difficult because DRN and MRN 5-HT terminal fields are often overlapping. However, in a recent microdialysis study, following up on earlier neuroanattomical findings, we identified the frontal cortex (FCx) and dorsal hippocampus (DH) of the rat as receiving a selective 5-HT input from the DRN and MRN, respectively (McQuade et al., 1995). Here we compare the 5-HT releasing action of d-FEN in the FCx and DH.

Male S-D (270-300 g) rats were anaesthetised with chloral hydrate and a concentric microdialysis probe (2 mm) was stereotaxically implanted into each of the FCx and contralateral DH. Probes were perfused with artificial CSF at 2 µl/min, and dialysates were collected every 20 min and analysed for 5-HT using HPLC-EC. Once 5-HT levels were constant (2-3 h post implantation) d-FEN was injected (3 or 10 mg/kg i.v.) or high potassium (60 mM KCl) was added to the perfusion medium. Dialysates were collected for a further 120 min.

Basal levels of 5-HT in perfusates of the FCx and DH (0.014 ± 0.003 and 0.012 ± 0.002 pmol/20 min, respectively; n=14) did not differ significantly. Also, the amount of 5-HT (0.025-0.028 pmol) released by 60 mM KCl was similar in both regions (n=5). The 5-HT response to 3 and 10 mg/kg i.v. d-FEN (n=5 and 4 rats, respectively) is shown in figure 1.

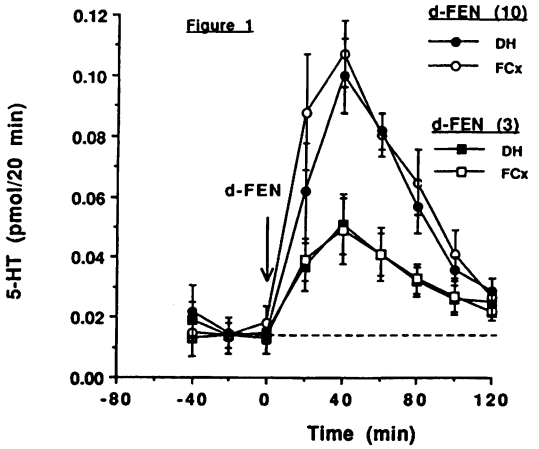

Figure 1 shows that d-FEN evoked a clear-cut and dose-related increase in 5-HT in both the FCx and DH. This effect of d-FEN was similar in magnitude and duration in both regions.

In summary, our microdialysis data show that, in the anaesthetised rat, d-FEN releases 5-HT to a similar degree in both the FCx and DH. On the basis of evidence that the FCx and DH receive 5-HT inputs from the DRN and MRN, respectively, our experiments indicate that d-FEN releases 5-HT from both DRN and MRN nerve terminals.

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221P 5-HT STIMULATES DOPAMINE RELEASE FROM RAT FRONTAL CORTEX

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Several "atypical" antipsychotics are potent antagonists of 5-HT_{2A} receptors as well as dopamine D_2 receptors. Recent evidence suggests a possible role for 5-HT in schizophrenia particularly in the frontal cortex (Laruelle *et al.* 1993; Joyce *et al.* 1993). The frontal cortex has been implicated in both the pathology of schizophrenia and in the therapeutic action of antipsychotic drugs, which may include effects on dopaminergic transmission in this region. We have previously shown (Jones *et al.* 1993) that glutamate agonists are able to stimulate the release of [^{3}H]dopamine from slices of rat frontal cortex. In this abstract we describe the effects of 5-hydroxytryptamine on [^{3}H]dopamine release in rat frontal cortex (Table 1.).

Minislices (350μm × 350μm) of rat frontal cortex (150g male Wistar) were pre-incubated with 22 nmol/L [^{3}H]dopamine for 15 minutes in the presence of desipramine (0.1μM) and pargyline (10μM). The tissue was then superfused with Krebs-phosphate buffer containing desipramine and pargyline as above and mazindol (10μM) at 400 μL/min. After 40 min of equilibration, fractions of superfusate were collected at 2 min intervals. Release of [^{3}H]dopamine was stimulated with a 2 min pulse of 5-HT. To determine the involvement of specific receptor sub-types in some experiments 5-HT antagonists were present from the beginning of tissue perfusion. [^{3}H]dopamine release was estimated by liquid scintillation counting . The responses to 5-HT were measured as the increase in release above basal for a period of 12 min after the pulse of 5-HT, and are expressed as a cumulative percentage of the total tissue [^{3}H]dopamine content at the time of collection.

Table 1.

The effects of 5-HT on [^{3}H]dopamine release from rat frontal cortex

5-HT Concentration	% [^{3}H]dopamine
100nM (n=6)	0.15 ± 0.07
1μM (n=12)	0.87 ± 0.15
10μM (n=36)	4.8 ± 0.3
100μM (n=6)	6.4 ± 0.5
200μM (n=6)	7.8 ± 1.1
500μM (n=12)	7.7 ± 0.4

Values are expressed as mean ± s.e.mean

When the specific 5-HT_{2A} receptor antagonist ketanserin (100nM) was placed in the perfusate there was a reduction in the response to 10μM 5-HT from 2.86 ± 0.29 to 1.94 ± 0.27 (n=6). There was also a small increase in basal release from 0.67 ± 0.03 to 0.77 ± 0.04. In both cases $P<0.05$ using t-test analysis. At 10μM ketanserin the response to 5-HT was reduced from 4.8 ± 0.3 to 0.12 ± 0.07 and there was a large increase in basal release from 0.77 ± 0.04 to 2.3 ± 0.1. This would be consistent with 5-HT stimulation of the release of dopamine primarily via 5-HT_{2A} receptors.
Thus one action of the recently introduced D_2/5-HT_{2A} antagonist antipsychotics may be to diminish 5-HT mediated cortical dopamine release.

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222P ELECTROPHYSIOLOGICAL ACTIONS OF 5-HT IN THE HUMAN CEREBRAL CORTEX *IN VITRO*

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Much is known about the electrophysiology and pharmacology of 5-HT in rodent brain tissue and in cultured cells containing endogenous and transfected 5-HT receptors. However, there is a paucity of data on the electrophysiology of 5-HT in the human cerebral cortex (McCormick & Williamson, 1989) because of the obvious difficulties in acquiring viable human cortical neurones. In this study we have attempted to determine which receptors mediate the actions of 5-HT in human cerebral cortical neurones *in vitro*.

After local ethical committee approval, 350 - 500 μm slices were prepared from samples of temporal, parieto-occipital and frontal cortex removed from 10 patients undergoing craniotomies for a variety of conditions. The slices were superfused *in vitro* with an artificial CSF at 30°C (see Newberry, 1992). Intracellular recordings were made with 3M K acetate (or 2M KCl)-filled microelectrodes. The apparent resistance of the cell membrane was tested with constant current pulses delivered through the recording electrode.

Stable intracellular recordings were obtained in 11 neurones from 7 slices. These cells were 0.6 mm (n = 1) and (1.4-1.5 mm, n = 10) down from the pial surface and their electrophysiological characteristics indicated that they were likely pyramidal cells. Their membrane potential was -70 mV (-62 to -79) (median, range) and their apparent input resistance was 40 MΩ (26 to 110). Superfused 5-HT (30 -50 μM) evoked a depolarization (+3 to +7 mV, n = 6), a hyperpolarization (-2 & -4 mV, n = 2), a hyperpolarization followed by depolarization (-3 & +6 mV, n = 1) or no effect (n = 2). The hyperpolarizing and depolarizing responses could be evoked reproducibly on individual cells. The depolarization was associated with an increase in the excitability of the cell and an increase in the apparent resistance of the cell membrane whether recorded with a K acetate or a KCl-filled electrode. The hyperpolarization was associated with a decrease in apparent membrane resistance. The depolarization was reduced by the 5-HT_2 receptor antagonists ketanserin (50 - 75 %, 0.1 - 1 μM, n = 3) and spiperone (80%, 1 μM, n = 1). The hyperpolarization was abolished on one neurone by 100 nM of the 5-HT_{1A} receptor antagonist WAY-100635 (Fletcher *et al.*, 1994).

These results indicate that these human neurones were depolarized by 5-HT_2 receptors (possibly 5-HT_{2A}) and hyperpolarized by 5-HT_{1A} receptors.

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223P THE 5-HT$_{1A}$ RECEPTOR ANTAGONIST WAY-100635 SELECTIVELY INCREASES DA SYNTHESIS RATE IN THE RAT PRE-FRONTAL CORTEX

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The proliferation of CNS 5-hydroxytryptamine (5-HT) receptor sub-types and the known interaction between 5-HT and dopamine (DA) systems has stimulated investigation of the relationship between 5-HT receptors and DA turnover (Nissbrandt *et al.*, 1992; Benloucif et al., 1993). Definitive studies are however dependent upon the availability of selective agonists and antagonists. Recently we reported that the silent and selective 5-HT$_{1A}$ receptor antagonist WAY-100635 (Fletcher *et al.*, 1994) significantly increased striatal DA synthesis and release (Brazell *et al.*, 1995). We now report the effects of WAY-100635 on DA synthesis rate in 11 rat brain regions. As the assay allows simultaneous assessment of 5-HT synthesis rate, this was also examined for comparison.

Male Sprague-Dawley rats (280 - 350 g, Charles River) were used. Animals received a single s.c. injection of vehicle or WAY-100635 (1.0 mg/kg), and brains were removed 1h later and dissected on ice, into the regions of interest (see Table 1). DA and 5-HT synthesis rates were assessed by measuring tissue 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) levels using HPLC-ED after L-aromatic amino acid decarboxylase inhibition by NSD-1015 (100 mg/kg, i.p.) given 45 min before the rats were killed. (For comparative purposes the previously reported striatal synthesis rate data is included in Table 1.)

WAY-100635 significantly increased DA synthesis rate in the pre-frontal cortex and striatum, but had no effect on DA synthesis in any of the other regions examined (see Table 1). 5-HT synthesis rate was not significantly effected by WAY-100635 in any of the regions studied. These results suggest that 5-HT$_{1A}$ receptor regulation of DA synthesis is regionally specific, and indicate that in the pre-frontal cortex, as in the striatum, 5-HT$_{1A}$ receptors have a tonic inhibitory influence on DA synthesis rate.

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Table 1. L-DOPA and 5-HTP concentrations (fmole/mg protein) in various rat brain regions after vehicle or WAY-100635 (1.0 mg/kg s.c.) administration

brain region	L-DOPA after vehicle	L-DOPA after WAY-100635	5-HTP after vehicle	5-HTP after WAY-100635
pre-frontal cortex	143.9 ± 13.6	192.2 ± 14.4 *	185.2 ± 18.0	182.8 ± 17.8
frontal cortex	1631.4 ± 193.1	1690.6 ± 234.1	1269.7 ± 156.3	1199.1 ± 121.9
nucleus accumbens	30767.3 ± 2984.2	40381.4 ± 5458.8	3510.1 ± 267.6	3075.1 ± 408.3
striatum	10954.7 ± 476.0	16921.1 ± 1002.4 **	1540.8 ± 84.6	1635.9 ± 137.9
hypothalamus	20239.3 ± 1286.1	20563.4 ± 845.3	20354.0 ± 1121.4	21381.7 ± 757.8
amygdala	6567.3 ± 590.6	6601.8 ± 416.8	7049.6 ± 546.5	6505.1 ± 257.3
hippocampus	1120.3 ± 75.0	1239.6 ± 35.0	2282.0 ± 201.2	2189.9 ± 261.6
ventral tegmental area	46249.4 ± 6903.9	34354.0 ± 6932.8	21266.6 ± 5448.4	21723.8 ± 4343.1
substantia nigra	22950.1 ± 2757.8	23454.7 ± 1556.8	12297.4 ± 701.6	14929.8 ± 914.8
medial raphe nucleus	4389.6 ± 724.4	3383.6 ± 543.9	29132.7 ± 2779.2	34478.1 ± 4714.4
dorsal raphe nucleus	14987.7 ± 1362.2	13892.9 ± 914.6	69432.0 ± 7513.8	89889.0 ± 9248.7

Significance was determined using ANOVA followed by Student's t-test. Values are mean ± s.e.m. n = 7 - 9. *p<0.05, **p<0.01

224P EFFECTS OF THE 5-HT$_{1A}$ RECEPTOR AGONIST, 8-OH-DPAT, AND RESTRAINT STRESS ON BLOOD PRESSURE AND HEART RATE IN THE CONSCIOUS RAT

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8-OH-DPAT and other 5-HT$_{1A}$ receptor agonists have been reported to lower mean arterial blood pressure (BP) and decrease heart rate (HR) in the conscious rat (McCall & Clement, 1994). This action is thought to be mediated by 5-HT$_{1A}$ receptors in the brain, stimulation of which inhibits sympathetic nerve activity (Fozard *et al.*, 1987). Previous studies have used conventional blood vessel cannulation in restrained animals to measure BP and HR, a procedure that is potentially stressful to the animals. Sympathetic nerve activity is reported to be elevated by stress (see Chaouloff, 1993), so we examined the possible contribution of stress to the cardiovascular effects of 8-OH-DPAT by comparing the effects of the drug on BP and HR in restrained rats with rats free to move in their home cages.

Male Sprague-Dawley rats (275-362g) were cannulated in the carotid artery and jugular vein, under halothane anaesthesia. 16h after recovering from surgery, rats were assigned to one of 2 groups: restrained, rats were confined to a blackened tube (diameter of 64mm), or non-restrained, rats had extensions attached to their external cannulae so that they were free to move in their home cages. After 30min habituation, 3 baseline readings of BP and HR were taken over 30min. Rats in each group were then injected with either 8-OH-DPAT (30, 100, or 300µg/kg iv), or saline (0.5ml/kg iv), and BP and HR recorded continuously for a further 2h.

Restrained rats had significantly greater baseline BP and HR than the non-restrained rats (Table 1). This may be due to increased sympathetic tone induced by stress. Saline did not change BP or HR in either group. 8-OH-DPAT dose dependently decreased BP and HR in the restrained rats at significantly lower doses than in the free moving rats (Table 1).

These results show that restraint stress enhances the cardiovascular effects of 8-OH-DPAT. This may be due to an increased sympathetic tone in the restrained rats.

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Table 1: Baseline values and maximum changes in BP and HR (mean±s.e.mean) induced by saline and 8-OH-DPAT in restrained and non-restrained cannulated rats (values in parentheses represent time to maximum effect in min).

Group		Base line values	Saline (0.5ml/kg iv)	8-OH-DPAT (µg/kg iv) 30	100	300
Non-restrained	Δ BP (mmHg)	129±2 †	-3±1 (45)	-6±3 (30)	-13±3 (10)	-16±1 (15)*
	Δ HR (bpm)	374±5 †	-6±9 (60)	-26±9 (15)	-53±14 (15)	-60±13 (15)*
Restrained	Δ BP (mmHg)	135±2	+3±1 (60)	-15±4 (15) *	-17±5 (15) *	-14±3 (15)
	Δ HR (bpm)	412±6	+14±6 (2)	-63±16 (10)	-97±8 (15) *	-107±16 (30) *

* represent significant changes from baseline values (1 factor ANOVA, Bonferroni/Dunn(control), p<0.05, n=6)
† represent significant differences in baseline values of non-restrained rats from restrained rats (1 factor ANOVA, p<0.05, n=24)

225P EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONIST, 8-OH-DPAT, AND SOCIAL ISOLATION ON BLOOD PRESSURE AND HEART RATE MEASURED BY RADIOTELEMETRY IN THE CONSCIOUS RAT

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8-OH-DPAT and other 5-HT_{1A} receptor agonists have been reported to lower mean arterial blood pressure (BP) and decrease heart rate (HR) in the conscious rat by a central site of action. The cardiovascular effects of 8-OH-DPAT are enhanced by restraint stress (Hallett *et al.*, 1995). Stress can also be induced in rats by keeping them in social isolation (Parra *et al.*, 1994). In this study, we used radiotelemetry to investigate the effects of 8-OH-DPAT on BP and HR in rats housed in pairs with rats housed in social isolation.

12 male Sprague-Dawley rats (143-170g), implanted with TA11PA-C40 radiotelemetry transmitters (Data Sciences, (Brockway *et al.*, 1991)) were divided into 2 groups of 6. One group of rats were individually housed (IH group) and the other group housed in pairs (PH group) for 2 weeks. At the start of each experiment, rats were transferred to clean cages for an hour to habituate before taking 4 baseline readings of BP and HR over 60min. Each rat was then injected with either 8-OH-DPAT (50, 100, 300, 1000, or 3000µg/kg sc) or saline vehicle (0.5ml/kg sc), and BP and HR were recorded for a further 2h. A crossover design with 1 week between each dose was used and each rat received all treatments.

The IH group had significantly greater baseline BP and HR than the PH group (Table 1). This may be due to increased sympathetic tone induced by the stress of social isolation. These values are lower than those of 129mmHg and 374bpm reported for free moving cannulated rats in our laboratory (Hallett *et al.*, 1995). This may reflect lower levels of stress, and hence sympathetic tone, in the radiotelemetered rats.

Saline injection caused significant increases in HR in the IH and the PH group, and an increase in BP in the PH group. This may be due to stress or increased activity caused by the injection. 8-OH-DPAT dose dependently decreased HR and BP in the IH group at lower doses than in the PH group (Table 1). These results show that isolation stress enhances the cardiovascular effects of 8-OH-DPAT. This may be due to the increased basal sympathetic tone in the isolated rats.

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Table 1: Baseline values and maximum changes in BP and HR (mean± s.e. mean) induced by saline and 8-OH-DPAT in rats housed individually or in pairs as measured by radiotelemetry (values in parentheses represent time to maximum effect in min).

Group		Baseline values	Saline (0.5ml/kg sc)	8-OH-DPAT (µg/kg sc) 50	100	300	1000	3000
PH	Δ BP	98±1 †	+15±4 (10) *	+6±3 (5)	+6±4 (60)	-6±2 (30)	-8±2 (30)	-11±2 (30) *
	Δ HR	325±3 †	+99±24 (10) *	+38±18 (5)	+28±15 (5)	-36±11(30)	-50±11 (30) *	-59±13 (90) *
IH	Δ BP	102±1	+10±3 (5)	-12±3 (60) *	-10±1 (15)	-21±5 (30) *	-14±2 (30) *	-12±3 (15)
	Δ HR	360±4	+81±29 (5) *	-49±20 (15)	-38±6 (15)	-78±17 (45) *	-62±18 (30) *	-56±16 (15) *

* represent significant changes from baseline values (1 factor ANOVA, Bonferroni/Dunn(control), $p<0.05$, n=6)
† represents significant differences in baseline values of PH rats from IH rats (1 factor ANOVA, $p<0.05$, n=36)

226P HYPERPHAGIA INDUCED BY THE NMDA RECEPTOR ANTAGONISTS, MK-801 AND CGS 19755, IN THE RAT IS BLOCKED BY THE SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST, WAY-100635

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There is increasing evidence of a functional interaction between glutamate neurotransmission and 5-HT_{1A} receptors. For example the 5-HT_{1A} receptor antagonist, WAY-100135 attenuated the behavioural syndrome induced by the non-competitive NMDA receptor antagonist, MK-801 (Loscher & Honack, 1993). Competitive and non-competitive NMDA receptor antagonists (Wirtshafter & Trifunovic, 1988) and 5-HT_{1A} receptor agonists induce feeding in rats. Here we have examined the effects of the selective and potent 5-HT_{1A} receptor antagonist, WAY-100635 (WAY635) (Fletcher et al., 1994), on feeding induced by MK-801 and a competitive NMDA receptor antagonist, CGS 19755 (Lehmann et al., 1988). Effects of WAY-100635 on feeding induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT were examined as a positive control.

The intake of a standard pelleted diet was assessed for 3h starting immediately after injection (s.c.) in free-feeding male Lister hooded rats (250-350g; n≥8) maintained in their home cages. The rats received MK-801 (0.01-0.1mg/kg), CGS 19755 (1-10 mg/kg), 8-OH-DPAT (0.3mg/kg) or vehicle, and at the same time WAY-100635 (0.3mg/kg) or vehicle.

MK-801 and CGS 19755 induced dose related increases in food intake, which were blocked by WAY-100635 (see Table 1). This contrasts with our previous finding that WAY-100635 does not block the enhancement of sucrose consumption induced by MK-801 (Laird et al., 1995). Thus, this interaction may be influenced by diet palatability. In agreement with previous findings, WAY-100635 also antagonised 8-OH-DPAT-induced hyperphagia (Hartley et al., 1994).

Hyperphagia induced by 8-OH-DPAT is mediated by activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe (Hutson et al., 1986). It is interesting that direct administration of NMDA antagonists into the raphe can also induce a feeding response (Wirtshafter & Trifunovic, 1988), suggesting that this may be one site of glutamate/5-HT_{1A} receptor interactions.

