312 P EFFECT OF CANNABINOID AGONISTS AND SR141716A ON ACETYLCHOLINE RELEASE FROM THE MYENTERIC PLEXUS OF THE GUINEA-PIG SMALL INTESTINE

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Cannabinoid receptor agonists inhibit electrically-evoked contractile responses of the myenteric plexus-longitudinal muscle preparation of the guinea-pig small intestine and this action can be reversed or prevented by the selective cannabinoid CB1 receptor antagonist, SR141716A, (Coutts *et al.*, 1995). It is assumed that the mechanism of action of cannabinoids is prejunctional since these drugs have no significant effect on the sensitivity of the preparation to exogenously applied acetylcholine (ACh). In the present experiments, we sought to demonstrate the presynaptic localisation of cannabinoid receptors by direct measurement of changes in evoked ACh release following exposure to cannabinoids.

Strips of myenteric plexus-longitudinal muscle were dissected from the small intestines of male albino Dunkin-Hartley guinea-pigs (300-800 g) as described by Pertwee *et al.* (1992). Donor preparations were suspended in 3ml organ baths containing choline chloride (20 μM) in modified Krebs solution at 37° C and bubbled with 95% O_2 and 5% CO_2. Tissues were stimulated continuously at supramaximal voltage (110%) with 0.5 ms pulses at 0.1 Hz and isometric contractions were recorded until they were constant. Subsequently, the preparations were incubated without stimulation for 1 h in the presence of the acetylcholinesterase inhibitor, eserine (7.7 μM). Samples of donor bath fluid were collected at the end of 4-min collection periods during which the preparations were stimulated at 0.1 Hz. ACh was assayed against standard solutions of ACh, on strips of myenteric plexus-longitudinal muscle mounted in 3 ml organ baths to measure ACh-induced isotonic contractions (Waterfield, 1973). Donor preparations were preincubated for 8 min in the presence of test drugs before the start of collection of samples.

The cannabinoid receptor agonists, WIN 55212-2 and CP 55940 (15 to 200 nM), caused a dose-related inhibition of ACh release which was reversed by SR141716A (100 to 400 nM) but not by an equivalent amount of its vehicle, Tween 80 (n=3 to 14). In the presence of a maximal concentration (200 nM) of WIN 55212-2 or CP 55940, ACh release fell from its predrug level ($P < 0.05$; Student's paired t test) by 35.2 ± 4.2% and 34.7 ± 4.4% respectively (mean ± s.e.m.; n=4 or 14). The cumulative dose response curves for WIN 55212-2 on both twitch response (n=8) and ACh output (n=6) were shifted to the right ($P < 0.05$; symmetrical 2+2 dose assay) in the presence of SR141716A (100 to 400 nM). In concentrations of up to 1000 nM WIN 55212-3, the (–)-enantiomer of WIN 55212-2, was devoid of activity as a twitch inhibitor (n=6) and had little effect on ACh release (n=4). It is concluded that WIN 55212-2 and CP 55940 inhibit electrically-evoked contractions of the myenteric plexus-longitudinal muscle preparation through prejunctional cannabinoid CB1 receptors. By itself, SR141716A (10 to 160 nM) caused small but significant increases in both ACh release ($P < 0.05$; Student's paired t test; n=9) and the amplitude of electrically-evoked contractions ($P < 0.01$; Student's unpaired t test; n=11 or 12). This suggests that SR141716A may be acting as an inverse agonist at cannabinoid receptors in the myenteric plexus-longitudinal muscle preparation or antagonising an endogenously-released cannabinoid.

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313 P LIPOPOLYSACCHARIDE-INDUCED NITRITE FORMATION IN RAT ANOCOCCYGEUS CULTURED SMOOTH MUSCLE CELLS

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Treatment with bacterial lipopolysaccharide (LPS) leads to expression of inducible nitric oxide synthase (iNOS) in rat aortic cultured smooth muscle cells (Szabo *et al.*, 1993). Similar mechanisms for NO formation have been implicated in producing vasodilation leading to severe hypotension in patients with septic shock (Moncada & Higgs, 1995). Here we have investigated if LPS treatment can also induce NO formation in non-vascular cultured smooth muscle cells (SMC) from the rat anococcygeus muscle.