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Table 1. The effect of WAY-100635 (0.3mg/kg) on feeding induced by MK-801, CGS 19755 and 8-OH-DPAT in Lister hooded rats.

	MK-801				8-OH-DPAT	CGS 19755				8-OH-DPAT
mg/kg	0	0.01	0.03	0.1	0.3	0	1	3	10	0.3
vehicle	1.5±0.4	2.8±0.5*	3.1±0.6*	3.7±0.5*	2.9±0.6*	1.6±0.5	2.6±0.3*	3.3±0.5*	4.2±0.9*	5.1±0.7*
WAY635	1.3±0.5	1.2±0.2#	1.5±0.4#	1.6±0.7#	1.8±0.4#	1.3±0.4	1.9±0.3	1.9±0.4#	1.9±0.6#	2.1±0.5#

The data are reported as the mean 3h food intake (g) ± s.e.mean
* or # =$P<0.05$: * cf. vehicle group, # cf. vehicle/NMDA antagonist or 8-OH-DPAT group

227P THE DOSE-RELATED EFFECTS OF THE 5-HT_{1A} AGONIST GEPIRONE ON OPERANT AND NON-OPERANT FOOD INTAKE IN FOOD-DEPRIVED RATS

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$5HT_{1A}$ receptors agonists, such as 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT), buspirone and gepirone, have previously been shown to increase food intake in non-deprived rats (see Ebenezer, 1992a). In contrast, it has recently been demonstrated that 8-OH-DPAT decreases feeding in food deprived rats (Ebenezer, 1992b; Ebenezer and Parmar, 1993). The present study was undertaken to investigate if another $5HT_{1A}$ agonist gepirone also inhibited feeding in food-deprived rats.

Experiment 1. Wistar rats (n=8, b. wt. 330 - 400g) were fasted for 22h and injected s.c. with saline or gepirone (0.5 - 2 mg kg^{-1}). 15 min later they were placed individually in experimental cages in which they had free access to food and water. The amount of food consumed was measured 15, 30 and 60 min after presentation. A repeated measures design was used with each animal receiving all doses of drug. 4 - 5 days separated successive trials.

Experiment 2. Female Wistar rats (n=8, b. wt. 190 - 240g) were deprived of food for 22h a day and trained to press a lever in an operant chamber for food pellets on a fixed ratio of 5 (FR-5) as described previously (Ebenezer, 1992a). During the sessions that followed, the rats were injected s.c. with either saline or gepirone (0.25 - 1 mg kg^{-1}) , and placed seperately in the operant chamber for 60 min. Operant food intake was measured in 20 min bins during this period.

The results obtained in Experiments 1 and 2 were analysed by ANOVA and are illustrated in Tables 1 and 2 respectively.

The results of this study extends previous findings with other $5HT_{1A}$ agonists (Ebenezer, 1992b; Ebenezer and Parmar, 1993), and show that gepirone has acute dose-dependent depressant effects on food intake in food-deprived rats in both operant and non-operant paradigms. It is of interest to note that similar doses of the drug have previously been found to increase food intake in non-deprived rats (Ebenezer, 1994). Further work is necessary to identify the mechanisms involved.

Table 1. Effects of gepirone (G) on non-operant food intake. n = 8 rats
Dunnett's t-test: **P<0.01

	Mean Food Intake (g)± s.e.m.		
	0-15 min	0-30 min	0-60 min
Saline	4.5±0.2	7.4±0.3	12.5±0.6
G (0.5 mg kg^{-1})	3.9±0.3	6.7±0.4	11.3±0.8
G (1.0 mg kg^{-1})	3.1±0.2**	5.8 ±0.4**	10.1±0.7**
G (2.0 mg kg^{-1})	2.4±0.3**	5.2 ±0.4**	9.1±0.6**

Table 2. Effects of gepirone on operant food intake. n = 8 rats.
Dunnett's t-test: *P<0.05 **P<0.01

	Mean Food Intake (g) ± s.e.m.		
	0-20 min	0-40 min	0-60 min
Saline	6.6±0.2	7.7±0.3	8.3±0.4
G (0.25 mg kg^{-1})	5.5±0.6	7.5±0.6	8.4±0.6
G (0.5 mg kg^{-1})	4.9±0.7*	7.9±0.6	9.1±0.5
G (1.0 mg kg^{-1})	1.9±0.5**	5.3±0.7*	8.1±0.6

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228P EFFECT OF CHRONIC ADMINISTRATION OF QUININE AND QUINIDINE ON 5-HT TURNOVER AND 5-HT_{2A} RECEPTOR NUMBER IN RAT FRONTAL CORTEX

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We have previously shown that administration of quinine or quinidine to rats following inhibition of monoamine oxidase activity induces the classical serotonin behavioural syndrome (Wang & Grahame-Smith, 1992). Further studies into the mechanism underlying this response demonstrated that, *in vitro*, both quinine and quinidine induced 5-HT release from synaptosomes and inhibited 5-HT reuptake into brain slices (Clement et al., 1991). In the present study we have examined the effects of chronic administration of both these compounds to rats on 5-HT turnover and 5-HT_{2A} receptor number in rat frontal cortex and compared their actions to those of the SSRI citalopram and the tricyclic antidepressant desipramine.

Male SD rats (250g) were anaesthetized and Alzet osmotic mini-pumps implanted (s.c.) which released saline (240µl/day), quinine, quinidine, desipramine or citalopram at a rate equivalent to 10mg/kg/day. After either 4 or 14 days, rats were killed and the frontal cortex rapidly removed. The right half was immediately plunged into 10 vol. cold perchloric acid (0.1M), $Na_2S_2O_3$ (0.4mM) and homogenised using a Polytron. The tissue was then centrifuged (13,000g, 2min) and the supernatant tested for 5-HT and 5-HIAA content by HPLC with electrochemical detection. The left frontal cortex was homogenised in 5mM Tris/EDTA and washed twice by centrifugation (30,000g, 15min) before resuspension in assay buffer (50mM Tris, 5mM $MgCl_2$, 1mM EGTA, pH7.4). 5-HT_{2A} receptor density was measured using [^{3}H]ketanserin with non-specific binding defined by 10µM methysergide. Each group comprised 4 animals and statistical comparison between groups was made using ANOVA and Dunnett's t-test.

After 4 days administration, neither quinine nor quinidine caused any significant change in 5-HT or 5-HIAA levels. Desipramine similarly had no effect whereas citalopram significantly reduced 5-HIAA level by 26% (control 1.03 ± 0.07 nmol/g; mean ± s.e.m.; citalopram 0.76 ± 0.08 nmol/g ;$p<0.05$). 5-HT_{2A} receptor number was not significantly altered by any of the drugs. After 14 days administration, quinine, quinidine and desipramine again had no significant effect on 5-HT or 5-HIAA content, whereas 5-HIAA levels were still significantly decreased (39%) by citalopram (0.67 ± 0.09 nmol/g ; control 1.09 ± 0.07 nmol/g; $p<0.05$). 5-HT_{2A} receptor number was similarly unchanged following quinine, quinidine or citalopram but was significantly decreased (24%) by desipramine (172 ± 14 fmol/mg protein ; control 227 ± 16 fmol/mg protein; $p<0.05$).

These data indicate that in contrast to citalopram neither quinine nor quinidine modified 5-HT turnover in rat frontal cortex following administration for 4 or 14 days. Furthermore neither quinine nor quinidine induced down-regulation of cortical 5-HT_{2A} receptors at either time-point, unlike desipramine. Further investigation will therefore be needed to determine the mechanism underlying the action of quinine and quinidine to induce the serotonin behavioural syndrome

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229P FUNCTIONAL PROPERTIES OF A HUMAN RECOMBINANT 5-HT_3 RECEPTOR EXPRESSED IN *XENOPUS LAEVIS* OOCYTES

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The 5-hydroxytryptamine$_3$ (5-HT_3) receptor subtype mediates rapid neuronal signalling *via* the opening of an integral cation conducting channel (Peters *et al.*, 1992). The functional characterization of 5-HT receptors native to human neural tissue has been hampered by the lack of suitable experimental preparations. The well documented species differences in 5-HT_3 receptor pharmacology (Peters *et al.*, 1992) prompted the present study in which we report the cloning of a human 5-HT_3 receptor subunit and the results of initial studies into its pharmacological properties.

A cDNA encoding a 5-HT_3 receptor subunit was isolated from a human amygdala cDNA library. The mature protein is predicted to comprise 457 amino acids and demonstrates 85% sequence identity to an alternatively spliced form (5-HT_3R-A_s) of the mouse 5-HT_3 receptor subunit (Hope *et al.*, 1993). Stage V and VI *Xenopus laevis* oocytes were injected with *in vitro* RNA transcripts of the human cDNA at least two days prior to experimentation. Electrophysiological studies of the homo-oligmeric receptor subsequently expressed were conducted under two electrode voltage-clamp at a holding potential of -60 mV. Injected, but not control, oocytes responded to bath applied 5-HT (0.6 - 100μM) with a concentration-dependent inward current. The 5-HT concentration-effect relationship yeilded an EC_{50} of 3.1 ± 0.1 μM (mean ± s.e.m.) and a Hill coefficient (nH) of 1.9 ± 0.1 (n = 4). Currents were also elicited by the 5-HT_3 receptor selective agonists 2-methyl-5-HT (EC_{50} = 5.3 ± 0.5 μM; nH = 2.1 ± 0.04; n = 4) *m*-chlorophenylbiguanide (EC_{50} = 3.6 ± 0.4 μM; nH = 1.8 ± 0.1; n = 4) and 1-phenylbiguanide (EC_{50} = 84 ± 9 μM, nH = 2.1 ± 0.2; n = 4) although the maximum current produced by these agonists was only 87 ± 3% (n = 4), 81 ± 2% (n = 4) and 68 ± 3% (n = 4) respectively of that produced by a saturating concentration (100 μM) of 5-HT. Responses evoked by 3 μM 5-HT were unaffected by the bath application of methysergide or ketanserin at a concentration (1 μM) which would block certain G protein coupled 5-HT receptor subtypes. By contrast, the current was blocked (IC_{50} values in parenthesis) in a concentration-dependent and reversible manner by the selective 5-HT_3 receptor antagonists ondansetron (303 ± 98 pM) and granisetron (314 ± 36 pM) and the non-selective antagonists metoclopramide (177 ± 13 nM) cocaine (756 ± 132 nM), and (+)-tubocurarine (2.6 ± 0.2μM) (all n = 4). A reversible and monophasic inhibition was also observed with Zn^{2+} (30 nM - 30 μM; IC_{50} = 1.05 μM; n = 4).

A comparison of the pharmacological profile of the human and mouse (Hope *et al.*, 1993) recombinant 5-HT_3R-A_s receptors reveals several interesting differences. These are (i) a 1500-fold discrepancy in the antagonist potency of (+)-tubocurarine; (ii) differences in the maximal current response to 2-methyl-5-HT (relative to 5-HT) between the two clones and (iii) the absence, at the human receptor, of potentiation by *low* (*ie.* 300 nM - 10 μM) concentrations of Zn^{2+} (*cf.* Gill *et al.*, 1995). Alignment of the primary amino acid sequence of these species homologues reveals several potentially important differences within the extracellularly located N-terminal domain that might underlie such divergent properties. Site directed mutagenesis experiments designed to investigate the significance of these substitutions are currently in progress.

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230P SOLUBILISATION OF THE 5-HT_3 RECEPTOR RECOGNITION SITE EXPRESSED IN PIG CEREBRAL CORTEX

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We report presently the solubilisation of the 5-HT_3 receptor expressed in pig brain, which is a necessary prerequisite before purification of the receptor.

To prepare the radioligand binding homogenate, pig brain tissue frozen at -80°C was defrosted and homogenised in ice-cold Tris/Krebs buffer (mM; Tris, 50; NaCl, 118.0; KCl, 4.75; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11.0; pH 7.4; 500 mg wet weight tissue/ml) using a Polytron blender (full power; 10s). The homogenate was washed twice by centrifugation/resuspension in Tris/Krebs buffer and finally resuspended at a concentration of 500 mg wet weight/ml. Assay tubes contained 50 μL of competing drug or vehicle (Tris/Krebs; total binding) and 100 μL of radioligand ([^{3}H]-(S)-zacopride, 0.16-21.4 nM final concentration for saturation studies or approximately 1 nM for competition studies). 50 μL of homogenate was added to initiate binding which was allowed to proceed at 37 °C for 60 mins before termination by rapid filtration. Bound radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy.

[^{3}H]-(S)-Zacopride labelled a single, saturable specific binding site (defined by 10 μM ondansetron) in homogenates of pig cerebral cortex (K_d = 1.62 ± 0.35, B_{max} = 54 ± 6 fmol/mg protein, mean ± SEM, n = 6). Potent 5-HT_3 receptor antagonists displayed nM affinities for [^{3}H]-(S)-zacopride binding sites with the following rank order of potency: granisetron >SDZ 206-830 >BRL 46470 >ondansetron >tropisetron >MDL 72222 >d-tubocurarine. Metoclopramide and cocaine displayed micromolar affinity. The rank order of potency of agonists was: meta-chlorophenylbiguanide >5-HT >phenylbiguanide. Hill coefficients for agonist and antagonist inhibition of binding were close to unity.

[^{3}H]-(S)-Zacopride inhibition studies using a range of detergents indicated that Triton X-100 and GENAPOL displayed the least inhibition of [^{3}H]-(S)-zacopride binding at concentrations exceeding their critical micellular concentration. 5-HT_3 receptor binding sites were solubilised from homogenates of pig cerebral cortex (1 g original wet weight/mL in 25 mM Tris, pH 7.4) by addition of an equal volume of buffer (25 mM Tris, 2 mM EDTA, 100 μM PMSF, 10 μg/mL bacitracin, 10 μg/mL sodium azide, 10 μg/mL soybean trypsin inhibitor, pH 7.4) containing 0-4% Triton X-100 (final concentrations). Maximum yield (35.8% ± 3.2, % mean ± SEM, n=5) was obtained using Triton X-100 at a final concentration of 0.4%.

Preliminary studies suggest that the pharmacological profile of 5-HT_3 receptor sites solubilised from pig brain is similar to that of the receptor sites in the crude homogenates. For example, the antagonists ondansetron and tropisetron, and the agonist 5-HT, compete for [^{3}H]-(S)-zacopride binding to solubilised receptor preparations giving similar pK_i values to those obtained in crude homogenate preparations; 8.09 ± 0.08, 7.33 ± 0.21 and 6.43 ± 0.12 (mean ± SEM, n=3) respectively, compared to values of 7.71 ± 0.16, 7.44 ± 0.05 and 6.28 ± 0.02 (mean ± SEM, n=3-6) obtained in crude homogenates (in calculating the K_i values it was assumed that the K_d of [^{3}H]-(S)-zacopride for the solubilised preparation was the same as for the membrane preparation).

We conclude that the 5-HT_3 receptor recognition site expressed in pig brain has been successfully solubilised, which is an essential prerequisite for the purification of the receptor.

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231P NO EVIDENCE THAT (S)-ZACOPRIDE DISPLAYS INTRINSIC ACTIVITY AT THE 5-HT$_3$ RECEPTOR IN THE RAT VAGUS NERVE

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Trichloroethanol (TCE) selectively enhances the affinity and intrinsic activity of agonists for the 5-HT$_3$ receptor without modification of 5-HT$_3$ receptor antagonist affinity (Downie et al., 1995). The majority of functional studies indicate that quipazine behaves as an antagonist of the 5-HT$_3$ receptor although this agent evokes the Bezold-Jarisch reflex (Robertson et al., 1992) and activates the 5-HT$_3$ receptor in the presence of TCE (Downie et al., 1995). Studies have suggested that (S)-zacopride may display intrinsic activity at the 5-HT$_3$ receptor *in vivo* (Barnes et al., 1992; Middlefell et al., 1990; Sancilio et al., 1990). In the present study we assess whether TCE reveals an intrinsic activity for (S)-zacopride to depolarise the rat isolated vagus nerve.

Vagus nerve from female Wistar rats (150-200g) were prepared for extracellular recording as described previously (Johnston et al.,1995)

5-HT (10 nM - 30 μM) induced depolarizations of the vagus nerve (EC_{50} = 0.97 ± 0.13 μM, (mean ± SEM, n = 5)) that were antagonized by the selective 5HT$_3$ receptor antagonist ondansetron (20 nM; pK_B = 8.9, mean, n=2). TCE (5 mM) significantly increased the potency and maximal response of 5-HT to depolarize the vagus nerve (5-HT; EC_{50} = 0.50 ± 0.12 μM, mean ± SEM, n = 5,). TCE (0.1-10 mM) maximally increased the response to a submaximal concentration of 5-HT (0.3 μM), TCE pEC_{50} = 2.55 ± 0.13, (mean ± SEM, n = 3). 5-HT (0.3 μM)-induced depolarisations in the presence of TCE at concentrations above 10 mM were sub-maximal.
The selective 5-HT$_3$ receptor agonist, phenylbiguanide (PBG) induced depolarizations with near the same potency as 5-HT (EC_{50} = 1.49 ± 0.33 μM, mean ± SEM, n = 3). The PBG-induced depolarization of the rat isolated vagus nerve was also potentiated by TCE (5 mM), PBG; EC_{50} = 0.28 ± 0.07 μM, maximal response relative to PBG in the absence of TCE = 124 ± 20% mean ± SEM, n = 3, $p < 0.05$, Students paired t-test.
Similarly, TCE (5 mM) significantly potentiated the depolarisation induced by quipazine (300 nM) from 0.04±0.01 mV in the absence of TCE to 0.25±0.04 mV in the presence of TCE, an increase of 700±231% (mean±SEM, n=3) whereas (S)-zacopride (300 nM) in the presence of the selective 5-HT$_4$ receptor antagonist GR113808 (500 nM), failed to depolarise the vagus nerve even in the presence of TCE (5 mM).
The present study has identified that trichloroethanol enhances the potency and the intrinsic activity of 5-HT$_3$ receptor agonists to depolarize the isolated rat vagus nerve. In addition, no evidence was found to indicate that (S)-zacopride displays intrinsic activity at the 5-HT$_3$ receptor.

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232P 5-HT$_4$ RECEPTOR-MEDIATED MODULATION OF EXTRACELLULAR LEVELS OF 5-HT IN THE RAT HIPPOCAMPUS *IN VIVO*

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5-HT4 receptor binding sites have recently been identified in various regions of the brain including the hippocampus (e.g. Grossman et al., 1993). In the present study, we investigate the ability of the 5-HT4 receptor to modulate basal 5-hydroxytryptamine (5-HT) release in the rat hippocampus estimated using the *in vivo* microdialysis technique.

For microdialysis studies, female Wistar rats (150-250 g) were anaesthetised with ketamine (60 mg/kg, i.p.) and medetomidine (250 μg/kg, i.p.) before chronic indwelling guide cannula were stereotaxically inserted. At least 14 days after surgery, a microdialysis probe was inserted (4 mm dialysis tip; for probe construction see Barnes et al., 1992) and perfused with artificial cerebro-spinal fluid (aCSF; mM: NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28 $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2HPO_4 0.48, glucose 7.1, pH 7.4) at 2 μl/min. Dialysate samples collected for at least the first 100 min were discarded and subsequent samples were collected every 20 min. Drugs were administered via the perfusing aCSF and dialysate 5-HT levels were analysed immediately using HPLC-ECD (ANTEC working electrode +700 mV versus Ag/AgCl reference electrode) similar to our previous methodology (Barnes et al., 1992).

Administration of 5-methoxytryptamine (5-MeOT, 10 μM; in the continued presence of, and 80 min following, the perfusion of the 5-HT1/5-HT2 receptor antagonists, methysergide (10 μM) and pindolol (10 μM)) and renzapride (100 μM), maximally enhanced dialysate levels of 5-HT by 451±40% and 214±30% of basal values, respectively (mean±S.E.M., n=3-5). Lower concentrations of renzapride (1.0 - 10 μM) were less effective. In the presence of the selective 5-HT reuptake inhibitor paroxetine (1.0 μM), renzapride (100 μM) still enhanced dialysate levels of 5-HT (maximally 246±15%, mean±S.E.M., n=3). The selective 5-HT4 receptor antagonist, GR118303 (1.0 μM), reduced dialysate levels of 5-HT (maximally 50±8%, mean±S.E.M., n=4) and also completely antagonised the elevation of dialysate 5-HT levels induced by either 5-MeOT (10 μM; co-perfusion of methysergide (10 μM) and pindolol (10 μM) with GR118303) or renzapride (100 μM).

In the present studies, the 5-HT4 receptor agonists 5-MeOT and renzapride (Ford and Clarke, 1993) increased extracellular levels of 5-HT in the hippocampus of conscious rats. The selective 5-HT4 receptor antagonist GR118303 (Grossman et al., 1993), prevented both the 5-MeOT and the renzapride-induced responses which indicates that the response was receptor mediated. Furthermore, it is unlikely that the elevation in extracellular levels of 5-HT induced by renzapride was due to competition for the 5-HT reuptake channel since the renzapride-induced response remained in the presence of the selective 5-HT reuptake inhibitor, paroxetine. The ability of GR118303 to reduce extracellular levels of 5-HT when perfused alone suggests that their is an endogenous tone on the 5-HT4 receptor modulating this response.

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233P FURTHER CHARACTERISATION OF THE 5-HT4 RECEPTOR MODULATING EXTRACELLULAR LEVELS OF DOPAMINE IN THE RAT STRIATUM *IN VIVO*

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5-HT4 receptor binding sites have recently been identified in various regions of the brain including the striatum (e.g. Grossman et al., 1993). Follwing our earlier studies where the 5-HT4 receptor appears to enhance dopamine release (Steward et al., 1995), we further investigate the ability of the 5-HT4 receptor to modulate basal dopamine (DA) release in the rat striatum *in vivo* estimated using the microdialysis technique.

For microdialysis studies, female Wistar rats (150-250 g) were anaesthetised with ketamine (60 mg/kg, i.p.) and medetomidine (250 μg/kg, i.p.) before chronic indwelling guide cannula were stereotaxically inserted. At least 14 days after surgery, a microdialysis probe was inserted (4 mm dialysis tip; for probe construction see Barnes et al., 1992) and perfused with artificial cerebro-spinal fluid (aCSF; mM: NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28 $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2HPO_4 0.48, glucose 7.1, pH 7.4) at 2 μl/min. Dialysate samples collected for at least the first 100 min were discarded and subsequent samples were collected every 20 min. Drugs were administered via the perfusing aCSF and dialysate DA levels were analysed immediately using HPLC-ECD (ANTEC working electrode +700 mV versus Ag/AgCl reference electrode) similar to our previous methodology (Andrews et al., 1993).

Administration of renzapride (10-100 μM), maximally enhanced dialysate levels of DA by 140±10 % and 161±10%, respectively (mean±S.E.M., n=4). Administration of 5-methoxytryptamine (5-MeOT) (10 μM, in the continued presence of, and 80 min following, the perfusion of the 5-HT_1/5-HT_2 receptor antagonists, methysergide (10 μM) and pindolol (10 μM)), maximally increased the extracellular levels of DA by 220±20% (mean±S.E.M., n=3). The selective 5-HT4 receptor antagonist, GR118303 (1.0 μM), reduced dialysate levels of DA (maximally to 83±8%, mean±S.E.M., n=3) and also completely antagonised the elevation of dialysate DA levels induced by either renzapride (100 μM) or 5-MeOT (10 μM). The non-selective cAMP-dependent protein kinase inhibitor H7 (1.0 μM) reduced the extracellular levels of DA in rat striatum (maximally to 54±5%, mean±S.E.M., n=4) and compeletely prevented the renzapride (100 μM)-induced increase in the dialysate DA levels.

In the present studies, the 5-HT4 receptor agonists 5-MeOT and renzapride (Ford and Clarke, 1993) increased extracellular levels of DA in the striatum of conscious rats. The selective 5-HT4 receptor antagonist GR118303 (Grossman et al., 1993), prevented both the 5-MeOT and the renzapride-induced increase in dialysate DA levels which indicates that the response was receptor mediated. The ability of GR118303 to reduce extracellular levels of DA when perfused alone suggests that their is an endogenous tone on the 5-HT4 receptor mediating this response. The ability of H7 to prevent the renzapride-induced elevation of the extracellular levels of DA indicates that the response is mediated via a metabotropic transduction system consistent with previous work with the 5-HT4 receptor (Fagni et al., 1992).

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234P FURTHER CHARACTERISATION OF THE 5-HT_4 RECEPTOR MODULATING DOPAMINE RELEASE FROM RAT STRIATAL SLICES

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The initial demonstration of relatively high levels of 5-HT4 receptor binding sites in the rat striatum (e.g. Grossman et al., 1993) prompted our investigations to identify a neurochemical role for this receptor in this brain region. Our *in vitro* release studies indicate that the 5-HT4 receptor enhances dopamine (DA) release in the striatum (Steward and Barnes, 1994; Steward et al., 1995). In the present study, we further investigate the ability of the 5-HT4 receptor to modulate basal DA release from rat striatal slices.

Female Wistar rats (180-220g) were killed by cervical dislocation. The dissected striata were sliced (350 μm; Vibratome slicer) before being placed in gassed (95/5 O_2/CO_2) Krebs buffer (mM; NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11) plus tyrosine (50 μM), GBR12909 (1.0 μM; Heikkila et al., 1984) and (-)-sulpiride (1.0 μM) at 37°C for at least 60 min. Striatal slices (2-3) were carefully placed into wells containing 120 μl of Kreb's buffer which was exchanged for fresh buffer every 2 min for 16 min. Drugs were usually applied after 8 min and were subsequently present for the remainder of the experiment. For antagonism/inhibition of renzapride-induced responses, the antagonist/inhibitor was present throughout the 16 min collection period. DA levels and protein content of the striatal slices were assayed as described previously (Steward et al., 1995).

The 5-HT4 receptor agonist renzapride (3 - 10 μM), enhanced dopamine release from rat striatal slices in a concentration dependent manner (maximal increase after 2 min drug application, 214±35% of basal values, ANOVA $P<0.05$, $P<0.01$ Dunnett's t test, mean±SEM, n=14). The drug-induced responses were maximal within the first 2 min of drug application, with the response subsequently being reduced. The stimulation of DA release induced by renzapride (10 μM), was completely prevented by the selective 5-HT4 receptor antagonist, GR113808 (100 nM; Grossman et al., 1993) and the non-selective protein kinase A inhibitor, H7 (100 nM) with values 2 min after renzapride (10 μM) administration being 89±8 and 88±21% of basal values, respectively (ANOVA $P>0.05$, mean±SEM, n=3-6). Neither GR113808 (100 nM) nor H7 (100 nM) significantly altered the basal release of DA. Over the course of the study, basal DA release = 2.78±0.29 pmol/min/mg protein (mean±SEM, n=62).

The ability of the selective 5-HT4 receptor antagonist GR118303 to antagonise the renzapride-induced increase in DA release from rat striatal slices provides good evidence that this response is mediated via the 5-HT4 receptor. The ability of H7 to prevent the renzapride-induced response indicates that the receptor is coupled to a metabotropic transduction system consistent with previous work with the 5-HT4 receptor (Fagni et al., 1992).

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235P EFFECTS OF A SELECTIVE AND POTENT 5-HT$_4$ RECEPTOR AGONIST, RS-67333, AND ANTAGONIST, RS-67532, IN A RODENT MODEL OF SPATIAL LEARNING AND MEMORY

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5-HT$_4$ receptors are located in rat brain structures associated with learning and memory (Waeber et al., 1993). 5-HT$_4$ agonists also enhances acetylcholine release in rat hippocampus; an effect reversed by selective 5-HT$_4$ antagonists (Consolo et al., 1994). We have studied the effects of a selective, novel, 5-HT$_4$ receptor agonist, RS-67333 (pEC_{50} = 8.4; Eglen et al., 1995) on rat performance in a model of learning and memory, the Morris Water maze (Morris, 1984). In addition, the effects of a novel, selective 5-HT$_4$ receptor antagonist, RS-67532 (pK_B = 8.8; 4-amino-5-chloro-2-(3,5-dimethoxybenzyloxyphenyl)-5-5-(l-piperidinyl)-1-pentan-one; Clark et al., 1994) have also been studied, either alone, or against behavioural responses to this agonist.

Rats (male, 250-300 g) were placed in a circular pool, containing opaque water. Animal performance, assessed by means of a HSV image tracking system, was determined as the time taken by animal to locate the submerged platform. Central cholinergic function was disrupted by administration atropine (30 mg.kg^{-1}) 15 min prior to the onset of the trial. Compounds were administered, i.p., 30 min prior to initiating the trial, to either atropine or vehicle pretreated animals. The assay was undertaken on two consecutive days and the data quoted (mean ± s.e. mean, n=6) relate to the second day.

In animals pretreated with vehicle, but not atropine, neither RS-67333 (0.1 mg.kg^{-1}), nor RS-67532 (1 mg.kg^{-1}) affected the time taken to locate the submerged platform. Furthermore, RS-67532 (1 mg.kg^{-1}) did not affect the increase in time to platform induced by atropine pretreatment (time to platform, vehicle, 20 ± 2.9; atropine, 56 ± 5.6; atropine + RS-67532, = 61 ± 5.9s). In contrast, the 5-HT$_4$ agonist, RS-67333 (0.1 mg.kg^{-1}) significantly ($p<0.05$) reduced the deficit induced by atropine (time to platform; vehicle, 19 ± 2.1; atropine, 50 ± 8.4; atropine + RS-67333, 30 ± 4.7s). These data suggest that selective 5-HT$_4$ receptor agonism may reverse decrements in spatial learning and memory induced by atropine.

The improvement induced by RS-67333 was reversed by RS-67532 (1 mg.kg^{-1}; time to platform, atropine, 50 ± 8.4, RS-67333 + RS-67532, 59 ± 8.7s). Moreover, this reversal, by RS-67532 (1 mg.kg^{-1}) did not, affect improvements in the deficit induced by ondansetron (0.1 mg.kg^{-1}; time to platform, ondansetron 33 ± 2.7; ondansetron + RS-67532, 36 ± 6.4s).

In summary, administration of a selective, hydrophobic 5-HT$_4$ receptor agonist, RS-67333 (Eglen et al., 1995) reversed the deficit induced by atropine in this rat model. This improvement was blocked by the selective 5-HT$_4$ antagonist, RS-67532, at a dose that failed to reverse improvements seen with ondansetron. It is concluded that the data are consistent with a role of 5-HT$_4$ receptors in rodent cognition.

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236P LACK OF EFFECT OF A LESION OF CORTICAL NORADRENERGIC NEURONES ON INHIBITION OF SYNAPTOSOMAL [^{3}H]NORADRENALINE UPTAKE BY 5-HT UPTAKE INHIBITORS *EX VIVO*

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It has been suggested that uptake of noradrenaline (NA) into non-noradrenergic neurones is important in clearance of extracellular transmitter in rat cerebral cortex (Michel *et al.*, 1984; Dalley & Stanford, 1995). The present experiments investigated, first, whether a lesion of cortical noradrenergic neurones affects NA uptake in this brain region. To achieve this, synaptosomal uptake of [^{3}H]NA *ex vivo* was measured after administration of the neurotoxin, N-(2-chloroethyl)-N-2-bromo-benzylamine (DSP-4), *in vivo*. Secondly, the effects of the selective serotonin reuptake inhibitors (SSRIs) citalopram or fluoxetine, on uptake of [^{3}H]NA were measured in synaptosomes from intact and lesioned tissues.

Male SD rats (300-400g) were used. Lesions were induced by injection of DSP-4 (40mg kg^{-1}i.p.); control rats received an equivalent injection of saline. Animals were killed 5 days later and synaptosomes prepared from the cerebral cortex. These were incubated at 37°C, with and without test drugs, in Tris-Krebs buffer (pH 7.4) containing (mM): NaCl 136, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, *d*-glucose 10, *l*-ascorbate 1, Tris base 20, pargyline 0.25 and [^{3}H]NA (50nM). Assays included the selective NA uptake blocker, desipramine, as an active control. Duplicate samples were incubated at 4°C to estimate specific uptake of [^{3}H]NA. Results were analysed statistically using the Mann-Whitney U-test, 2-way ANOVA (main factors: drug concentration and lesion) or 1-way ANOVA with the Tukey test as appropriate.

5 days after injection of DSP-4, there was a significant reduction in cortical NA content (ng g^{-1} tissue: control, 279.8 ± 39.6; DSP-4, 153.6 ± 22.0; P=0.004). However, [^{3}H]NA uptake after the lesion was unchanged (Table 1). Both citalopram (Table 1) and fluoxetine significantly reduced uptake of [^{3}H]NA with IC_{50} values of 32 µM ($F_{3,40}$=8.27; P<0.001) and 12 µM ($F_{3,12}$=78.43; P<0.001), respectively. The IC_{50} of desipramine was 19 µM. The reduction in [^{3}H]NA uptake in the presence of citalopram was also unaffected by a DSP-4 lesion (drug *x* lesion interaction: $F_{1,14}$=0.657; P=0.581).

Table 1. The effect of a lesion of cortical noradrenergic neurones on the inhibition of synaptosomal [^{3}H]NA uptake by citalopram.

	CONTROL		DSP-4	
Control (buffer)	0.995 ± 0.1	(11)	0.835 ± 0.046	(11)
Citalopram 2.5µM	1.006 ± 0.108	(10)	0.782 ± 0.047	(10)
Citalopram 25µM	0.643 ± 0.067*	(11)	0.575 ± 0.047*	(11)
Citalopram 50µM	0.472 ± 0.074*	(11)	0.464 ± 0.044*	(11)

Values show mean ± s.e.mean uptake of [^{3}H]NA (pmol mg^{-1} prot.). Sample size shown in parentheses. * P< 0.05 *cf* equivalent control.

It is concluded that NA uptake is unaffected by a partial lesion of noradrenergic neurones in rat cerebral cortex. In view of the appreciable inhibition of [^{3}H]NA uptake by SSRIs, which is also unaffected by such a lesion, it is possible that serotonergic neurones contribute to this uptake. These findings support the suggestion that uptake of NA by neurones arising from the locus coeruleus has a minor role in regulation of extracellular levels of NA in the rat cerebral cortex (Thomas & Holman, 1991).

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237P CHANGES IN CORTICAL 5-HT1$_A$ RECEPTOR BINDING IN LOBAR ATROPHIES (PICK'S DISEASE AND DEMENTIA OF THE FRONTAL LOBE TYPE)

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The neurochemical profile of non-Alzheimer's dementia due to lobar atrophy (Pick's disease and Dementia of the Frontal Lobe Type (DFT)) appears to be distinct from that of Alzheimer's disease (Francis *et al.*, 1993; Qume *et al.*, 1994). We have examined $5HT_{1A}$ receptor binding (2nM 8-hydroxy-2-(n-dipropylamino)-tetralin ([^{3}H] 8-OH-DPAT), non-specific binding determined in the presence of 10μM serotonin) in the frontal (Brodmann Area, (BA) 9), temporal (BA21) and parietal (BA7) lobes of the cortex of previously frozen human brains from control patients (n=28), or patients suffering from histologically confirmed Pick's disease (n=10) or DFT (n=6). Subjects were matched for age, pH, sex and *post-mortem* delay.

	Frontal	Temporal	Parietal
Control	34 ± 4	44 ± 4	50 ± 3
Pick's Disease	17 ± 11*	15 ± 4*	47 ± 5
DFT	24 ± 6	25 ± 7*	67 ±12

Table 1: $5HT_{1A}$ binding (2nM [^{3}H] 8-OH-DPAT) in control, Pick's disease and DFT. Values are fmole/mg protein, mean ± s.e.mean. * indicates a significant difference from control, one-way ANOVA followed by LSD, P ≤0.05.

Table 1 shows that $5HT_{1A}$ receptor binding was significantly reduced (p ≤0.05) in the frontal and temporal lobes of Pick's disease cases compared with control. In DFT however, a significant reduction (P ≤0.05), compared with control, was observed in the temporal lobe only.

Scatchard analysis of the $5HT_{1A}$ receptor was performed (0.05-4nM, [^{3}H] 8-OH DPAT) on the temporal lobes of a sub-group of the above patients. Receptor affinities (Kd) of both Pick's disease (1.00 ± 0.21, mean ± s.e.mean, n=5) and DFT (1.20 ± 0.09, n=5) cases were unaltered compared with control values (1.01 ± 0.16nM, n=4). The B_{max} values were significantly reduced compared with control values (133 ± 24 fmole/mg protein, mean ± s.e.mean. n=4) in both DFT (65 ± 16, n=5) and Pick's disease (46 ± 14, n=5).

Pick's disease and DFT are characterised by loss of pyramidal neurones in the frontal and temporal lobes (Mann *et al.*, 1994). It is considered therefore that $5HT_{1A}$ receptors may provide a biochemical index of this cell loss.

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238P CHOLECYSTOKININ-A RECEPTOR ANTAGONISM SELECTIVELY REDUCES SPONTANEOUS PREFERENCE FOR ETHANOL IN NAIVE RATS

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Naive adult male Wistar rats (300g) were investigated for their preference to ethanol by exposure to a free choice paradigm, i.e. rats were free to choose between bottles containing either water or 10% ethanol (v/v). After 4 - 5 weeks, a clear identification of rats drinking mainly water (WD rats, alcohol intake: 3.1 ± 1.5 dg kg^{-1} daily) and rats drinking also ethanol (about 36 ± 7 dg kg^{-1} daily; ED rats) was made.

The neuropeptide cholecystokinin (CCK) is a neurotransmitter involved in anxiety pathways, as selective CCK agonists are anxiogenic in rats and man and selective CCK antagonists exert anxiolytic activity in rodents and primates (Crawley, 1988). In order to observe if a link between the neurochemical mechanisms beyond anxiety/stress and ethanol preference could exist, treatments with pentagastrin, an anxiogenic compound, or with L-365,260 a CCK-B receptor antagonist (Gaviraghi et al., 1992), anxiolytic compound, were performed daily for 4 days.

In ED rats, pentagastrin (2mg kg^{-1} i.p., n = 6) did not modify significantly the drinking behaviour. In contrast, six WD rats submitted to the same treatment showed a substantial increase (800 ± 180%) of their ethanol intake. Moreover, when ED rats were treated with high doses of the CCK-B antagonist L-365,260 (5 mg kg^{-1} i.p., n = 6) a large, but transient decrease of ethanol intake was detected; to about 58 ± 21% and 18 ± 14% of preinjection values 3 and 4 days after the beginning of the treatment, respectively. By contrast, L-365,260 was without effect when used at doses selective for the CCK-B receptor (0.5 μg kg^{-1}, Gaviraghi *et al.*, 1992). The selective CCK-A receptor antagonist L-364,718 at doses specifically affecting the CCK-A receptor (5μg kg^{-1} i.p., n = 6, daily, 4 days) halved the consumption of alcohol of the ED rats without modifiying their total liquid intake. This effect lasted at least for the three weeks post-treatment.

These data indicate the presence of a higher tone of the endogenous CCK system in ED rats compared to the WP rats. This tone could play a crucial role in sustaining the ethanol preference. Therefore, a potential therapeutic role for CCK-A antagonists in the treatment of ethanol abuse could be proposed.

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239P THE SUPPRESSANT EFFECT OF ATROPINE ON FOOD INTAKE IN RATS IS ATTENUATED BY PRETREATMENT WITH THE CCK_A RECEPTOR ANTAGONIST DEVAZEPIDE

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It has previously been demonstrated that systemic administration of the muscarinic antagonist atropine reduces food intake in rats (Stein, 1963). However, not much is known about the mechanisms involved. As the gut/brain neuropeptide cholecystokinin (CCK) has been implicated in satiety, the present study was undertaken to investigate whether the hypophagic effect of atropine may be linked with a CCK mechanism.

Experiment 1: Male Wistar rats (n=8, body weight 260 - 320g) that were deprived of food for 22h were injected i.p. with either vehicle solution (veh) followed by saline (sal), veh followed by atropine (0.6 mg kg^{-1}), devazepide (dev) (250 µg kg^{-1}) followed by sal, or dev (250 µg kg^{-1}) followed by atropine (0.6 mg kg^{-1}). A period of 15 min separated the two injections. The rats were presented with food and water 30 min after the 2nd injection, as described previously (Ebenezer, 1992). Food intake was measured 30 min after presentation. A repeated measures design was used with each animal receiving all treatments. A period of at least 2 days was allowed between successive trials. The data were analysed by ANOVA with repeated measures and *post-hoc* tests were carried out using the Newman-Keuls test.

Experiment 2: A similar experimental protocol as that described for Experiment 1 was used, except that the rats (n=8, body weight 290 - 370g) were injected i.p. with methyl atropine (0.6 mg kg^{-1}) instead of atropine.

Analysis of the data from Experiment 1 showed a significant main effect of drug treatment on food intake ($F_{(3,21)}$=27.2390, P<0.001). Atropine (0.6 mg kg^{-1}) significantly reduced food intake from a control mean ± s.e. mean of 6.9 ± 0.3g to 2.4 ± 0.4g (P<0.01). Dev (250 µg kg^{-1}) did not affect food intake (7.0 ± 0.3g) on its own, but pretreatment with dev significantly attenuated the suppressant effect of atropine (0.6 mg kg^{-1}) on feeding (food intake: 5.2 ± 0.5g, P<0.01). The results obtained in Experiment 2 showed that the quaternary analogue of atropine, methyl atropine (0.6 mg kg^{-1}), which does not cross the blood brain barrier, also significantly reduced food intake from a control mean ± s.e. mean of 8.6 ± 0.4g to 3.6 ± 0.6g. However, pretreatment with dev (250 µg kg^{-1}) did not significantly attenuate the depressant effect of methyl atropine on food intake (food consumed = 3.7 ± 0.7g).