Primary cultures of SMC were obtained by collagenase digestion of rat anococcygeus muscles or aortae, and by growth of cells in Medium 199, 10% foetal bovine serum and antibiotics/amphotericin. Cells were identified as SMC by immunofluorescence after treatment with an anti-smooth muscle actin antibody (from mouse), followed by a fluorescently-labelled anti-mouse IgG (from goat). Confluent cells were trypsinized and passaged into 12 well plates. LPS or drugs were added in culture medium to confluent cells, and the medium was sampled from wells at designated times for determination of nitrite by the Griess reaction, as an index of NO production. None of the drugs affected cell viability under the conditions used.

In initial experiments, anococcygeus SMC were treated with LPS (1, 10 and 100 μg/ml) for 96h, involving media/LPS replacement every 24h with fresh solutions immediately after media samples from the cells had been taken for nitrite analysis. Nitrite concentrations (μM) per 24h sample increased with concentration and duration of LPS treatment, reaching an apparent maximum around 72 - 96h. At 24h these nitrite values were: No LPS, 0.6 ± 0.2; 1 μg/ml, 1.2 ± 0.6; 10 μg/ml, 1.6 ± 0.7; 100 μg/ml, 3.3 ± 0.9. At 96h, corresponding values were: No LPS, 1.0 ± 0.4; 1 μg/ml, 2.7 ± 0.6; 10 μg/ml, 5.6 ± 1.3; 100 μg/ml, 8.5 ± 1.8 (mean ± s.e. of 4 cultures from different rats).

Pyrrolidine dithiocarbamate (PDTC), an antioxidant which acts as an inhibitor of transcription factor NF-κB activation, blocks iNOS expression in rat aortic smooth muscle cells (Hattori *et al.*, 1996). Phenylarsine oxide (PAO), a protein-tyrosine phosphatase (PTP) inhibitor, also prevents NF-κB activation implying a role for PTP in the pathway for this activation (Singh & Aggarwal 1995). After 24h treatment, PDTC significantly ($P < 0.05$, t-test) inhibited LPS-induced nitrite formation in SMC from rat aorta (1 μg/ml LPS, 4.8 ± 0.8; LPS + 25 μM PDTC, 0.4 ± 0.1, n=5) and anococcygeus (100 μg/ml LPS, 4.7 ± 0.9; LPS + 12.5 μM PDTC, 1.4 ± 0.8, n=6). Similarly, PAO (65 nM) treatment also significantly inhibited nitrite formation in aortic (1 μg/ml LPS, 7.8 ± 0.8; LPS + PAO, 3.3 ± 1.1, n=7) and anococcygeus SMC (10 μg/ml LPS, 3.8 ± 0.9; LPS + PAO, 1.0 ± 0.3, n=5).

These studies suggest that iNOS expression in both aortic and anococcygeus SMC may be linked to NF-κB activation, although more direct methods of determining iNOS protein and nuclear NF-κB translocation will be required to confirm this.

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314 P EFFECTS OF SIGNAL TRANSDUCTION INHIBITORS UPON LIPOPOLYSACCHARIDE-INDUCED NITRITE FORMATION IN RAT AORTIC CULTURED SMOOTH MUSCLE CELLS

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Although various cell types express inducible nitric oxide synthase (iNOS) when exposed to bacterial lipopolysaccharide (LPS) and some cytokines, the transduction mechanisms producing iNOS gene activation remain to be defined. Here, we have used some putative inhibitors of LPS and cytokine signalling pathways, to investigate their effects upon LPS-induced nitrite formation in rat aortic cultured smooth muscle cells (SMC).

Primary cultures of SMC, obtained by collagenase digestion of rat aortae, were grown in Medium 199, 10% foetal bovine serum and antibiotics/amphotericin. Cells were subcultured by trypsinization and used from passage 2 - 24 for experiments. For these, cells were passaged and grown to confluence in 12 well plates, after which LPS (1 μg/ml) and test drugs in culture medium were added to wells. Medium was sampled from wells, at 20h in most studies, for determination of nitrite by the Griess reaction, as an index of NO production. None of the drugs affected cell viability at the concentrations used. Table 1 shows nitrite concentrations (μM) as mean ± s.e. values from 4-5 cultures. Each drug significantly inhibited ($P<0.05$, t-test) LPS-induced nitrite formation.

Table 1. Effects of drugs upon LPS-induced nitrite formation.