The results of this study indicate that the CCK_A receptor antagonist dev attenuates the hypophagic effects of atropine, but not that of its quaternary analogue methyl atropine. As dev enters the brain from the systemic circulation, the results of this study suggests that there may be an interactive role between central cholinergic systems and CCK in the control of food intake.

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240P RECEPTOR BINDING CHARACTERISTICS OF THE SELECTIVE CCK-B ANTAGONIST GV 150013

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GV 150013 ((+)-N-{1-(1-Adamantane-1-methyl)-2,4-Dioxo-5-Phenyl-2,3,4,5 tetrahydro-1H, 1,5 Benzodiazepin-3-yl}-N'-Phenylurea) is a new, highly selective CCK_B antagonist (Corsi et al., 1995). The present study was aimed at exploring the binding affinities of GV 150013 at central CCK_B receptors from rat, guinea pig and human tissue as well as determining the selectivity against rat pancreas CCK_A receptors.

Tissue membrane preparations were obtained essentially as described before by Innis and Snyder (1980) for CCK_A receptors, and by Van Dijk et al., (1984) for CCK_B receptors in rat and guinea pig (GP). HeLa cells, expressing human temporal cortex CCK_B receptors (Denyer et al., 1994), were kindly provided by Glaxo Group Research. The cells were grown to confluency in standard culture flasks, scraped, homogenized, centrifuged for 10 min at 50,000 x g, and resuspended to a final protein concentration of 25-30 µg/ml. The binding conditions in all CCK_B assays were 60 min at 25 °C. In the CCK_A binding assay, at 37 °C, receptor density was reduced by pancreatic enzyme activity at a rate of 15 to 50 %/hr, depending on the specific membrane preparation. The binding was performed by incubation for 90 min at 37 °C. In both assays [^{3}H]-CCK8s was used at a concentration of 0.2 nM.

K_D-values obtained for [^{3}H]-CCK8s were 0.3 (0.24 - 0.35) nM (GP), 0.53 (0.35-0.67) nM (rat) and 89 (81-97) pM ($hCCK_B$); Bmax amounted to 57 (51-63) (GP), 27 (25-29) (rat), and 200 (169-231) ($hCCK_B$) fmol/mg protein (range from 3-4 experiments in triplicate). The K_D of CCK8s obtained in rat pancreas was 0.2 nM, and the approximate Bmax value 200-600 fmol/mg, depending on intrinsic pancreatic enzyme activity. Changes in Bmax did not modify Ki values obtained. Table 1 shows that all compounds had their highest CCK_B affinity in $hCCK_B$ expressing HeLa cell membranes, whereas they were lowest in rat cerebral membranes, suggesting that species differences may exist between CCK_B receptors. GV 150013 was the most selective CCK_B antagonist (at least 500-fold) when intraspecies selectivity was considered. This was clearly higher than for PD 134308 (75-fold) and for the less potent R-L365260 (25-fold).

It is concluded that GV 150013 is a selective CCK_B antagonist with high affinity, both for rodent and human cerebral CCK_B receptors.

Table 1: pK_i values of GV 150013 and reference compounds obtained in CCK_A and CCK_B binding assays. (mean ± s.e.m., n = 3-6 experiments).

Compound	GP-B	Rat-B	$hCCK_B$	Rat-A
GV 150013	**9.15±0.04**	**8.55±0.05**	**9.43±0.12**	**5.83±0.05**
R-L365260	8.53±0.11	7.91±0.05	8.80±0.06	6.48±0.09
PD 134308	8.73±0.06	8.03±0.04	9.45±0.06	6.16±0.06
S-L364718	7.06±0.02	6.57±0.10	7.53±0.10	9.83±0.05
CCK8s	9.83±0.05	9.10±0.08	10.1±0.02	9.45±0.10
CCK4	7.45±0.15	7.71±0.03	8.22±0.07	4.27±0.03

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P. Bertrand, A. Böhme, O. Piot, C. Durieux[1], B. Jeantaud, P. Boudeau, C. Pauchet, F. Manfré, C. Guyon, M.C. Dubroeucq & A. Doble, Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, Vitry-sur-Seine, and [1]Laboratoire de Pharmacochimie Moléculaire, CNRS URA 1500/INSERM U-266, Faculté de Pharmacie, Paris, France.

Cholecystokinin is an important gastrointestinal hormone which predominantly occurs in the mammalian brain as a sulphated octapeptide (CCK_8). Significant progress in the understanding of its biological roles has been initiated by the use of non-peptide antagonists selective for each CCK receptor subtype (CCK_A or CCK_B/gastrin sites). Here, we describe the binding profile and pharmacological properties of a novel CCK_B receptor antagonist, RPR 101367 [(+)-(2R, 4R)-3-{-3-{-2-[4-*ter*-butoxycarbonyl -2-(-2-fluoro-phenyl)-3-thiazolidinyl]-2-oxo-ethyl} ureido} phenylacetic acid].

In vitro binding assays performed as described by Bertrand *et al.* (1994) demonstrated that RPR 101367 potently inhibited CCK_B receptor binding in guinea pig brain membranes (Ki = 3 nM). Its affinity for CCK_A receptors in guinea-pig and rat pancreas corresponded to Ki values of 139 and 608 nM respectively. The apparent ability of RPR 101367 to penetrate into the mouse brain was demonstrated by an *ex vivo* binding assay similar to that reported by Bertrand *et al.* (1994) with a transcardiac perfusion step. A dose-dependent inhibition of cerebral [^{3}H]propionyl-CCK_8 binding was observed after i.p. and p.o. administrations of RPR 101367 (ID_{50} values of 9 and 12 mg/kg respectively). In rat hippocampal slices prepared as described by Böhme *et al.* (1988), RPR 101367 potently antagonized CCK_B receptor-mediated excitatory effects of CCK_8 with an IC_{50} value of 0.18 µM; in this bioassay, RPR 101367 was 4 to 10 times more potent than CI-988 (IC_{50} = 0.8 µM) and L-365,260 (IC_{50} =1.8 µM), two known and selective CCK_B receptor antagonists.

These compounds were previously shown to be active in behavioural models of anxiolytic drug action (Rataud *et al.*, 1991; Singh *et al.*, 1991). In this respect, RPR 101367 displayed an anxiolytic-like effect in the elevated plus-maze test performed as described by Rataud *et al.* (1991). The number of entries into the open arms of the maze and the time spent in these arms by CD_1 mice (n≥8) were significantly increased following i.p administration of RPR 101367 (3 mg/kg). This increase in exploratory behaviour was comparable to that observed with diazepam (1 mg/kg i.p., n≥8), and was not due to an increase in locomotor activity. Moreover, RPR 101367 was tested in the mouse marble-burying paradigm, a model responsive to clinically used anxiolytics, by using minor modifications of the method of Njung'e & Handley (1991). The number of marbles buried by treated CD_1 mice (n≥8) was significantly and dose-dependently decreased after i.p. administration of RPR 101367 (1, 3 and 10 mg/kg). By comparison, diazepam (0.5 mg/kg, n≥8) potently reduced marble burying behaviour after oral administration.

Interestingly, RPR 101367 could also induce promnesic-like effects in the rat social recognition test performed as described by Lemaire *et al.* (1992). Adult Wistar rats (n=10) treated by i.p. administration of RPR 102681 still remembered the odour of a juvenile 120 min after an initial exposure, and took significantly less time to investigate it during the second contact. RPR 102681 (0.3-3.0 mg/kg) thus reduced the ratio of investigation duration (R.I.D) of the second to the first contact when compared to control group. A significant decrease of R.I.D values were also observed at very low doses of L-365,260 (0.03-3 mg/kg i.p.) and CI-988 (0.01-3 mg/kg i.p.).

Taken together, these results indicate that RPR 101367 is a novel and useful tool to examine the involvement of CCK pathways in brain disorders.

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242P PHARMACOLOGICAL ANALYSIS OF THE EFFECT OF SOMATOSTATIN AND RELATED ANALOGUES ON NORADRENALINE RELEASE AND FIRING RATE IN THE RAT LOCUS COERULEUS *IN VITRO*

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Somatostatin (SRIF) has a wide distribution throughout the CNS and has been shown to affect electrical activity and neurotransmitter release in a number of systems. In this study we have used novel methodology to study the effect of SRIF and some related analogues on spontaneous firing rate and noradrenaline release, simultaneously, in the rat locus coeruleus (LC).

Brain slices (350µm) containing the LC were prepared from male AHA Sprague-Dawley rats (150-200g). Slices were placed in a perspex bath superfused with oxygenated artificial CSF at 32°C (flow rate = 1.2ml min^{-1}). Measurement of noradrenaline release was performed using fast cyclic voltammetry, together with simultaneous unit recording from the same carbon electrode (Stamford *et al.*, 1993). The LC was identified as a translucent area, ventrolateral to the fourth ventricle, where a regular firing rate (0.5-3Hz) with a spike duration of 1-2msec could be recorded.

At the commencement of each experiment the pharmacological profile of the LC site was tested with 30µM NA; this caused an abolition of the firing rate and a substantial increase in the voltammetry signal (beyond the maximum recordable), both of which were reversible upon washing. The cumulative application of SRIF caused a concentration-dependent decrease in the firing rate (EC_{50} = 13.4nM [8.3-21.5], Hill slope = 1.33±0.1, n= 23), which was reversible upon washing. On no occasion did the voltammogram record indicate that SRIF caused a change in NA levels in the LC. A second concentration-effect curve to SRIF was highly reproducible 45min after the end of the first (EC_{50}= 14.6nM [3.9-53.5], n= 4). The equipotent molar ratios (EMR) of some SRIF analogues (see Raynor *et al.*, 1993) were therefore compared with those of SRIF (see Table 1).

Table 1. Potencies of SRIF and related analogues on the firing rate of LC neurones. Values are the geometric means (EC_{50} and EMR) and arithmetic mean (Hill slope) of *n* observations.

	EC_{50} (nM)	EMR	Hill slope	*n*
SRIF	14.6 [3.9-53.5]	1.35 [0.4-4.7]	1.7±0.3	4
SRIF28	16.1 [1.8-148]	0.96 [0.5-1.9]	1.9±0.3	3
BIM23027	9.3 [0.6-143]	0.23 [0.04-1.2]	1.2±0.1	4
MK678	6.8 [0.8-55.1]	0.37 [0.1-1.1]	1.6±0.2	3
L362855	16.4 [0.5-488]	3.69 [0.3-40.5]	1.5±0.4	3
Octreotide	2.6 [1.3-5.3]	0.31 [0.2-0.4]	1.3±0.1	3
BIM23056	inactive	-	-	3

The inhibitory effect of SRIF on the firing of LC neurons was not tachyphylatic, which contrasts with other effects of SRIF (Feniuk *et al.*, 1993). The high potency of BIM23027 and MK678 (seglitide) suggests that this inhibition is mediated via stimulation of sst_2 receptors (Raynor *et al.*, 1993); and it does not involve the release of noradrenaline. However, the high potency of L362855 raises the possibility that an sst_5, or other SRIF receptor might also be involved (Raynor *et al.*, 1993). In addition, this study demonstrates that rapid changes in firing rate and neurotransmitter release from CNS nuclei can be measured and quantified using the carbon electrode system introduced by Stamford and colleagues (1993).

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243P ELEVATION IN THE LEVELS OF AT_2 RECEPTORS IN THE TEMPORAL CORTEX (BRODMANN AREA 21) OF PATIENTS WITH ALZHEIMER'S DISEASE

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Our previous studies have demonstrated that angiotensin-converting enzyme density is significantly increased in the temporal cortex from patients with Alzheimer's disease (Barnes et al., 1991). This is consistent with elevations in the activity of this enzyme in the brain of patients with Alzheimer's disease (Arregui et al., 1982). In the present study, we report changes in the levels of angiotensin receptor subtypes in brain regions from patients with Alzheimer's disease (AD).

Human brain tissues were obtained at autopsy from male and female patients with clinically diagnosed Alzheimer's disease and age-, sex- and post-mortem delay-matched (control) patients who had died without a neurological or psychiatric disorder. Individual brain tissues were homogenized in ice-cooled incubation buffer (mM: sodium chloride 150; sodium dihydrogen phosphate 50; magnesium chloride 10; EGTA 5 and 0.4 % w/v bovine serum albumin, final pH 7.4) and washed (see Barnes et al., 1991 for methodology). For [^{125}I][Sar^1, Ile^8]angiotensin II binding, 50 µl competing compound (1.0 µM of either losartan or PD123,177) or vehicle (incubation buffer), 50 µl [^{125}I][Sar^1, Ile^8]angiotensin II (0.1 nM) and 250 µl brain homogenate were incubated for 90 min at 25°C before termination by rapid filtration. Radioactivity remaining on the filters was quantified using a gamma-counter. Protein content of the brain homogenates was assayed by the Bio-Rad Coomassie Brilliant Blue method (Bradford, 1976).

In temporal cortex (Brodmann area 21) homogenates from control patients, [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 15 % and 10 % of the total binding, respectively (specific AT_1 and AT_2 receptor binding=0.35±0.04 and 0.08±0.01 fmol/mg protein, respectively, mean±S.E.M., n=8). In temporal cortex (Brodmann area 21) homogenates from patients with AD, specific AT_2 receptor binding was increased by approximately 300 % ($P<0.01$, Student's t test) when compared to the levels in tissue from control patients, whilst specific AT_1 receptor binding remained unaltered. In substantia nigra homogenates from control patients, [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 70 % and 3% of the total binding, respectively (specific AT_1 and AT_2 receptor binding=7.95±0.95 and 0.21±0.07 fmol/mg protein, respectively, mean±S.E.M., n=10). In substantia nigra homogenates from patients with AD, specific [^{125}I][Sar^1, Ile^8]angiotensin II binding to AT_1 receptor was reduced by approximately 30 % ($P<0.05$, Student's t test) whilst specific AT_2 receptor binding was similar to the levels in tissue from control patients. In homogenates of caudate nucleus, putamen, frontal cortex (Brodmann area 10), temporal cortex (Brodmann area 22), cerebellum and hippocampus, the levels of both AT_1 and AT_2 receptors were not significantly altered in tissues from patients with AD.

The present finding further implicates an elevation in the central angiotensin systems in Alzheimer's disease.

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244P REDUCTIONS OF BOTH AT_1 AND AT_2 RECEPTOR LEVELS IN THE CAUDATE NUCLEUS OF PATIENTS WITH PARKINSON'S DISEASE

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Previous studies have demonstrated that the angiotensin system is altered in Parkinson's disease (e.g. Allen et al., 1992). In the present study, we investigated the levels of angiotensin receptor subtypes in brain tissues from patients with Parkinson's disease (PD) and control subjects.

Human brain tissues were obtained at autopsy from male and female patients with clinically diagnosed Parkinson's disease and age-, sex- and post-mortem delay-matched (control) patients who had died without a neurological or psychiatric disorder. Individual brain tissues were homogenized in ice-cold incubation buffer (mM: NaCl 150; NaH_2PO_4 50; $MgCl_2$ 10; EGTA 5 and 0.4 % w/v bovine serum albumin, final pH 7.4) and washed (see Barnes et al., 1991 for methodology). For [^{125}I][Sar^1, Ile^8]angiotensin II binding, 50 µl competing compound (1.0 µM of either losartan or PD123,177) or vehicle (incubation buffer), 50 µl [^{125}I][Sar^1, Ile^8]angiotensin II (0.1 nM) and 250 µl brain homogenate were incubated for 90 min at 25°C before termination of the binding by rapid filtration through GF/B filters. Radioactivity remaining on the filters was quantified using a gamma-counter. Protein content of the brain homogenates was assayed by the Bio-Rad Coomassie Brilliant Blue method (Bradford, 1976).

In homogenates of caudate nucleus from control patients, the [^{125}I][Sar^1, Ile^8] angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 75 % and 10 % of total binding, respectively (specific AT_1 and AT_2 receptor binding=35.7±6 and 3.3±0.7 fmol/mg protein, respectively, mean±S.E.M., n=10). In caudate nucleus homogenates from patients with PD, specific [^{125}I][Sar^1, Ile^8]angiotensin II binding to AT_1 and AT_2 receptors was decreased by approximately 70 % and 60 %, respectively ($P<0.01$, Student's t test). In putamen homogenates from control patients, the [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 70 % and 5 % of total binding, respectively (specific AT_1 and AT_2 receptor binding=42.4±4.9 and 1.6±0.5 fmol/mg protein, respectively, mean±S.E.M., n=10). In putamen homogenates from patients with PD, specific [^{125}I][Sar^1, Ile^8]angiotensin II binding to AT_1 receptors was decreased by approximately 70 % ($P<0.01$, Student's t test), whereas AT_2 receptors levels were unaltered. In substantia nigra homogenates from control patients, the [^{125}I][Sar^1, Ile^8] angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 80 % and 7 % of total binding, respectively (specific AT_1 and AT_2 receptor binding=9.9±1.2 and 0.22±0.06 fmol/mg protein, respectively, mean±S.E.M., n=10). In substantia nigra homogenates from patients with PD, specific [^{125}I][Sar^1, Ile^8]angiotensin II binding to AT_1 receptors was decreased by approximately 90 % ($P<0.01$, Student's t test), whereas AT_2 receptor levels were not altered.

The principle findings suggest that significant populations of both AT_1 and AT_2 receptors within the terminal regions of the nigro-striatal pathway are located on neurones that degenerate in Parkinson's disease (dopamine neurones?).

We thank the MRC Brain Bank (Cambridge) for the gift of tissue.

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245P ALTERATION IN THE LEVELS OF AT_1 AND AT_2 RECEPTOR LEVELS IN BRAIN TISSUES FROM PATIENTS WITH HUNTINGTON'S DISEASE

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Previous studies have indicated that the angiotensin-converting enzyme (ACE) activity is decreased in the striatum from patients with Huntington's disease (e.g. Arregui et al., 1979). The present studies investigated the levels of angiotensin receptor subtypes in caudate nucleus, putamen and substantia nigra from patients with Huntington's disease (HD).

Human brain tissues were obtained at autopsy from male and female patients with clinically diagnosed Huntington's disease and age-, sex- and post-mortem delay-matched (control) patients who had died without a neurological or psychiatric disorder. Individual brain tissues were homogenized in ice-cold incubation buffer (mM: NaCl 150; NaH_2PO_4 50; $MgCl_2$ 10; EGTA 5 and 0.4 % w/v bovine serum albumin, final pH 7.4) and washed (see Barnes et al., 1991 for methodology). For [^{125}I][Sar^1, Ile^8]angiotensin II binding, 50 µl competing compound (1.0 µM of either losartan or PD123,177) or vehicle (incubation buffer), 50 µl [^{125}I][Sar^1, Ile^8]angiotensin II (0.1 nM) and 250 µl brain homogenate were incubated for 90 min at 25° C before termination by rapid filtration. Radioactivity remaining on the filters was quantified using a gamma-counter. Protein content of the brain homogenates was assayed by the Bio-Rad Coomassie Brilliant Blue method (Bradford, 1976).

In caudate nucleus homogenates from control patients, [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 70 % and 4 % of the total binding, respectively (specific AT_1 and AT_2 receptor binding=12.0±1.6 and 0.67±0.12 fmol/mg protein, respectively, mean±S.E.M., n=10). In the caudate nucleus homogenates from patients with HD, specific [^{125}I][Sar^1, Ile^8]angiotensin II binding to AT_2 receptor was increased by approximately 200 % ($P<0.05$, Student's t test) compared to the values obtained from the control patients, whilst specific AT_1 receptor binding levels were unaltered. In putamen homogenates from control patients, [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 70 % and 5 % of the total binding, respectively (specific AT_1 and AT_2 receptor binding=12.8±1.2 and 0.50±0.12 fmol/mg protein, respectively, mean±S.E.M., n=10). In putamen homogenates from patients with HD, specific [^{125}I][Sar^1, Ile^8] angiotensin II binding to AT_1 receptor was reduced by approximately 30 % ($P<0.05$, Student's t test) whilst specific AT_2 binding was not altered. In substantia nigra homogenates from control patients, [^{125}I][Sar^1, Ile^8]angiotensin II specific AT_1 and AT_2 receptor binding represented approximately 80 % and 5 % of the total binding, respectively (specific AT_1 and AT_2 receptor binding=22.8±2.8 and 0.69±0.12 fmol/mg protein, respectively, mean±S.E.M., n=10). In substantia nigra homogenates from patients with HD, specific AT_1 and AT_2 receptor binding levels were not different to levels in tissue from control patients.

The present studies further implicates an abnormality of the central angiotensin systems in HD.