Drug treatment	Medium alone	LPS alone	LPS + drug
Apocynin (5 mM)	0.37 ± 0.03	6.6 ± 1.9	0.47 ± 0.05
Curcumin (35 μM)	0.47 ± 0.07	8.7 ± 1.6	1.37 ± 0.30
NDGA (10 μM)	0.30 ± 0.03	4.9 ± 0.4	0.67 ± 0.09
Catalase (800 U/ml)	0.12 ± 0.04	4.4 ± 0.3	0.83 ± 0.03
TPCK (40 μM)	0.24 ± 0.20	5.7 ± 1.2	0.23 ± 0.05
D609 (110 μM)	0.21 ± 0.08	3.8 ± 1.0	0.13 ± 0.07

Cytokine-induced activation of the transcription factor NF-κB can be inhibited by (i) apocynin, an inhibitor of superoxide generating NADPH oxidase (Satriano & Schlondorff 1994), (ii) by the antioxidants nordihydroguaiaretic acid (NDGA) and curcumin (Israel *et al.*, 1992; Singh & Aggarwal 1995), the latter also inhibiting iNOS expression in macrophages (Brouet & Ohshima 1995) (iii) by the protease inhibitor N-p-tosyl-L-phenylalanine chloromethylketone (TPCK) (Griscavage *et al.*, 1996). Catalase, which degrades H_2O_2, was proposed to inhibit macrophage iNOS activity by preventing cofactor (tetrahydrobiopterin) synthesis (Li *et al.*, 1992) and D609 (tricyclodecan-9-yl xanthogenate), an inhibitor of phosphatidylcholine-specific phospholipase C, also blocked iNOS induction in macrophages (Kengatharan *et al.*, 1996).

Although the exact mechanism of action of these compounds needs confirmation, our studies suggest that LPS-induction of iNOS in rat aortic SMC may involve not only NF-κB, but also signalling mechanisms in which reactive oxygen species may contribute.

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315 P RYANODINE, CAFFEINE AND INTRACELLULAR FLASH PHOTOLYSIS OF DM-NITROPHEN AND DIAZO-2 MODULATE EXCITABILITY OF RAT CULTURED SENSORY NEURONES

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The whole cell patch clamp technique was used to measure the effects of altering the intracellular Ca^{2+} concentration on membrane potential and the properties of action potentials. Intracellular Ca^{2+} concentration of cultured dorsal root ganglion neurones was manipulated either by extracellular application of caffeine (1mM) or ryanodine (10μM) or by intracellular flash photolysis of caged Ca^{2+} (DM-nitrophen, 4mM with 2mM $CaCl_2$) or caged Ca^{2+} chelator (diazo-2, 2mM). Initial experiments were carried out to investigate the effects of removing 1.1-5mM EGTA from the KCl-based patch pipette solution on electrophysiological events. With EGTA present the membrane potential was stable, but when EGTA was excluded from the patch pipette solution the resting membrane potential depolarised by 10 ± 4mV (n=13, ± SEM) during the first 5 minutes of recording. Action potentials were evoked from a clamp potential of -75mV by 5ms depolarising current step commands. When EGTA was excluded from the patch pipette solution the action potential peak amplitude was reduced from 38 ± 2 mV (n=56) to 21 ± 4mV (n=12, $P<0.001$) and the action potential duration measured at 0mV was reduced from 2.7 ± 0.3ms (n=56) to 0.7 ± 0.1ms (n=12, $P<0.005$). Action potential after-depolarisations and after-hyperpolarisations were also significantly increased in amplitude and duration. Ryanodine or caffeine which are known to liberate Ca^{2+} from intracellular stores, evoked membrane potential depolarisations of 14 ± 2mV (n=9) and 21 ± 3mV (n=6) respectively, in 15 out of 26 neurones. Intracellular photorelease of about 20μM Ca^{2+} from DM-nitrophen also depolarised the resting membrane potential by 26 ± 6mV (n=8 out of 12 neurones). Caffeine and intracellular flash photolysis of DM-nitrophen also suppressed multiple firing evoked by 0.5-1s depolarising current step commands and significantly increased action potential duration and decreased action potential peak amplitude. It was apparent that caffeine and ryanodine failed to produce changes in membrane potential in some neurones. This same group of cultured DRG neurones also did not respond to intracellular photorelease of Ca^{2+}, suggesting that they did not express a functional population of Ca^{2+}-activated ion channels. Intracellular photolysis of diazo-2 to yield 200 to 800μM of Ca^{2+} chelator evoked hyperpolarising responses in cells which had been depolarised by raising intracellular Ca^{2+} with caffeine or ryanodine or by activating 20-50 action potentials at 2Hz. Photorelease of diazo-2 had no effect on neurones with hyperpolarised resting membrane potentials or on neurones which were depolarised to -35mV by injection of depolarising current. We conclude that a rise in intracellular Ca^{2+} activates Ca^{2+}-dependent conductances which result in depolarisation of the membrane potential and that intracellular photolysis of diazo-2 can chelate Ca^{2+} and reverse this effect. Attenuation of action potentials by Ca^{2+} may occur as a result of Ca^{2+}-induced voltage-activated Ca^{2+} channel inactivation.