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246P THE APPARENT MOLECULAR SIZE OF ANGIOTENSIN RECEPTOR SUBTYPES IN THE RAT AND HUMAN BRAIN

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The radiation inactivation method provides a means for estimating the molecular mass of protein either purified or bound in cellular membrane (e.g. Nielsen and Braestrup, 1988). The present studies have investigated the molecular size of protein for both AT_1 and AT_2 receptor in rat and human brain homogenates using the radiation inactivation technique.

Whole rat brain (without cerebellum) and rat cerebellum from female Wistar rats (one week old) and human cerebellum (from patients died from non-neurological and non-psychiatric disorders) were irradiated with the 10 MeV linear accelerator at Risø, Denmark, as described by Nielsen and Braestrup (1988). Enzymes of known molecular weight were added to tissues prior to freezing. The tissues were kept on ice bags at -15°C during irradiation and were cooled for 2 min at -20°C after each dose of 2 Mrad. For binding studies, tissues were homogenised in ice-cooled incubation buffer (mM: sodium chloride 150; sodium dihydrogen phosphate 50; magnesium chloride 10; EGTA 5 and 4 % w/v bovine serum albumin, final pH 7.4; see Barnes et al., 1991 for methodology) and the supernatants from first time centrifugation were removed for enzyme assays. For [^{125}I][Sar^1, Ile^8]angiotensin II binding, 50 µl competing compound (final concentration 1.0 µM of either losartan or PD123,177) or vehicle (incubation buffer), 50 µl [^{125}I][Sar^1, Ile^8]angiotensin II (final concentration 0.1 nM) and 250 µl brain homogenate were incubated for 90 min at 25°C before termination by the rapid filtration. Radioactivity remaining on the filters was quantified using a gamma-counter. Protein content of the brain homogenates was assayed by the Bio-Rad Coomassie Brilliant Blue method (Bradford, 1976). For calculation of the receptor molecular masses it was assumed that the radioation did not alter the affinity of the radioligand for the AT_1 and AT_2 receptors.

The present studies indicated that the specific [^{125}I][Sar^1, Ile^8]angiotensin II labelled AT_1 (defined by losartan 1.0 µM) and AT_2 (defined by PD123177, 1.0 µM) receptor recognition sites were decreased by high-energy radiation in a dose dependent manner. The estimated molecular masses of AT_1 receptor recognition sites were 97, 139 and 107 kDa (mean±S.E.M., n=3) in the rat cerebellum, rat whole brain (without cerebellum) and human cerebellum, respectively. The estimated molecular masses of AT_2 receptor recognition sites were 93, 110 and 86 kDa (mean±S.E.M., n=3) in the rat cerebellum, rat whole brain (without cerebellum) and human cerebellum, respectively.

The estimated molecular mass for AT_1 and AT_2 receptors by the present studies are similar. The molecular weight for AT_1 receptors are slightly higher in the rat whole brain than in the rat and human cerebellum. The molecular mass for both AT_1 and AT_2 receptors estimated by the present studies are approximately double those obtained from molecular biological studies (e.g. Bunnemann et al., 1993; Mukoyama et al., 1993).

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247P VOLTAMMETRIC MONOAMINERGIC AND PEPTIDERGIC DYNAMICS IN THE NUCLEUS ACCUMBENS OF RATS DURING REWARD OR DRUG (COCAINE) SEEKING BEHAVIOUR

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Rodents can exhibit spontaneously different behaviours when submitted to identical experimental procedures. Therefore, one aim of this study was to develop a simple animal model to study the putative individual sensitivity to psychotropic drugs such as cocaine. A "free choice" two bottle drinking test was then implemented in naive adult male Wistar rats (300 g.) as described previously (Falk *et al.* 1990). Ex vivo voltammetric analysis of neurotransmitter activities were successively performed in the nucleus accumbens, limbic area, mainly involved in the brain reward system (Koob & Goeders, 1989).

During the first hour of the active (dark) period only a limited amount of food (pellets) was available (45 mg). During the next hour, the rodents revealed two main behaviours in the concomitant presence of water and a solution of cocaine (0.5mg/ml). The "water preferring" rats (WP) were consuming exclusively water (approximately 25ml), while the "cocaine drinking" animals (CD) were also consuming a significant amount of cocaine solution. In particular, the liquid consumption of the CD rats followed a circadian rhythm, i.e. they consumed mainly water one day (about 22ml), while the following day they consumed mainly cocaine (approximately 8 ± 2 mg, mean ± s.e.m., n = 10). This behaviour indicated that the CD animals were one day without need for the drug so that they might be considered to be in a "reward state". Their maximum intake of cocaine observed the following day suggest that the rats were then in a drug seeking state (craving?). Therefore, this cycle of consumption permitted the prediction of the subsequent "state" and allowed the sacrifice of the CD rats during the "reward" or the "craving" phase, so that their brains could be submitted to biochemical studies. In particular, the nucleus accumbens was prepared as described (Hassoni & Crespi, 1990) and analysed using voltammetry in conjunction with treated micro-biosensors, in order to measure dopaminergic, serotonergic and peptidergic activities (Crespi, 1990).

Results revealed that the activity of the dopaminegic system, which had basal levels of $30.4 \pm 4.5\mu M$ (n = 4 mean ± s.e.m.), was selectively increased during the reward state to approximately $350 \pm 55\%$ of controls ($p < 0.05$) but unchanged during the craving state (n = 4). By contrast, when brains from four animals under the craving phase were examined, only the peptidergic signal was increased to $210 \pm 28\%$ (n = 4, mean ± s.e.m., $p < 0.05$) of control values (basal levels were $1.8 \pm 0.3\mu M$). The serotoninergic signal was not significantly changed in both conditions. Statistical comparison between groups was made using ANOVA and Tukey test.

These data support the proposed main role of the dopaminergic system within the reward state (Koob & Goeders, 1989) and gives rise to the original hypothesis of the involvement of one (or more) peptidergic system(s) in the modulation of the drug seeking (craving?) state.

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248P FUNCTIONAL INVESTIGATION OF HT-29 EPITHELIA STABLY TRANSFECTED WITH THE HUMAN Y_1-NEUROPEPTIDE Y RECEPTOR

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Neuropeptide Y (NPY) and peptide YY (PYY) are broad spectrum antisecretory agents in gastrointestinal preparations from different species (Cox, 1993). The cDNA sequence of the Y_1 receptor (originally an orphan G protein-linked receptor called FC5, Eva *et al.*, 1990) has been stably transfected into a human colonic epithelial cell line, HT-29. The wild-type (wt) cell line responds to secretagogues (forskolin and vasoactive intestinal polypeptide, VIP) and antisecretory agents (somatostatin, SOM and clonidine) but not to NPY and PYY. Three clones expressing high levels of ^{125}I-NPY binding have been isolated and used in voltage-clamp studies. Y_1 receptor cDNA was stably transfected into HT-29 cells by calcium-phosphate precipitation and glycerol shock using the construct pTEJ-8FC5, and resultant clones were identified by geneticin resistance conferred by the expression vector (Johansen *et al.*, 1990). Three clones were selected (FC5-4, -7 and -16) grown to confluence on collagen-coated filters (covering an area of $0.2cm^2$) and voltage-clamped at 0 mV as described by Cuthbert *et al.* (1987). Peak changes in short-circuit current (s.c.c.) were recorded (and are quoted below as $\mu A.cm^{-2}$) in response to basolateral additions of various peptides or forskolin.

Basal s.c.c. and resistances of confluent layers of FC5-4 and FC5-16 clones were not significantly different from wt HT-29 ($3.2 \pm 0.9\ \mu A.cm^{-2}$ and $25.0 \pm 1.9\ \Omega.cm^2$, n=68) but were significantly greater (unpaired Student's *t*-test throughout) in FC5-7 cells ($11.6 \pm 1.5\ \mu A.cm^{-2}$, $P < 0.001$ and $38.8 \pm 2.7\ \Omega.cm^2$ $P < 0.001$, n=49). PYY (100 nM) reduced basal s.c.c. in all three clones (-1.3 ± 0.4, n=3 in FC5-4; -3.3, n=1 in FC5-7 and -1.0 ± 0.3, n=3 in FC5-16) and as expected, there was no response to PYY in wt HT-29 cells. VIP produced a long lasting increase in s.c.c. with EC_{50} values (in nM) of 18.3 in wt, 13.6 in FC5-4 and 9.2 in FC5-16 cells. Following 30 nM VIP responses to PYY (100 nM) were -1.7 ± 0.2 (n=5) in FC5-4; -4.4 ± 1.2 (n=4) in FC5-7 and -2.4 ± 0.5 (n=4) in FC5-16 epithelia. [Leu^{31},Pro^{34}] NPY (1 μM) was similarly effective, reducing the s.c.c. by -0.5 ± 0.3 in FC5-4; -2.2 ± 1.0 in FC5-7 and -1.6 ± 0.3 in FC5-16 (n=3 throughout) and significantly attenuating the response to further addition of PYY (100 nM) in FC5-7 and FC5-16 ($P < 0.05$). SOM (100 nM) responses in each clone (after VIP and PYY) were not significantly different from those in wt HT-29 (-5.8 ± 2.9, n=3). Increases in s.c.c. following forskolin (10 μM) or VIP (100 nM) were significantly larger in FC5-7 (39.8 ± 4.7, n=17, and 38.5 ± 3.4, n=20) compared to wt controls (16.9 ± 2.1, n=31 and 19.5 ± 3.3, n=21, $P < 0.001$ in both cases) and also when compared to FC5-4 and -16 responses. The effectiveness of both PYY and SOM (at 100 nM) was reduced when these agonists were applied after forskolin rather than VIP.

Stable expression of the Y_1 receptor cDNA in HT-29 epithelia therefore confers sensitivity to PYY under basal and stimulated conditions. The Y_1-selective agonist [Leu^{31},Pro^{34}]NPY also reduced VIP elevated s.c.c. in all three clones (this agonist is inactive in jejunum mucosa, Cox & Krstenansky, 1991). These studies provide a foundation for comparing the functional and biochemical consequences of transfecting native and mutated Y_1 receptor cDNA into epithelia.

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249P AUTORADIOGRAPHIC DISTRIBUTION OF SUBSTANCE P BINDING SITES IN THE BRAINSTEM OF RAT AND FERRET

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It has been shown that injection of tachykinin NK_1 receptor antagonists into the region of the nucleus tractus solitarius (NTS) of the ferret brainstem has powerful anti-emetic effects (Gardner *et al.*, 1994), prompting interest in the role of tachykinins in the emetic reflex. The distribution of tachykinin recognition sites in rat and ferret brainstem has been examined by *in vitro* autoradiography using [^{125}I] Bolton-Hunter substance P (BHSP, 0.05nM). Segments of brainstem approx. 4mm caudal and rostral to the obex were obtained from Wistar rats and Fitch ferrets of either sex. Coronal sections (20μm) were cut and binding carried out according to the procedure of Helke *et al* (1984). Radiolabelled tissue sections were exposed to radiation-sensitive ^{3}H-hyperfilm for 3 days and the resulting autoradiograms were analysed using a computer-assisted image analysis system.

In rat brainstem (n=3), two distinct regions showing high BHSP specific binding (nCimg^{-1}) were identified. These regions were within the NTS, the dorsal NTS (dNTS, 4.95) and the ventral NTS also containing the dorsal motor nucleus of the vagus (vNTS, 5.91). In addition there was extensive binding in the inferior olive (2.90), nucleus ambiguus (2.12) and the hypoglossal nucleus (2.49). In contrast, the cerebellum (-0.02), spinal trigeminal nucleus (0.34) and area postrema (0.25) displayed low levels of specific binding. In general, this distribution is consistent with previous findings in rat brain (Helke *et al.*, 1984). In the ferret brainstem (n=3) a similar distribution of BHSP binding was observed. High binding was observed in both regions of the NTS (3.60 and 4.10, dNTS and vNTS respectively), and also in the inferior olive (1.08), nucleus ambiguus (1.73) and the hypoglossal nucleus (1.41). As in the rat, low binding was observed in the cerebellum (0.13), spinal trigeminal nucleus (0.30) and the area postrema (0.69).

The ability of SP (1μM) and the NK_1 receptor antagonist CP-99,994 (1μM) to inhibit BHSP binding to slices of both rat and ferret brainstem were also determined. These cold ligands competed for BHSP binding in most regions of rat brainstem with approximately equal potencies. However, a component of the BHSP binding in both regions of the NTS inhibited by SP was unaffected by CP-99,994. The NTS was subdivided in the coronal plane for further analysis. The resultant reduction in specific binding using CP-99,994 was 26 and 23% (dNTS and vNTS respectively, caudal region), 15 and 15% (postremal region) and 4 and 9% (rostral region). These data suggest that CP-99,994-resistant binding sites for SP exist in the rat NTS, predominantly located caudally. In contrast, there was no significant difference in the ability of SP and CP-99,994 to inhibit BHSP binding in any areas studied in ferret brainstem. Electrophysiological studies have demonstrated an absence of NK_2 and NK_3 receptors in the rat NTS (Maubach *et al.*, 1994) and thus, it is possible that this CP-99,994-resistant site represents an atypical receptor.

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250P EFFECTS OF RACEMIC CP-99,994, A TACHYKININ NK_1 RECEPTOR ANTAGONIST, ON MOTION-INDUCED EMESIS IN *SUNCUS MURINUS*

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We have shown previously that in the ferret, the tachykinin NK_1 receptor antagonist, CP-99,994 inhibits emesis induced by cisplatin, cyclophosphamide, radiation, morphine, copper sulphate and ipecacuanha (Bountra *et al.*, 1993). The present experiments have been carried out to determine the efficacy of this agent against emesis induced by motion.

Suncus murinus, the house musk shrew, is a small Asiatic insectivore that is susceptible to motion sickness, which can be antagonised by a variety of agents known also to be effective in man (Ueno *et al.*, 1988). Emesis was induced by exposing the animals to sinusoidal acceleration using linear oscillation in a horizontal plane at 1Hz, with an excursion of 4cm for a period of 5min. Six adult male (body weight range 53-87g) and five adult female (33-42g) animals were used for these experiments. The animals received a subcutaneous dose of either saline, 4ml kg^{-1}, or racemic CP-99,994 (CP-93,009), 10 or 30mg kg^{-1}, administered 15min before motion-testing. Numbers of emetic episodes and (in the case of responders) latency to first emesis were recorded. Experiments were of a 'cross-over' design, with an interval of 13 days between treatments. All the animals received saline pre-treatment on one occasion - four on the first exposure to motion, and the remainder on the second exposure. Following administration of saline, 9 of the 11 animals vomited. The mean number of episodes was 3.7±1.30 and mean latency to first emesis was 131±20.3s. The two animals that did not respond were excluded from further analysis. At the doses used, racemic CP-99,994 significantly attenuated motion-induced emesis in the house musk shrew, suggesting a role for substance P and NK_1 receptors in this response.

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Table 1. Effects of ±CP-99,994 on motion-induced emesis in *Suncus murinus*.

	Control			Treated		
±CP-99,994	Responders	Episodes	Latency (s)	Responders	Episodes	Latency (s)
10mg kg^{-1} s.c.	4/4	3.8±1.49	123±30.1	2/4	0.8±0.47*	175±85.0
30mg kg^{-1} s.c.	5/5	5.2±2.46	138±29.7	1/5	0.2±0.20†	110

Values are means and SE means. * p=0.04 paired t test. † p=0.06 paired t test

251P AN ANTAGONIST OF TACHYKININ NK_1 RECEPTORS INHIBITS CYTOTOXIC-INDUCED PLASMA PROTEIN EXTRAVASATION IN THE FERRET

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Neurogenic inflammation has been demonstrated in many tissues, including the bladder and gastrointestinal and respiratory tracts (Maggi & Meli, 1988). A cardinal feature is plasma protein extravasation (PPE). Many anti-neoplastic therapies induce adverse effects that involve inflammation. For example, haemorrhagic cystitis following cyclophosphamide (CYP) administration or gastrointestinal damage after radiotherapy. In the present study, the effects of a tachykinin NK_1 receptor antagonist (CP-99,994) and two $5\text{-}HT_3$ receptor antagonists (granisetron and ondansetron) on PPE induced by CYP, radiation (Rx) or cisplatin (CP) were investigated in a range of tissues from the ferret.

Adult male ferrets (0.85-1.50kg) were given a subcutaneous dose of either saline (1ml kg^{-1}) or an antagonist 5min before administration of either intraperitoneal CYP or CP, or whole-body Rx at previously determined $ED_{90\text{-}100}$ emetogenic doses. Anaesthesia was induced with pentobarbitone sodium (60mg kg^{-1} i.p.) and Evans blue dye (50mg kg^{-1}) was administered intravenously fifteen minutes before the blood was flushed out with saline (time of sacrifice: see Table 1). The bladder, portions of the duodenum and jejunum, a kidney and lobe of lung were removed and the dye was extracted with formamide (24h at 60°C), and quantified by spectrophotometry (at 620nm).

Pre-treatment with granisetron or ondansetron (1mg kg^{-1} s.c.) had no effect on cytotoxic-induced PPE, except in the duodenum of irradiated animals (Control, 135.8±16.1; granisetron, 97.5±5.7; ondansetron, 75.3±3.1µg Evans blue g^{-1} tissue). However, CP-99,994 (5mg kg^{-1} s.c.) significantly inhibited the PPE induced in the bladder after CYP, as well as in the small intestine of ferrets treated with Rx or CP (see Table 1).

These data indicate that a tachykinin NK_1 receptor antagonist attenuated some of the adverse inflammatory effects induced by cytotoxic agents in the ferret.

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Table 1. Major effects of CP-99,994 on cytotoxic-induced PPE in ferrets. (Values are means and SE mean, n=3-5 per group)

Cytotoxic	Time*	Tissue	PPE (µg Evans blue g^{-1} tissue)	
			Control	CP-99,994
CYP (125mg kg^{-1} i.p.)	1h	Bladder	157.8 ± 9.7	63.1 ± 2.3†
Rx (2Gy)	2h	Duodenum	135.8 ± 16.1	91.4 ± 9.8†
		Jejunum	103.0 ± 8.4	66.8 ± 6.6†
CP (10mg kg^{-1} i.p.)	6h	Jejunum	104.3 ± 11.4	69.0 ± 3.8†

* Time between administration of cytotoxic agent and exsanguination. †$p<0.05$, compared with. control, unpaired Student's *t* test.

252P ESTIMATION OF BRAIN PENETRATION FOLLOWING ORAL ADMINISTRATION OF THE NK_1 ANTAGONIST RPR 100893 USING AN *EX VIVO* BINDING ASSAY IN THE GUINEA-PIG

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Since the initial discovery of CP-96,345 and RP 67580, several non-peptide antagonists of the NK_1 receptor have been described. Most of them show variations in affinity and therefore in antagonist potency across species. However, if these antagonists have been fully characterized *in vitro*, little information is available on their ability to cross the blood brain barrier. We recently reported that RPR 100893, a triarylperhydroisoindolol derivative, is a new selective NK_1 antagonist having high and preferential affinity for the guinea-pig and human receptor as compared to rat and mouse (Fardin *et al.*, 1994). This work studies the occupancy of the NK_1 receptor in the brain after administration of RPR 100893 to conscious guinea-pig, using an *ex vivo* binding assay. In these assays, RPR 100893 was compared to (±) CP-99,994, another potent antagonist of the human and guinea-pig NK_1 receptor (Mc Lean *et al.*, 1993).

Male Dunkin-Hartley guinea-pigs (300 - 400 g, Charles River) were treated with RPR 100893 (dissolved in PEG 400), (±) CP-99,994 (dissolved in water) or their vehicles. Animals were killed by decapitation at various times after administration of drugs and their brains rapidly removed. Binding assays were performed in crude homogenates (6.7 mg of wet tissue/tube) of striatum, a cerebral structure particularly rich in NK_1 receptors, using [^{3}H]-Substance P (SP) as the ligand. Assays were performed at 25°C during 20 min as previously described (Fardin & Garret, 1991) except that the final volume was 0.2 ml. Non specific binding was defined in presence of 10 µM SP.

Under these experimental conditions, [^{3}H]-SP binds to an apparently homogeneous population of sites with a K_D value of 2.4 ± 0.3 nM. (n=3). Thus, a ligand concentration of 2.5 nM was used for further studies.

One hour after its oral administration, RPR 100893 significantly inhibited the [^{3}H]-SP specific binding in comparison with vehicle treated animals, at doses of 10 mg/kg (53.4 ± 6.5%, n=9) and 30 mg/kg (57.4 ± 5.3%, n=6). In comparison, CP-99,994 inhibited the binding by 39.5 ± 3.6% and 57.4 ± 5.0% at 10 and 30 mg/kg p.o., respectively. Furthermore, at doses of 10 mg/kg p.o., in contrast to CP-99,994, significant inhibition was still observed 6 hours (57.3 ± 2.2%, n=6) and 24 hours (26.0 ± 3.4%, n=6) post administration of RPR 100893.