316 P EFFECTS OF THE CANNABINOID RECEPTOR AGONIST, WIN 55,212-2, ON FAST SYNAPTIC TRANSMISSION IN MYENTERIC PLEXUS NEURONES OF THE GUINEA-PIG ILEUM

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Cannabinoids inhibit electrically-induced contractions of the guinea-pig myenteric plexus-longitudinal muscle preparation by activation of cannabinoid CB1 receptors (Pertwee *et al.*, 1996). To determine the mechanisms of this neuromodulatory effect, the actions of the cannabinoid receptor agonist, WIN 55,212-2 [(+)-WIN] (Pertwee *et al.*, 1996), its inactive enantiomer (–)-WIN and an antagonist selective for cannabinoid CB1 receptors, SR141716A (Pertwee *et al.*, 1996), were examined by means of conventional intracellular recording techniques.

Strips of myenteric plexus-longitudinal muscle dissected from the ileum of adult male Dunkin-Hartley guinea-pigs were pinned to the base of a recording chamber. The electrical properties of 60 neurones were recorded with intracellular glass electrodes (74-130 MΩ) filled with 2M KCl. Synaptic responses were elicited with micropipettes (10 μm dia.) filled with modified Krebs solution and placed on the surface of the ganglion or on interconnecting strands. The drugs used were mixed with two parts of Tween 80 by weight, dispersed in saline. They were applied by superfusion and protected from the direct light of the microscope. Mean values (± s.e.mean) have been compared by analysis of variance followed by Newman-Keul's test (ANOVA) or by Student's paired *t*-test ($P > \text{or} < 0.05$).

In the presence of (+)-WIN, fast excitatory postsynaptic potential (fEPSP) amplitudes fell significantly below their pre-drug values. The decreases were 18 ± 10% at 1 nM in 8/10 neurones, 46 ± 4% at 100 nM in 4/5 neurones and 46 ± 21% at 1000 nM in 4/5 neurones ($P < 0.05$; ANOVA). The depressant effects of (+)-WIN (1, 100 and 1000 nM) on fEPSP amplitude, expressed as the area above the amplitude-time curve (mV.s), were 5,420 ± 1,650 (n=5), 14,069 ± 4,774 (n=5) and 14,145 ± 5,828 (n=4), respectively, and were all significantly different from the Tween control value (1,047 ± 2,423, n=8; ANOVA).

In 3 neurones, (–)-WIN (100 nM) did not produce a reduction in fEPSP amplitude (11 ± 1 mV), whereas subsequent application of (+)-WIN to the same neurones produced a significant decrease (8 ± 2 mV) compared with the control response (12 ± 2 mV) (ANOVA).

In 11 neurones the effects of (+)-WIN (100 nM) on fEPSPs and on depolarisations induced by acetylcholine (ACh) were compared. In 5/11 neurones, 100 nM (+)-WIN reduced the amplitudes of fEPSP and ACh-induced depolarisation to 54 ± 11% and 63 ± 10% of the pre-drug values respectively, there being no significant difference between these values (*t*-test). In 3 neurones (+)-WIN had no detectable effect on fEPSPs or on ACh-induced depolarisation and in 3 other neurones, (+)-WIN induced a reduction only in the amplitude of fEPSPs (53 ± 15%; $P < 0.05$; *t*-test).

In some neurones, SR141716A (1 μM) antagonised the inhibitory effect of (+)-WIN (100 nM) on fEPSP amplitude (3/8 neurones) and, in a different group of neurones, ACh-induced depolarisations (5/12 neurones). However, in 6/8 neurones, SR141716A (1 μM) by itself inhibited fEPSP amplitude by 38 ± 10% ($P < 0.05$; *t*-test).

It is concluded that WIN 55,212-2 acts stereospecifically to inhibit fast synaptic transmission in guinea-pig myenteric neurones, acting presynaptically in some neurones and postsynaptically in others. Certain neurones seem to be devoid of receptors for this cannabinoid receptor agonist.