This study shows that RPR 100893, administered orally to guinea-pigs, is able to act in the central nervous system with a good duration of action. This result is in agreement with behavioural studies performed in the same species (see Piot *et al.*, this meeting). Furthermore, this *ex vivo* binding assay could be useful to select an NK_1 antagonist of the human receptor with higher brain penetration.

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253P AN AUTORADIOGRAPHIC COMPARISON OF NK3 RECEPTOR BINDING SITES IN GUINEA-PIG BRAIN USING [^{125}I]-[MePhe^7]NEUROKININ B AND [^{3}H]SENKTIDE

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The distribution of tachykinin NK_3 receptors in rodent brain has previously been demonstrated using the selective agonist [^{3}H]-Senktide (succinyl-[Asp^6, MePhe^8]substance P(6-11) (Guard *et al.*, 1990; Stoessl and Hill, 1990). Recently, [^{125}I]iodohistidyl [MePhe^7]-Neurokinin B ([^{125}I]-[MePhe^7]NKB) has been shown to be a highly selective radioligand for the NK_3 receptor (Suman-Chauhan *et al.*, 1994).The aim of this study was to compare the autoradiographic distribution of NK_3 binding sites in guinea pig brain using [^{3}H]-Senktide and [^{125}I]-[MePhe^7] NKB.

Male Dunkin-Hartley guinea pigs (300-450g) were killed by cervical dislocation, the brain carefully removed and frozen in iso-pentane at -35°C. 10μm coronal sections were cut and mounted onto gelatin subbed slides and stored at -70°C until required. Thawed, dried sections were incubated with [^{125}I]-[MePhe^7]NKB (50-80 pM), or [^{3}H]-Senktide (2-3nM), in 50mM Tris-HCl (pH7.4 @ 21°C, containing enzyme inhibitors, $MnCl_2$ and BSA) at 21°C for 90 and 60 min, respectively. Non-specific binding was defined using 1μM senktide or 1μM Cam-4659 (Suman-Chauhan *et al.*,1994a). The sections were apposed to [^{3}H]-Hyperfilm for 4 days or 8 weeks for sections labelled with [^{125}I]-[MePhe^7]NKB and [^{3}H]-Senktide, respectively. Films were analysed using an MCID analysis system.

A summary of binding densities observed for a number of brain areas are shown in Table 1. Both radioligands gave highest levels of specific binding in various levels of the cortex, amygdala, habenula and the ventromedial hypothalamus (VMH), whilst little binding was seen in the caudate putamen and cerebellum. Additional areas (e.g. nucleus accumbens, substantia nigra, hippocampus and pons) were also labelled by [^{125}I]-[MePhe^7]NKB, but not [^{3}H]-Senktide. In regions differentiated by both [^{125}I]-[MePhe^7] NKB and [^{3}H]-Senktide, the rank order of binding densities were similar. The higher specific activity of [^{125}I]-[MePhe^7] NKB renders this radiolabel a more sensitive tool in autoradiographic studies of the NK_3 binding site.

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Table 1. Binding densities (dpm/mm^2, mean and range, n=2-3) of [^{125}I]-[MePhe^7] NKB and [^{3}H]-Senktide at the NK_3 binding site in guinea pig brain.

Brain Region	[^{125}I]-[MePhe^7]NKB	[^{3}H]-Senktide
Cingulate Cortex	216 (201-225)	36 (33-47)
Amygdala	233 (227-245)	29 (29-29)
Habenula	158 (89-236)	43 (37-49)
VMH	99 (26-138)	3.5 (2.2-4.7)
Caudate putamen	6.5 (5.5-7.0)	0.4 (0.2-0.6)
Cerebellum	3.0 (1.3-6.3)	0.6 (0.6-0.6)

254P CHARACTERISATION OF BOMBESIN RECEPTORS IN HUMAN ASTROCYTOMA U373MG CELLS

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Recent studies indicate that the multiple biological actions of bombesin and related peptides may be mediated through two distinct receptor types, neuromedin B (NMB) and gastrin-releasing peptide (GRP). Both receptors are expressed in central and peripheral tissues, and in a variety of human tumour cell lines. In this study we demonstrate that bombesin receptors are also present in human astrocytoma U373MG cells, and have characterised the type expressed using radioligand binding.

In vitro binding assays using [^{125}I][Tyr^4]bombesin were carried out as described by Guard *et al.* (1993). Non-specific binding was defined by 1μM bombesin. Functional assays measuring changes in intracellular calcium levels ($[Ca^{2+}]_i$) using the fluorescent indicator Fura2 were carried out as described by Pinnock *et al.* (1994). Association studies indicated that binding reached a steady-state within 60 minutes, and was maintained for up to 2 hours. Attempts to effect dissociation with addition of 1μM bombesin were unsuccessful, with less than 10% dissociation occurring even up to 2 hours after addition of bombesin, suggesting internalisation of the radioligand during the incubation period. Binding of [^{125}I][tyr^4]bombesin was saturable, yielding linear scatchard plots, indicating binding to a single population of binding sites with a K_D and B_{max} value of 0.10 nM (range 0.09-0.11), and 1.3 ± 0.2 fmol/10^6 cells (mean ± s.e.m.;n=3) respectively. Competition studies with a range of bombesin receptor ligands yielded IC_{50} values of 0.2 (0.09-0.15), 0.32 (0.23-0.54), 0.42 (0.13-0.85), 0.28 (0.22-0.43), 0.48 (0.20-0.82), 4.6 (2.3-9.3) and 1.0 (0.4-1.4) nM for GRP, bombesin, NMC, [D-Phe^6]BN_{6-13}EtOH, [D-Phe^6]$BN_{6-13}EtNH_2$, NMB and litorin respectively (geometric mean and range; n=3-6). Comparison with previously published data on rat bombesin receptors (Guard *et al.*, 1993) suggest that the bombesin receptor expressed by this cell line is of the GRP type. The structure activity requirements for the GRP receptor expressed by U373 MG cells was investigated by carrying out an alanine scan on the minimum active fragment of bombesin which retains high affinity for bombesin receptors, acetyl-$bombesin_{7-14}$ (IC_{50} = 0.9nM; range 0.4-1.8nM, n=4). In agreement with data for rat GRP receptors (Guard *et al.*, 1993), and for cloned human GRP receptors expressed in CHO cells (Suman-Chauhan *et al.*, this meeting), the Leu and Trp residues are of primary importance for high affinity binding to the GRP receptor present on U373 MG cells, as substitution with Ala of these residues resulted in the greatest loss of affinity (>1000-fold).

Application of bombesin, NMC and NMB increased $[Ca^{2+}]_i$ in a concentration dependent manner with EC_{50} values of 1-10nM for bombesin and NMC, and ~50-100nM for NMB respectively. The rank order of affinity for increasing $[Ca^{2+}]_i$ confirmed data obtained in binding studies. In summary, human astrocytoma U373MG cells naturally express the GRP bombesin receptor and provide a useful system for *in vitro* studies of this bombesin receptor type.

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255P THE PHARMACOLOGY OF CGRP AND AMYLIN RECEPTORS ON GUINEA-PIG VAS DEFERENS

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Calcitonin gene-related peptide (CGRP) and islet amyloid polypeptide (amylin) are bioactive peptides with significant sequence homology. Recently it has been shown that these peptides promote relaxation in a number of smooth muscle preparations including rat vas deferens and guinea-pig urinary bladder (Giuliani *et al.*,1992). It has been suggested that distinct receptors exist for each peptide (Dennis *et al.*,1989) but cross reactivity at a distinct CGRP receptor has also been reported (Chantry *et al.*,1991).

The present study was carried out to establish the classification of CGRP and amylin receptors on the guinea pig isolated vas deferens from Duncan Hartley animals, 300-400g. These were transmurally stimulated, with trains of rectangular pulses at 0.04Hz. Each train was of 1s duration consisting of 20 pulses of 0.5ms pulse width, 70V stimulation amplitude (adapted from Kerr *et al.*, 1990). Cumulative concentration-response curves were constructed.

The relaxation induced by human (h) and rat (r) - alpha CGRP was insensitive to the CGRP receptor antagonist CGRP-(8-37) (1 - 3μM) but relaxation induced by human and rat amylin was antagonised by this compound (see table 1). This tissue appears slightly different to the rat vas deferens (Giuliani et *al*, 1992) where CGRP-(8-37) is more effective against CGRP. This suggests that there is a species difference. However, both tissues have distinct amylin and type 2 CGRP receptors (Dennis *et al.*,1990). Further studies using the novel amylin receptor antagonist AC187 (acetyl-[asn^{30}, tyr^{32}]-salmon calcitonin (8-32), Young *et al.*, 1994) show that it significantly antagonised rat amylin but has no effect on hCGRP (see table 1). These findings provide additional evidence for distinct CGRP and amylin receptors.

Table 1.

Peptide	EC_{50} (nM)	Peptide	EC_{50} (nM)
hCGRP	9±2.8 (n=47)	hCGRP+3μM CGRP(8-37)	11± 3.9 (n=5)
rCGRP	105±46 (n=33)	rCGRP+1μM CGRP(8-37)	108±63 (n=10)
rAmylin	77±8.8 (n=38)	rAmylin +1μM CGRP(8-37)	174±22.0* (n=4)
hAmylin	213±22.0 (n=24)	hAmylin +3μM CGRP(8-37)	450±98.0* (n=5)
hCGRP +10μM AC187	11.4±3.1 (n=4)	rAmylin +10μM AC187	610±22* (n=5)

Values are means ± s.e.means. *denotes significant difference from agonist alone, $P \leq 0.05$ (paired student's T-test).

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256P PACEMAKER POTENTIALS FROM THE GUINEA-PIG PROXIMAL AND DISTAL RENAL PELVIS: DIFFERENT SENSITIVITY TO CGRP

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We have recorded spontaneous (pacemaker) action potentials and contractions from the proximal (close to kidney) and distal (close to the ureter) regions of the guinea-pig renal pelvis (PRP and DRP, respectively) by single sucrose-gap. Pacemaker potentials recorded from the two regions of male guinea-pig (300-350 g) renal pelvis were significantly different ($p<0.05$, Student's t-test), as follows: frequency 4.53±0.19 and 1.14±0.1 cycles/min; maximal amplitude of depolarization 7.74±0.81 and 14.59±1.08 mV; rate of rise of depolarization 8.74±1.26 and 31.21±2.65 mV/s; time of repolarization (at 50% of repolarization) 989±86 and 2073±251 ms in the PRP and DRP, respectively (n=30 for each group). The amplitude of accompanying contraction was not significantly different between the PRP (1.86±0.16 mN) and DRP (2.23±0.19 mN). Superfusion with 3μM cromakalim promptly abolished pacemaker potentials and contractions from both the PRP and DRP. Superfusion with a concentration of calcitonin gene-related peptide (CGRP, 0.1μM for 5 min), producing maximal inhibitory effect on contractility of the whole renal pelvis in organ bath experiments, transiently inhibited (about 30%) the frequency of pacemaker potentials in the DRP while being totally ineffective in the PRP (Table 1). In organ bath experiments, 10μM glibenclamide did not affect the inhibitory action of 0.1μM CGRP (n=6) on the whole renal pelvis, while totally blocking the suppressant effect of cromakalim.

The present findings document the existence of remarkable regional differences in the effector mechanisms evoked by CGRP in the guinea-pig pyeloureteral tract. Contrary to the ureter (Santicioli & Maggi, 1994), CGRP appears to be unable to activate glibenclamide-sensitive potassium channels in the guinea-pig renal pelvis. Within the renal pelvis, the sensitivity to the inhibitory effect of CGRP appears in the more distal region, from which an "ureter-like" action potential is recorded.

Santicioli P. & Maggi C.A. (1994) *Br. J. Pharmacol.* 113, 588-592

Table 1. Effect of CGRP on pacemaker potentials and contractions of the proximal and distal guinea-pig renal pelvis

	Proximal Renal Pelvis (n=12)			Distal Renal Pelvis (n=10)		
	Before CGRP	After CGRP	%	Before CGRP	After CGRP	%
Frequency (cycles/min)	3.71±0.4	3.65±0.4	97±2	0.91±0.1	0.65±0.1 *	71±4 *
Potential (mV)	6.39±1	6.47±1	100±1	12.96±1.7	12.77±1.7	99±1
Contraction (mN)	1.94±0.32	1.88±0.31	96±2	1.61±0.2	1.56±0.19	97±1

Each value is the mean ± s.e.mean. (*) Significantly different from value before CGRP ($p<0.05$, Student's t-test for paired data)

257P *IN VITRO* COMPARISON OF 2-[^{125}I]IODOMELATONIN BINDING SITES IN THE CHICKEN BRAIN AND PERIPHERAL TISSUES

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The pineal hormone melatonin has been implicated in the regulation of seasonal changes in the reproductive axis of photoperiodic species, the entrainment of circadian rhythms in birds, reptiles and mammals and the modulation of some aspects of retinal physiology (Morgan *et al.*, 1994). Using both radioligand binding techniques, and *in vitro* autoradiography, specific, saturable, high affinity 2-[^{125}I]iodomelatonin (2-[^{125}I]aMT) binding sites have been identified in the pituitary and various brain areas of every species studied to date (Morgan *et al.*, 1994). More recently, 2-[^{125}I]aMT binding sites have been described in peripheral tissues of a variety of species, such as the kidney of the chicken (Song *et al.*, 1992) the human gastrointestinal tract (Pontoire *et al.*, 1993) and the caudal artery and circle of Willis of the rat (Viswanathan *et al.*, 1990). The characterization of 2-[^{125}I]aMT binding sites in peripheral areas is incomplete, as the location and pharmacological properties of the sites have not been fully established.

2-[^{125}I]aMT binding was compared in chicken brain, kidney, spleen and duodenum membranes (chickens of mixed sex, 80-120g, 14-21 days old, obtained from Orchard Farms, Bucks.), using both radioligand binding and *in vitro* autoradiography. Competition assays were performed using 20 melatonin analogues, of diverse chemical structure and varied affinity, in an attempt to identify potential heterogeneity of the binding site in these tissues.

For saturation studies, duplicate membrane aliquots were incubated with 2-[^{125}I]aMT (Dupont, UK; 4-800pM), and the equilibrium constant (K_d) and maximum number of binding sites (B_{max}) determined for each tissue. In competition studies, membranes were incubated with 80pM 2-[^{125}I]aMT and various concentrations of competing drug. For binding site autoradiography, tissue sections (20μm thick) were preincubated with assay buffer for 15 mins, followed by 80pM 2-[^{125}I]aMT for 1hr. Non-specific binding was determined in the presence of 1μM melatonin.

Saturation studies (n=1) revealed a single, saturable, high affinity binding site in each tissue, with a K_d (pM) of; brain 36.1$\pm$3.4, kidney 84.6$\pm$12.7, spleen 40.1$\pm$7.8 and duodenum 23$\pm$0.2. B_{max} values (fmol/mg protein) obtained were; brain 13.2$\pm$4.7, kidney 1.6$\pm$0.1, spleen 0.62$\pm$0.07 and duodenum 0.13$\pm$0.03. Errors given are computer derived estimates. Competition assays gave monophasic inhibition curves with pseudo Hill coefficients close to unity. A comparison of the affinity constants (K_i) gave highly significant correlation coefficients (r, $p<0.001$) of; brain/kidney 0.93, brain/spleen 0.96 and brain/duodenum 0.95. *In vitro* autoradiography revealed a widespread distribution of specific binding in all tissues, with some areas, such as the collecting tubules of the kidney, showing increased density. In all tissues, non-specific binding could not be distinguished from background.

These results indicate that a single, saturable, high affinity, 2-[^{125}I]aMT binding site is present in chicken brain, kidney, spleen and duodenum. Competition studies were unable to differentiate the 2-[^{125}I]aMT binding site in these four tissues, suggesting that the sites are pharmacologically identical.

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258P FUNCTIONAL PROPERTIES OF MURINE β1 HOMOMERIC GABA$_A$ RECEPTORS EXPRESSED IN *XENOPUS LAEVIS* OOCYTES

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The β subunit within the GABA$_A$ receptor complex is regarded as an essential structural component for the expression of functional ion channels. Interestingly, expression of bovine homomeric β1 subunits in *Xenopus* oocytes revealed a GABA-activated Cl^- ion channel (Blair *et al.*, 1988) whereas expression of rat β1 subunits produced anion-selective channels lacking GABA-gating properties (Sigel *et al.*, 1989). This study examined the function of homomeric murine β1 subunits expressed in *Xenopus laevis* oocytes, using a two-electrode voltage clamp technique.
At a holding potential of -40 mV, the membrane input conductance of oocytes expressing murine β1 subunits was unaffected by high concentrations of GABA (1 mM), muscimol (500 μM), isoguvacine (500 μM) or bicuculline (50 μM). Moreover, the resting membrane resistances were lower (0.2 ± 0.05 MΩ, mean ± s.e.m; n = 16) when compared to uninjected oocytes or oocytes expressing heteromeric α1β1 GABA$_A$ receptors (2.0 ± 0.5 MΩ; n = 22). Application of low concentrations of Zn^{2+} (1 μM), picrotoxin (10 μM; PTX) or penicillin G (1 mM) to oocytes injected with β1 cDNA, increased the resting membrane resistance concurrent with developing outward currents. The action of these antagonists suggested the presence of an underlying non-inactivating membrane current seen only in oocytes injected with the β1 subunit cDNA. PTX and Zn^{2+}-inhibition curves were constructed for the reduction in input conductance induced by these antagonists, providing IC_{50}s of 2.11 ± 0.6 and 0.23 ± 0.03 μM (n = 8) respectively. Current-voltage (I-V) relationships for this membrane current displayed slight outward rectification with reversal potentials estimated as -27.1 (for PTX) and -24.7 mV (Zn^{2+}). These values coincided with E_{Cl} and were in accordance with this current being carried by Cl^-. GABA$_A$ receptor modulators such as, pentobarbitone or propofol, induced an inward current (cf. Sanna *et al.*, 1995) associated with a conductance increase that was sensitive to 10 μM PTX. Construction of an equilibrium dose-response curve for pentobarbitone produced an EC_{50} and a Hill coefficient of 6.0 ± 0.34 μM and 1.9 ± 0.26, respectively (n = 4). The PTX-sensitive current was unaffected by flurazepam (10 μM), methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM; 10 μM), flumazenil (10 μM), chlormethiazole (100 μM) or pregnanolone (500 nM). To ascertain that the expression of the Cl^- channel originated from the injected β1 subunit cDNA, oocytes were incubated in the presence of a transcription inhibitor, actinomycin D (50 μg/ml) for 2-5 days, following an injection with either cRNA encoding for murine β1 subunits, or cDNAs encoding for α1β1 GABA$_A$ receptors. The β1 cRNA injection promoted the expression of ion channels with the same properties as those resulting from injections of β1 subunit cDNAs. In contrast, expression of α1β1 subunits following cDNA injection was not observed as GABA (10 to 500 μM) and PTX (50 μM) failed to elicit any response.

These results suggest the possibility that homomeric GABA$_A$ receptors, formed from β1 subunits, may adopt conformations which lead to apparently spontaneously opening ion channels. Furthermore, since the binding site for GABA has been shown to occur on two domains present in the β-subunit of heteromeric GABA$_A$ receptors (Amin and Weiss, 1993), it is presumably spatially discrete from the barbiturate binding site.

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259P DIFFERENTIAL EFFECT OF PHENYTOIN AND Na VALPROATE ON GENERALISED SEIZURE-INDUCED CHANGES IN HIPPOCAMPAL GABA AND GLUTAMATE RELEASE *IN VIVO*

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Previously we have shown that generalised tonic-clonic seizures, induced by maximal electroshock (MES), decreased the neuronal release of γ-aminobutyric acid (GABA) and glutamate (GLU) in rat hippocampus *in vivo* (Rowley *et al.*, 1995a). However, due to 20 min sampling times it was not possible to determine changes in amino acid release immediately after the seizure. In an attempt to resolve this issue we have reduced our sampling time (5 min) and now report the effects of MES and modification by anticonvulsant drugs.

In all studies MES (200V for 2 sec), was administered via ear-clip electrodes to conscious, male, Lister hooded rats (250-350g), 20 hours post-implantation of a microdialysis probe into the ventral hippocampus (Rowley *et al.*, 1995b). Controls were ear-clipped only. Microdialysis probes were perfused at a flow rate of 2.0µl/min and 5 min samples collected 20 min pre- and 40 min post-MES. Amino acid concentration in the samples was determined by HPLC with ECD (Rowley *et al.*, 1995b). MES administered to animals without pre-treatment induced tonic hind-limb extensions. Alternatively, MES was given 60 min after either phenytoin (20 mg/kg) or Na valproate (400 mg/kg; 1ml/kg i.p. volume) which abolished tonic hind-limb extension. Control animals received saline. All amino acid levels are expressed as pmol/20µl and values are mean ± S.E.M. (n=6).

In animals which received no pre-treatment, a single MES resulted in a significant increase of102 ± 16% ($p<0.01$; ANOVA with post hoc Dunnett's t-test) in basal GABA levels (0.21 ± 0.01), in the 5 min post-ictal period, compared to sham controls. Levels were then significantly ($p<0.01$) reduced to 0.11 ± 0.01 in the following 5 min and remained at this depressed level for the duration of the experiment. Basal GLU levels (0.61 ± 0.01) were significantly ($p<0.01$) increased by 26 ± 6% in the 5 min post-ictal period but subsequently decreased to 0.52 ± 0.02 over the next 15 min. There was then a gradual increase to 53 ± 8% above pre-intervention levels in the 20-40 min post-ictal period, compared to sham control.

Basal GABA (0.19 ± 0.01) and GLU (0.58 ± 0.02) levels were not altered by pre-treatment with phenytoin and there were no significant effects on MES-induced changes in GABA release. By contrast, the immediate MES-induced increase (30 ± 8%; $p<0.01$) in GLU levels, observed in saline-treated animals, was significantly ($p<0.05$) attenuated (46 ± 9%) by phenytoin. The secondary increase in GLU levels was unaltered compared to saline controls.

Treatment with Na valproate significantly ($p<0.001$; ANOVA) increased basal GABA levels (0.21 ± 0.01 to 0.31 ± 0.01) but did not alter the immediate post-ictal rise in GABA. However, its secondary reduction was prevented. Whilst Na valproate treatment had no effect on basal GLU levels (0.60 ± 0.02) or the MES-induced changes up to 25 min post-ictally, it did prevent the secondary increase observed from 25 min onwards in saline-treated controls.

These data confirm our earlier report that the neuronal release of GABA is decreased and GLU increased in the 40 min following a MES (Rowley *et al.*, 1995a). Furthermore, they extend these findings to show that in the 5 min period following a 30 sec generalised seizure there is a marked increase in both GABA and GLU levels. Although phenytoin and Na valproate both prevented tonic hind-limb extension, their effects on GABA and GLU release were markedly different. The experiments here suggest that the anticonvulsant effects of phenytoin may involve suppression of GLU release in the seizure period whereas valproate appears to enhance GABA release which may counteract the MES-induced increase in GLU. In addition it is noteworthy that Na valproate prevented the prolonged rise in GLU but that phenytoin was without effect. Whether this difference in effect on post-seizure GLU levels has any consequences remains to be determined.

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260P THE NEUROPEPTIDE Lys-Pro-Asn-Phe-Ileu-Arg-Phe-amide (KPNFIRFamide) HYPERPOLARISES THE SOMATIC MUSCLE CELLS OF THE PARASITIC NEMATODE *ASCARIS SUUM*

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A number of FMRFamide-like neuropeptides have recently been isolated from nematodes and one of these, KPNFIRFamide (KPN), relaxes the somatic muscle cells of the parasitic nematode *Ascaris suum* (Maule *et al*, 1995). Here we have used conventional intracellular electrophysiological techniques to determine the effects of this peptide on the electrical properties of the muscle.

Two-electrode intracellular recordings were made from *Ascaris suum* muscle cells using an Axoclamp 2A system. Drugs were bath applied to the muscle cells and the effect on membrane potential and input conductance monitored. As previously reported, GABA elicited a chloride-dependent hyperpolarization accompanied by an increase in input conductance, with an EC_{50} of 15 µM (Holden-Dye *et al*, 1989). KPN also hyperpolarized the muscle cells. The threshold for this effect was between 1 nM and 10 nM (n=5). The hyperpolarization was accompanied by an increase in input conductance with a value of 0.54 ± 0.20 µS (n=5) at 100 nM KPN. In two cells the hyperpolarization elicited by GABA and KPN was reversed to a depolarization when the extracellular chloride concentration was changed from 107 mM to 40 mM. The input conductance increase to a maximal concentration of GABA and 10 µM KPN was additive (n=3; Figure 1).

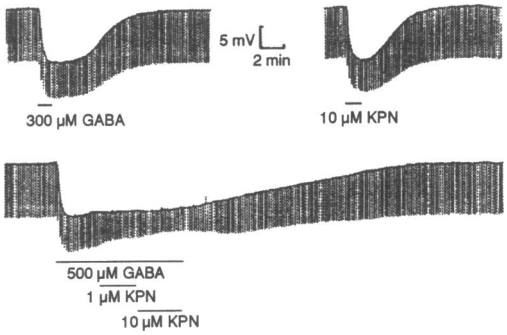

Figure 1. Intracellular voltage recording from an *Ascaris* muscle cell (resting membrane potential -34 mV). The downward deflections are elicited by current injection (20 nA, 500 ms, 0.1 Hz). The bars show the duration of application of drugs as indicated.

These data show that KPN hyperpolarizes *Ascaris* muscle cells by a chloride-dependent mechanism. It may do this by stimulating the release of GABA from the inhibitory motorneurones, however the observation that the effects of KPN and a maximal concentration of GABA are additive is not consistent with this.

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261P CHARACTERISATION OF THE 5-HT RECEPTOR MEDIATING CONTRACTION OF THE CIRCULAR MUSCLE OF HUMAN SMALL INTESTINE

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5-Hydroxytryptamine (5-HT) has been shown to influence motility in the small and large intestine of man. In the isolated human small intestine, application of 5-HT induces contraction of both circular and longitudinal muscle layers (Borman & Burleigh, 1994). 5-HT-induced contraction of longitudinal muscle strips has been shown to be mediated via a 5-HT_{2B} receptor (Borman & Burleigh, 1995), however the receptor mediating contraction of the circular muscle layer has proved more difficult to characterize. The response was found to be antagonised by methysergide (in a non-surmountable manner) and by ketanserin (non-surmountable at concentrations above 100nM; Borman & Burleigh, 1994). In the present study, we have evaluated the effect of a selective 5-HT_{1D} receptor antagonist, GR 127935T (Skingle *et al.*, 1993), on 5-HT-induced contraction of the circular muscle layer of human small intestine. Strips of circular muscle (1.5-2.5 x 15mm) were suspended under isotonic conditions and a preload of 1g in oxygenated Krebs solution at 37°C. Each preparation was subjected to two cumulative concentration-response curves to 5-HT, the second challenge being performed in the presence of antagonist or control vehicle (incubated for 30min). GR 127935T was initially dissolved in a few drops of acetic acid, then diluted in Krebs solution. Data were analysed using the Mann-Whitney U test, with $p<0.05$ taken to indicate a significant difference. EC_{50} values are given as geometric mean with 95% confidence limits (95% C.L.). Application of 5-HT produced a contraction of circular muscle strips of human small intestine, with an EC_{50} of 41.9nM (95% C.L. 29.7-59.2). Application of tetrodotoxin (3.1μM) had no significant effect on the response to 5-HT (n=4, $p>0.05$), indicating that the response was not neurogenic in nature. Application of GR 127935T (10-300nM, n=3-6 for each concentration) evoked a parallel, rightward displacement of the second response curve to 5-HT, with no alteration of the maximum response to 5-HT. Schild analysis of this antagonism gave a Schild plot with a slope of 0.80 (95%C.L. 0.60-1.05; Figure 1), and an apparent pA_2 estimate of 8.30±0.12 (slope constrained to unity).

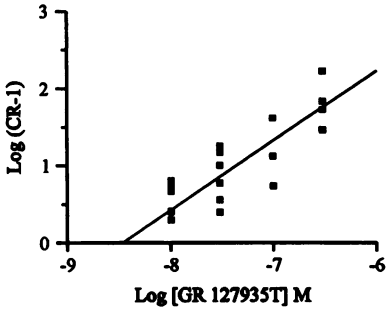

Figure 1. Schild plot for the antagonism by GR 127935T of 5-HT-induced contraction of human small intestinal circular muscle.

This estimate is in accordance with values reported elsewhere (8.5±0.1; Skingle *et al.*, 1993), and may indicate the involvement of a receptor of the 5-HT_{1D} sub-type in 5-HT-induced contraction of the circular muscle of human small intestine. The relatively high affinity of ketanserin at this receptor may support a role for the $5\text{-HT}_{1D\alpha}$ rather than the $5\text{-HT}_{1D\beta}$ receptor.

We are grateful to Glaxo for their generous donation of GR 127935T.

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262P THE USE OF PHENOXYBENZAMINE TO DISCRIMINATE 5-HT RECEPTORS MEDIATING ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASORELAXATION

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The presence of a vascular smooth muscle relaxant 5-HT receptor often confounds attempts to characterise operationally the orphan endothelial 5-HT receptor mediating relaxation indirectly through release of nitric oxide. This is particularly problematic when trying to obtain agonist potency data, and has lead some workers to draw ambiguous or incorrect conclusions about the actions of agonists at this endothelial site (Hoyer *et al.*, 1994). The activity of 5-carboxamidotryptamine (5-CT) at this receptor has been of particular interest as it is a potent agonist at several 5-HT receptors; however its actions at vascular endothelial relaxant 5-HT receptors has been a matter of debate because it has potent relaxant effects at the smooth muscle receptor. This study attempted to abolish agonist effects through the smooth muscle receptor by selective irreversible receptor alkylation with phenoxybenzamine (PBZ).

Endothelium-dependent and -independent relaxations were measured as isometric force changes in rings of rabbit external jugular vein (RbJV) prepared as detailed elsewhere (Martin *et al.*, 1987). For irreversible alkylation experiments PBZ (0.3μM) was incubated for 30min. followed by thorough washing. Cumulative agonist concentration-relaxation curves were then constructed in tissues contracted with the thromboxane mimetic, U46619 (10nM). Data is expressed as mean ± s.e.mean, $n \geq 4$.

5-CT produced concentration-dependent relaxation of endothelium-intact RbJV ($p[A_{50}]$ 7.51±0.10) which was unaffected by either endothelial denudation or the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME 100μM; $p[A_{50}]$ 7.60±0.08, 7.52±0.06 respectively), suggesting an interaction with the smooth muscle receptor alone. In endothelium-denuded preparations responses to 5-CT were abolished by 0.3μM PBZ. However, in PBZ treated endothelium-intact RbJV relaxations to 5-CT were largely preserved, with a small but significant decrease in potency ($\Delta p[A_{50}]$ -0.33, $p<0.005$) and a 37% reduction in maximum response ($p<0.05$). Under these conditions, responses were abolished by L-NAME (100μM), indicating action at an endothelial 5-HT receptor. To confirm that PBZ treatment did not affect responses via the endothelial receptor we studied the effect of (±)α-Me-5-HT, a potent agonist at this receptor but without affinity for the smooth muscle receptor (Martin *et al.*, 1987). The concentration-effect curve was unaffected by PBZ treatment, $p[A_{50}]$ 8.45±0.12 and 8.42±0.08 respectively, with no significant difference in maximum response.

In summary, pretreatment of RbJV with PBZ selectively inactivated the smooth muscle relaxant receptor revealing endothelium-mediated responses to 5-CT previously masked by effects at the smooth muscle receptor. In fact, 5-CT was almost equipotent at the two receptors in this preparation. Selective inactivation of the smooth muscle receptor allows definitive estimation of agonist potencies at the endothelial 5-HT receptor, and caution should be exercised when interpreting data which is generated in tissues where the two receptors remain functional.

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263P THE MECHANISM OF 5-HT-EVOKED CONTRACTION IN THE RABBIT MESENTERIC ARTERY

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In permeabilized strips of rabbit mesenteric resistance artery, application of 5-Hydroxytryptamine (5-HT) does not stimulate the release of intracellular calcium but does lead to a sensitization of the myofilaments to calcium. This sensitization is abolished by inhibition of protein kinase C (PKC; Seager *et al.*, 1994) and reduced by the phospholipase A_2 (PLA_2) inhibitor quinacrine (Parsons *et al.*, 1995). It is unclear whether the sensitization is sufficient to initiate and sustain contraction in intact arterial segments. In the present study, the ability of 5-HT to evoke contraction in this vessel and the mechanisms underlying this process have been investigated further.

Endothelium-denuded segments of rabbit mesenteric artery (mean diameter 283 ± 15 mm; n=22) were mounted in a Mulvany-Halpern myograph and maintained at 37°C in oxygenated Krebs buffer. Removal of the endothelial cell layer was confirmed by the lack of relaxation to acetylcholine (10 μM) following pre-constriction with noradrenaline (1 μM).

Application of 5-HT (10 nM-100 μM) evoked very small increases in tension (maximum change 0.6 ± 0.2 mN; n=6). However, in the presence of 25 mM K^+-Krebs buffer, application of 5-HT elicited concentration-dependent contraction with a maximum response of 25.1 ± 1.3 mN (n=12). Exposure to 25 mM K^+-Krebs buffer alone induced only a small contraction of 1.7 ± 0.4 mN (n=12).

Pre-incubation with nifedipine (1 μM; 10 mins) or the PKC inhibitor Ro31-8220 (10 μM; 10 mins), significantly inhibited contraction to all concentrations of 5-HT and the maximum response was reduced to 39.5 ± 5.5 % (n=4; $p<0.01$) and 23.0 ± 8.0 % (n=4; $p<0.01$) of the initial contraction, respectively. Exposure to nifedipine and Ro31-8220 together abolished 5-HT-evoked contractions (n=4).

Pre-exposure of arterial segments to the PLA_2 inhibitor quinacrine (10 mins) also inhibited 5-HT-evoked contraction. In the presence of 1 μM and 5 μM quinacrine, the maximum response to 5-HT was reduced to 68.4 ± 11.2 % and 38.6 ± 7.6 % (n=4; $p<0.01$) of control values, respectively.

Addition of nifedipine (1 μM), Ro31-8220 (10 μM) and quinacrine (5 μM) to arterial segments maximally contracted with 5-HT reduced the level of tone to a similar extent as pre-incubation (n=4).

These observations show that 5-HT can evoke contraction in segments of the rabbit mesenteric artery in the presence of 25 mM K^+-Krebs but not in normal Krebs buffer. An important component of the contraction appears to derive from calcium influx through L-type calcium channels. In addition, a large component of the response involves the activation of PKC and PLA_2 and may reflect myofilament sensitization.

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264P CHARACTERIZATION OF PUTATIVE 5-HT_7 RECEPTORS IN *CYNOMOLGUS* MONKEY JUGULAR VEIN, *IN VITRO*

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Receptors for 5-hydroxytryptamine (5-HT) have been classified by operational, transductional and structural criteria into four major subtypes, namely, 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (Hoyer et al., 1994). In addition, three other less well defined subtypes (5-ht5, 5-ht6 and 5-ht7) have also been proposed (Hoyer et al., 1994). Although these novel receptors have been cloned from central nervous system tissues, unambiguous functional equivalents in peripheral tissues have yet to be identified. In the present study, we describe a 5-HT receptor mediating endothelium-independent relaxation in *Cynomolgus* monkey jugular vein.

Jugular veins were isolated from monkeys of either sex (4 - 7 kg) and kept in modified Krebs solution that contained pindolol (3 μM), ketanserin (0.1 μM), ondansetron (1 μM), GR 113808 [[1-(2-methane-sulfonamido-ethyl)-piperidin-4-yl]-methyl-indole-3-carboxylate maleate] (1 μM), phentolamine (1 μM), cocaine (30 μM), corticosterone (30 μM) and indomethacin (3 μM). Ring segments of 3 mm length were suspended under 1 g resting tension in 10 ml organ baths maintained at 37°C and aerated with 95% O_2 5% CO_2.

5-Carboxamidotryptamine (5-CT) and 5-methoxytryptamine relaxed U46619 [9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$] precontracted jugular vein with potencies (pEC_{50} ± s.e. mean) of 7.5±0.1 (n=18) and 5.7±0.1 (n=4), respectively. The relaxation produced by 10 μM 5-CT was 87±3 % of the U46619 induced tone, and was not dependent on endothelium integrity as revealed by a lack of response to acetylcholine. The intrinsic activity of 5-methoxytryptamine was 0.9±0.1, relative to 5-CT. In contrast, 5-HT and α-methyl-5-HT caused an initial contraction (10 nM-1 μM) followed by relaxation at higher concentrations (1 μM-32 μM). The effects of 5-CT were antagonized by several 5-HT antagonists (Table 1) and the affinity estimates obtained were in good agreement with values obtained at cloned human 5-ht7 receptors (Table 1; Bard et al., 1993). It is concluded that relaxation of *Cynomolgus* monkey jugular vein is mediated by a functional correlate of the human 5-ht7 receptor.

Table 1

Antagonist [M]	methiothepin (10^{-8})	mesulergine ($3x10^{-7}$)	metergoline ($3x10^{-7}$)	clozapine (10^{-6})	mianserin (10^{-6})	spiperone (10^{-6})	ritanserin (10^{-6})	methysergide (10^{-6})	ketanserin (10^{-5})	8-OH-DPAT (10^{-6})
pK_B±s.e.mean	9.7±0.3	8.1±0.3	8.0±0.1	7.8±0.1	7.7±0.2	7.3±0.4	7.1±0.2	7.0±0.2	5.7±0.1	<6
(pK_I, human 5-ht7)	(8.4)	(7.7)	(8.2)	--	--	(7.0)	(7.3)	(7.1)	(5.9)	(6.3)

Values are mean ± s.e. mean, n=4-6.

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265P THE EFFECTS IN THE RAT OF THE 5-HT$_3$ RECEPTOR ANTAGONIST, WAY-SEC-579, ON INCREASED MICROVASCULAR BLOOD FLOW INDUCED BY INTRADERMAL INJECTION OF 5-HYDROXYTRYPTAMINE

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In man the wheal and flare reaction evoked by intradermal (i.d.) 5-hydroxytryptamine (5-HT) is blocked by 5-HT3 receptor antagonists (Richardson et al., 1985). This reaction is not readily seen in laboratory animals. We describe here an increase in local microvascular blood flow evoked by 5-HT (i.d.) in the rat and its blockade by the potent and selective 5-HT3 receptor antagonist, WAY-SEC-579, endo-N-(8-methyl-8-azabicyclo [3.2.1] octan-3-yl)-1-cyclohexyl-1,4-dihydro-4-oxoquinoline-3-carboxamide (Coleman et al., 1994).

Male Wistar rats (230-300 g) were sedated with Hypnorm (fentanyl/fluanisone, 0.3 mg/kg i.m.) and the dorsal skin shaved and washed. Room temperature was 23-25°C. After 2 h, basal blood flow was measured in the dorsal skin at 6-8 marked sites with a laser Döppler flow probe (Perimed II, Warren et al., 1993) set at 4 Hz gain 10. The mean of 3 readings (15 sec each) was taken. Data are shown as a percentage change (± s.e. mean) from this value at each site. The vasodilator dose-response and time course to 5-HT were established in 8 rats. Separate sites were injected i.d. with phosphate buffered saline (PBS) or 5-HT (3, 10 or 30 µM dissolved in PBS) in a volume of 100 µl. Blood flow was measured at 15, 30, 60 and 120 min after injection. In separate groups of rats the effect of WAY-SEC-579 (WAY; 1, 10 or 100 nM) was tested against a single dose of 5-HT (100 µl of 10 µM). Two sites on each rat were injected (100 µl i.d.) with either PBS, 5-HT alone, WAY alone or WAY and 5-HT. WAY was injected 30 min before 5-HT challenge. One concentration of WAY was tested in each animal. Blood flow was measured 30 min after 5-HT injection.

The time to maximum increase in blood flow was 30 min following injection of 5-HT. The mean increase (%) in flow was 22.7±10.2, 42.8±14.9, 76.0±18.6 and 43.1±17.4 at sites injected with PBS, 3, 10 or 30 µM 5-HT respectively (n=8). The 5-HT dose-response curve was thus bell-shaped with 10 µM 5-HT producing the only change significantly greater than that of the PBS controls ($P<0.05$, ANOVA). Results with WAY are shown in Table 1. 5-HT evoked a significantly greater increase in flow than PBS in 2/3 groups. No significant difference was detected between PBS control sites and sites treated with WAY. The response to 5-HT was significantly reduced at sites pretreated with 10 and 100 nM WAY.

Table 1. Blood flow increase(%)

WAY	PBS	5-HT	WAY	WAY+ 5-HT
1 nM	40.6±17.9	145.9±28.8*	58.7±24.4	105.4±34.1
10 nM	9.9±10.0	89.6±15.9*	39.6±15.5	36.6±13.1[a]
100 nM	84.4±27.5	146.7±56.9	37.7±44.5	-11.0±12.9*[a]

Mean values ± s.e. mean. Significantly different ($p<0.05$) to PBS controls*, or [a] to 5-HT , groups using two way ANOVA, n=5-6.

These results are consistent with a 5-HT3 receptor mediated vasodilator mechanism in rat skin. The bell-shaped dose-response curve for 5-HT may be due to vasoconstrictor effects of 5-HT via other receptor subtypes at higher concentrations.

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266P ATP-INDUCED Ca^{2+} ENTRY RELEASES Ca^{2+} FROM INTRACELLULAR STORE IN FRESHLY ISOLATED SMOOTH MUSCLE CELLS FROM HUMAN SAPHENOUS VEIN

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Venous tone strongly depends on sympathetic innervation and it is well known that ATP is released as an excitatory co-transmitter together with noradrenaline at sympathetic nerve terminals present in smooth muscle tissues (Von Kügelgen & Starke, 1991). Effects of exogenous ATP were studied in freshly isolated myocytes from human saphenous vein by measurement of intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) using indo-1 fluorescence, and whole-cell currents, using patch-clamp techniques.

At a holding potential of -60 mV, ATP (10 µM) elicited a transient inward current and increased $[Ca^{2+}]_i$. The P_{2X}-specific ATP analog AMP-C-PP (10 µM) activated a transient inward current and inhibited the response to a subsequent application of ATP. The ATP-gated current corresponded to a non-selective cation conductance allowing Ca^{2+} entry.

The ATP-induced rise in $[Ca^{2+}]_i$ was totally abolished in the absence of external Ca^{2+} although a large inward current was still recorded. The rise in $[Ca^{2+}]_i$ induced by ATP was reduced to 30.1 ± 5.5 % (n = 14) of the control response when it was applied immediately after caffeine. When the intracellular Ca^{2+} store has been depleted by 1 µM thapsigargin, which is known to inhibit sarcoplasmic reticulum Ca^{2+}-ATPase (Thastrup *et al.*, 1990), the increase in $[Ca^{2+}]_i$ induced by ATP was reduced to 23.7 ± 3.8% (n = 11) of control. However, intracellular application of the inositol 1,4,5-trisphosphate receptor antagonist, heparin (1 mM) (Worley *et al.*, 1987) did not affect the rise in $[Ca^{2+}]_i$ induced by ATP, suggesting that inositol 1,4,5-induced Ca^{2+} release was not involved in this response.

Therefore, we have examined the effect of the Ca^{2+}-induced Ca^{2+} release (CICR) antagonist tetracaine (Almers & Best, 1976) to assess the contribution of this mechanism to the transient rise in $[Ca^{2+}]_i$ evoked by ATP. Tetracaine inhibited the rise in $[Ca^{2+}]_i$ induced by both caffeine and ATP with apparent inhibitory constants of 70 µM and 100 µM, respectively. A component corresponding to 29.3 ± 3.9 % (n = 8) of the ATP-induced increase in $[Ca^{2+}]_i$ was tetracaine-resistant.

In conclusion, these data suggest that the effects of ATP in human saphenous vein myocytes are only mediated by activation of P_{2X} receptor-activated channels. The ATP-induced $[Ca^{2+}]_i$ rise is due to both Ca^{2+} entry and Ca^{2+} release activated by Ca^{2+} ions that enter the cell through P_{2X} receptor-activated channels.

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267P THE MAGNITUDE OF RELAXATION OF AORTIC SMOOTH MUSCLE BY THE A_2 RECEPTOR AGONIST, NECA, IS DEPENDENT ON THE α-ADRENOCEPTOR AGONIST USED FOR PRECONTRACTION

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We have previously shown that the degree of relaxation of guinea-pig aortic rings by the adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA), is dependent upon the α-adrenoceptor agonist used for contracting the tissue (Maddock & Broadley, 1995). The present study investigates possible mechanisms for these differences, related to intracellular and extracellular calcium mobilisation by these different α- adrenoceptor agonists.

From each guinea-pig, four 5mm rings of endothelium-denuded thoracic aorta, were mounted under a resting tension of 1g, in Krebs bicarbonate solution at 37°C gassed with O_2:CO_2 (95:5). Cumulative concentration-response curves (CCRC's) were constructed for noradrenaline (NA), phenylephrine (PE), adrenaline (ADR), methoxamine (ME) and (-)dobutamine (DOB) to determine a submaximal concentration suitable for preconstriction. Aortic rings were precontracted with NA, PE, ADR, ME or DOB either in the presence or absence of ryanodine 10^{-6}M, calcium free Krebs (2mM EDTA) or nifedipine 10^{-6}M, which were added 20 min beforehand. When the tension had plateaued, CCRC's were constructed to NECA. Statistical significance ($P < 0.05$) was tested using Student's t-test of paired data.

All the adrenergic agonists caused concentration-related increases in the aortic tension. NECA relaxed the aortic rings in a concentration-dependent manner, the maximum relaxation being dependent on the contractile agonist used. The maxima for NECA in PE, ADR, ME and (-)DOB precontracted aortic rings (61.8±9.5%, 44.4±4.7%, 59.8±5.4, 112.04±18.9% (n=4), respectively) were significantly greater ($P<0.05$) than with NA (10.9±3.8%, n=4). Contraction by the α_{1A}-adrenoceptor agonist DOB (Ruffolo *et al.*, 1991) which has reported action via translocation of extracellular calcium (Oriowo *et al.*, 1992), produced the largest relaxation by NECA.

Further experiments were conducted to determine whether the differences in magnitude of relaxation by NECA in NA and PE contracted tissues were due to variable involvement of intracellular or extracellular calcium. Ryanodine was used to inhibit intracellular calcium stores and had no significant effect ($P<0.05$, n=4) on the maximal response of NECA in NA (control 21.9±4.1%, test 31.3±2.7%) or PE (control 69.6±8.4%, test 55.3±5.1%) precontracted aortic rings. The maximal inhibition by NECA of NA contractions in the presence of calcium-free Krebs (18.9± 5.8%) or nifedipine (17.5±0.6%) did not alter significantly ($P<0.05$, n=4) compared to the controls (8.5±2.4% and 8.7±4.1%, respectively). The presence of calcium-free Krebs (19.3±8.7%) or nifedipine (34.5±4.02%), however, had significant ($P<0.05$, n=4) effect on the maximal inhibitory response of NECA in PE-precontracted aortic rings. These maxima were less than the paired controls (73.0±11.1% and 65.7±5.6%, respectively). The NECA inhibitory response therefore appears most effective when the aorta is contracted by PE rather than NA. The A_2 receptor-mediated relaxation by NECA of the guinea-pig aorta appears to be due to the inhibition of calcium influx when contracted with PE. The small maximum relaxation of NA-contracted tissues may arise because the contraction is predominantly due to release of intracellular calcium (Oriowo *et al.*, 1992).

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268P INWARD CURRENTS RECORDED ON P_{2X}-PURINOCEPTOR ACTIVATION IN GUINEA-PIG VAS DEFERENS ISOLATED SMOOTH MUSCLE CELLS

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Recently, the cloned P_{2X} purinoceptor from rat vas deferens has been studied in Xenopus oocytes (Valera et al., 1994) and found to be a cation selective ion channel, producing a large inward current on activation. The aim here was to study currents activated by ATP in isolated cells from the guinea-pig vas deferens and determine their characteristics.

Guinea-pig vas deferens cells were dispersed using a collagenase/pronase enzyme solution and perfused with a Ca^{2+}-free physiological salt solution (mM: NaCl 125, KCl 5, $MgCl_2$ 4, KH_2PO_4 1.2, HEPES 10 and glucose 11) until the whole cell recording mode was achieved, when the solution was changed to one containing Ca^{2+} (2.5 mM). The composition of the pipette solution was : (mM) NaCl 5, KCl, 120, KH_2PO_4 1.2, $MgCl_2$ 1.2, HEPES 10, glucose 11, oxalacetate 5, pyruvic acid 2 and succinic acid 5. ATP (1-100 μM) was applied by pressure ejection from a micropipette placed close to the cell. Except in desensitisation and antagonist studies, a single concentration of ATP was applied once to each cell. Responses were obtained to ATP (10 μM) after 8 min incubation with antagonist and then repeated 10 min after washout of antagonist.

The cells had a resting membrane potential of -46.0 ± 3.8 mV (n=22). When held at -70 mV (to minimise spontaneous outward K+ currents) ATP (1-100 μM) induced a transient, inward current (Table 1). The peak current response to ATP (10 μM) was significantly reduced to 51.0 ± 3.5 % ($P < 0.05$, n=5) of the initial value when repeated after 8 min.

ATP concentration	Peak current (pA)	Time to peak (ms)	$T_{1/2}$ (ms)
1μM (n=3)	-130 ± 60	300 ± 50	905 ± 300
3 μM (n=3)	-180 ± 35	210 ± 40	930 ± 80
10 μM (n=7)	-390 ± 150	285 ± 100	1405 ± 270
100 μM (n=4)	-80 ± 20	295 ± 30	1915 ± 360

Table 1. Characteristics of ATP-induced inward current (mean ± s.e.m.)

In the presence of suramin (1 mM) inward currents to ATP (10 μM) were -65 ± 65 pA (n=3) compared to -700 ± 125 pA in the absence of suramin. Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) has been reported to be a selective antagonist at P_{2X} purinoceptors (Ziganshin et al., 1994). PPADS (10 μM) abolished responses to ATP in three cells.

These results demonstrate the presence of ATP-induced inward currents in these cells. The currents recorded, due to their transient nature, rapid desensitisation and antagonist sensitivity, are likely to result from P_{2X}-purinoceptor activation.

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269P EFFECT OF N^6-CYCLOPENTYLADENOSINE, N^6-CYCLOHEXYLADENOSINE, AND N^6-2-PHENYLISOPROPYLADENOSINE ON RAT SUPERFICIAL AND DEEP FASCIA

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Fascia, both superficial fascia (SF) and deep fascia (DF), are generally considered to be a simple connective tissue. The most abundant cellular constituents are the fibroblasts and mast cells. Both SF and DF are capable of contraction in vitro (Masood & Naylor,1994 a). In a previous study (Masood & Naylor,1994 b)it was demonstrated that the cells present in rat fascia have receptors for adenosine. Since intact fibroblasts have been shown to contract to exogenous ATP (Joseph et al 1988), further investigation is required into the types of receptors, responding to adenosine.

Dorsal fascia (SF, n=7 and DF, n=7), were obtained from female, Hooded Lister rats (200-300g, Bradford strain). After killing the animal by cervical dislocation, tissue strips 2x1cm, were mounted in 2.5 ml baths containing oxygenated Krebs Henseleit solution(37^{0}C), under a resting tension of 2g. Their contractility to N^6- cyclopentyl adenosine (CPA),N^6-cyclohexyladenosine(CHA), and N^6-2-phenylisopropyl adenosine (PIA), was measured isometrically

CPA (fig 1), CHA (fig 2), & PIA (fig 3), elicited concentration dependent, reproducible and reversible contractile responses in both SF (n=7) and DF (n=7), over a dose range for CPA of (10^{-9}- 10^{-6}M), CHA (10^{-8}-10^{-4}M), and PIA (10^{-7}-10^{-5}M). The contractile responses of the DF to CPA and PIA were significantly greater than those of the SF. Using Wilcoxon Signed Rank test, for CPA (all concentrations) P<0.02, and for PIA (concentrations 10^{-6} and 10^{-5} M) P<0.05 and P<0.02 .

These results show that cells present in rat fascia respond to the agonists in the rank order of CPA, CHA, and PIA. The nature of this responsiveness suggests that the contractile cell in both types of fascia is the myofibroblast, and that DF has more of these cells than the SF. This suggest the presence of the A_1 receptor.

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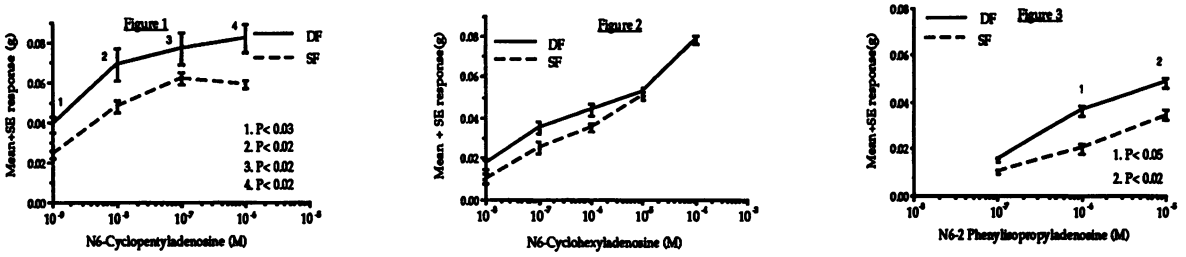

Response of rat SF and DF to CPA Fig1, CHA Fig 2, and PIA Fig 3.

270P EFFECTS OF P_{2Z}-PURINOCEPTOR-SELECTIVE AGENTS ON THE RAT PAROTID SALIVARY GLAND *IN VITRO*

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ATP stimulates elevation of intracellular Ca^{2+} ($[Ca^{2+}_f]_i$) and amylase secretion in rat parotid acini and it has been proposed that this effect may be mediated via an action of ATP^{4-} on P_{2Z}-purinoceptors (Soltoff *et al.*, 1992). The aim of this study was to further characterise these purinergic effects by investigating the actions of P_{2Z}-selective agonists and antagonists on these responses. Parotid acini were prepared from male Wistar rats, amylase secretion measured in parotid slices and $[Ca^{2+}_f]_i$ determined in fura-2-loaded acini as previously described (Arkle *et al.*, 1989).

Both ATP and the P_{2Z}-selective agonist, 2'&3'-O-(4-benzoylbenzoyl)-ATP (BBATP), stimulated amylase release and elevation of $[Ca^{2+}_f]_i$ with similar concentration-response relationships (table 1). The increase in $[Ca^{2+}_f]_i$ in response to 30μM ATP^{4-} and 30μM BBATP was inhibited by preincubation of acini for 60 min. with 300μM 2,3-dialdehyde ATP (oxidised ATP) prior to addition of agonist (72±4 and 70±8 % inhibition respectively, 4 observations). These responses were inhibited to a lesser extent by 5 min. preincubation with 100μM 5'-*p*-fluorosulphonyl benzoyladenosine (FSBA) (37±17 and 44±13 % inhibition respectively, 4 observations). The secretory response to 300μM ATP^{4-} was also inhibited by 300μM oxidised ATP and 100μM FSBA (33±9 and 49±13 % inhibition respectively, 4 observations). When the parotid tissues were preincubated for 5 min. with the phospholipase C inhibitor, U73122 (10μM), no significant change in 300μM ATP^{4-}-stimulated amylase secretion (47±11 i.u./min/g tissue, 4 observations) or 30μM ATP^{4-} -stimulated elevation of $[Ca^{2+}_f]_i$ (197±47nM, 4 observations) was seen.

Table 1. Concentration-response relationships for ATP^{4-} and BBATP-stimulated amylase secretion (i.u./min/g tissue) and elevation of $[Ca^{2+}_f]_i$ (nM) (mean ± s.e. mean, 4 observations).

		[Agonist] (μM)				
agonist		10	30	100	300	1000
ATP^{4-}	$[Ca^{2+}_f]_i$	109±31	208±47	374±64	611±99	-
ATP^{4-}	amylase	9±6	23±7	38±9	51±6	-
BBATP	$[Ca^{2+}_f]_i$	6±12	210±42	362±48	661±92	623±93
BBATP	amylase	5±3	15±6	33±9	55±11	49±9

We conclude that these data are consistent with the notion that the calcium-mobilising and secretory effects of ATP in the rat parotid gland are mediated via P_{2Z}-purinoceptors and that these effects are independent of phospholipase C activation.

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271P THE ADENOSINE A_{2B} RECEPTOR MAY MODULATE THE RELEASE OF TUMOUR NECROSIS FACTOR α (TNFα) FROM HUMAN THP-1 CELLS

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Inflammatory cytokines, including tumour necrosis factor-α (TNF-α), are released from monocytes and macrophages upon activation by various inflammatory stimuli, e.g bacterial lipopolysaccharide (LPS). It was reported that compounds acting via adenosine receptors can modify TNF-α release in peripheral macrophages (Reinstein *et al.*,1994).

Using the THP-1 cell line (of human leukaemic monocyte/macrophage lineage) we investigated the inhibition of LPS-stimulated TNF-α and interleukin-1β (IL-1β) release by adenosine agonists and antagonists.

THP-1 cells ($2x10^6$ cells/well) were cultured at 37 °C with 5% CO_2. Adenosine antagonists were added one hour prior to agonists followed by LPS (2.5μg/ml) one hour later. Media was collected after 18 hours incubation and the supernatant assayed for TNF-α and IL-1β by sandwich ELISA.

Results are expressed as percentage inhibition of LPS induced TNF-α release ± the standard deviation.
The LPS dose given resulted in a sub-maximal release of TNF-α, 3.45 ± 0.41ng/ml (3.05-4.14) and IL-1β, 2.13 ±1.07ng/ml (1.3-3.75).

5' N-ethylcarboxamidadenosine (NECA) and 2-chloroadenosine (2CA) inhibited (dose-dependently) LPS stimulated TNF-α release. The maximum inhibition of TNF-α release observed with NECA was 64.5 ± 3.29% (Range 59.67-77.47, n=5), 2CA 50.16% (Range 47.76-52.56, n=2) and CGS21680 (2-p-(2-carboxyethyl) phenethylamino-5'N-ethylcarboxamidoadenosine HCl) 19.15% (Range 12.15-26.15, n=2).
N^6-2(4-Aminophenyl)ethyladenosine (APNEA) and N^6-cyclopentyladenosine (CPA) produced non dose-dependent inhibition of less than 15% (n=2). None of the compounds tested inhibited IL-1β release.

The EC_{50} values for the inhibition of TNF-α release by NECA and 2CA were 0.39μM (0.21-0.58, n=5) and 2.2μM (1.8-2.6, n=2) respectively. The effect of NECA was antagonised by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) at 1 and 0.1μM, and by 8-phenyltheophylline (8PT) and xanthine amine congener (XAC) at 1μM (n=2).

The EC_{50} of NECA and the rank order of potency of adenosine agonists, NECA>2CA>>CGS21680=APNEA=CPA is consistent with effects at the A_{2B} receptor (Collis *et al.*,1993).These data suggest that A_{2B} receptors on THP-1 cells may mediate TNF-α but not IL-1β release.

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272P THE EFFECT OF THE NOVEL ECTO-ATPase INHIBITOR FPL 67156 ON CONTRACTILE RESPONSES TO NERVE STIMULATION AND TO AGONISTS IN GUINEA-PIG ISOLATED VAS DEFERENS

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The neurogenic contraction to nerve stimulation in the guinea-pig vas deferens is biphasic, the initial peak being predominantly purinergic and the smaller tonic component predominantly adrenergic. The novel ecto-ATPase inhibitor FPL 67156 (6-N,N,diethyl-D-β,γ-dibromomethylene ATP, Crack *et al.*, 1995) enhances contractile responses to sympathetic nerve stimulation in this tissue (Sneddon *et al.*,1995). We have now investigated further the selectivity of the ecto-ATPase inhibitor.

Guinea-pig isolated vas deferentia were bathed in Krebs' solution at 37°C and bubbled with 95% O_2 5% CO_2. Contractions were evoked by field stimulation (supramaximal voltage, 0.5ms pulse width) at 1-8 Hz for 20s or by adding ATP, α,β-methyleneATP (α,β-meATP), noradrenaline (NA), KCl, or carbachol to the bathing solution. Data were analysed using Student's paired t-test and $P<0.05$ was considered to be significant.

FPL 67156 (5-100μM) significantly enhanced contractile responses to nerve stimulation in a concentration-dependent manner. For example at 4 Hz, 100μM produced a significant increase in peak amplitude from 0.97±0.17g to 2.06±0.22g, n=8. In the presence of 10^{-7}M prazosin, neurogenic contractions are purely purinergic. Prazosin did not affect the ability of FPL 67156 to enhance responses from 1.26±0.12g to 2.23±0.17g, n=8.

The effect of FPL 67156 on responses to exogenously applied P_{2X}-purinoceptor agonists was examined. 100μM FPL 67156 significantly enhanced the effect of exogenously applied ATP (100μM) from 2.65±0.30g to 4.22±0.42g, n=11. In contrast, the response to α,β-meATP was not significantly affected by FPL 67156, from control values of 1.51±0.13g to 1.46±0.10g, n=6. Surprisingly, the responses to NA (10μM), carbachol (5μM) and KCl (40mM) were all significantly enhanced by FPL 67156 (100μM), from 1.16±0.25g to 3.41±0.44g, n=13; 2.4±0.13g to 3.08±0.29g, n=8; 3.08±0.30g to 4.2±0.34g, n=9, respectively. This could be due to an increase in endogenous ATP levels, increasing the excitability of the smooth muscle. This was tested by examining the effect of FPL 67156 after blocking P_{2X}-purinoceptors with the selective antagonist PPADS. When PPADS (100μM) was present, the responses to exogenous NA and KCl were not enhanced by FPL 67156 (100μM), from control values of 2.39±0.22g to 2.17±0.17g, n=7, and from 5.26± 0.32g to 5.19±0.34g, n=6, respectively.

These results indicate that FPL 67156 enhances the purinergic component of sympathetic neurotransmission in guinea-pig vas deferens. The ability of the ecto-ATPase inhibitor to enhance responses to other agonists may be due to an increase in endogenous ATP levels.

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