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317 P ANTAGONIST INHIBITION CURVES, DISSOCIATION CONSTANTS AND THE DEGREE OF AGONIST STIMULATION DURING PHYSIOLOGICAL RELEASE

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The inhibition of the effect of an agonist by a competitive antagonist can be calculated by combining the Gaddum-Schild equation:

$$DR = 1 + [B]/K_i \quad \ldots\ldots\ldots\ldots 1$$

with the relation between fractional inhibition, Q, and dose-ratio (Barlow, 1995):

$$Q = \frac{(DR^P - 1)}{DR^P + ([A]/[A_{50}])^P} \quad \ldots\ldots\ldots\ldots 2$$

where DR is the dose-ratio, [B] the concentration of antagonist, K_i its dissociation constant, P is the exponent of the agonist concentration- response curve and A_{50} is the concentration producing a half-maximum response. The ratio $[A]/[A_{50}]$ has been called the degree of agonist stimulation. An approximation for the relation between Q and [B] is given by:

$$Q = \frac{[B]^{P'}}{[B]^{P'} + IC_{50}^{P'}} \quad \ldots\ldots\ldots\ldots 3$$

where P' is an exponent and IC_{50} produces 50% inhibition. This can be used when the antagonist blocks the effects of nervous or hormonal stimulation and from values of P' and IC_{50} it should be possible to deduce the degree of agonist stimulation produced. When it is small, P' should be 1 and rise to P, the value for the agonist, when it is large. The value of IC_{50} is related to K_i by the equation:

$$IC_{50}/K_i = [2+([A]/[A_{50}])^P]^{1/P} - 1 \quad \ldots 4$$

so with high agonist stimulation, $IC_{50} > K_i$ but it can be less than K_i when the degree of agonist stimulation is low and the agonist exponent is large (P>1).

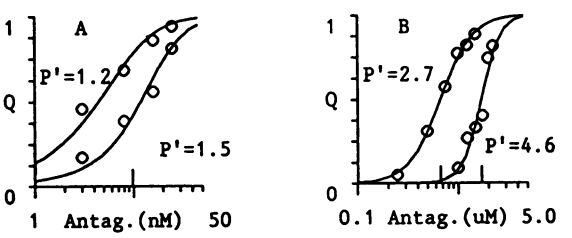

Figure 1 Inhibition (Q) vs antagonist conc.
A. Guinea-pig ileum: diphenyl- acetoxydimethyl ethyl- ammonium bromide and responses to carbachol ($[A]/[A_{50}]$=1.0 and 2.0: P=3.1); the lines show a least- squares fit to the combination of equations 1 and 2 and the estimate of K_i.
B. Rat phrenic nerve-diaphragm: (+) tubocurarine vs nervous stimulation (0.3Hz and 0.2Hz). The lines show a least- squares fit to equation 3.

Experiments with carbachol and the competitive antagonist diphenyl- acetoxyethyl dimethyl ethyl-ammonium bromide (Abramson et al., 1969) on isolated guinea-pig ileum in Krebs solution at 37°C show the expected relations between Q and the degree of agonist stimulation (Figure 1A). The results justify the use of IC_{50} and P' values to assess the degree of agonist stimulation during physiological release, such as inhibition curves obtained with (+)tubocurarine chloride on the rat phrenic nerve-diaphragm preparation (Figure 1B).

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318 P CONSERVATION OF ENDOTHELIAL CELL RESPONSE IN RAT MESENTERIC ARTERY STORED IN PHYSIOLOGICAL SALT SOLUTION (PSS) AT 4° C

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It is generally accepted that vascular tissue can be stored overnight in PSS at 4°C without loss of viability (Flanders et al.1996) but it is not known whether vessels will remain viable if stored for longer periods. The aim of this investigation was to determine whether rat mesenteric arteries show altered responsiveness to endothelium-dependent and -independent vasodilators, and vasoconstrictors when stored in PSS at 4°C for up to 4 days.

Third order mesenteric arteries (mean internal diameter 250±7.4μm, n=72) were dissected from the mesenteric bed of male Cob-Wistar rats (wt 150-250g). One was used immediately whilst the others were stored separately in PSS at 4°C for up to 4 days. The vessels were mounted as ring preparations on a small vessel myograph, capable of measuring isometric tension, and equilibrated at their optimum resting force ($0.9L_{100}$;Mulvany&Halpern,1977). Concentration response curves to acetylcholine (ACh; 10^{-9}-$3x10^{-5}$M), SIN-1 (10^{-9}-10^{-4}M), potassium chloride (KCl; 2.5-140mM) and endothelin-1 (ET-1; 10^{-11}-$3x10^{-7}$M) were obtained. Vasodilator responses were assessed by adding ACh and SIN-1 to vessels preconstricted with a sub-maximal concentration (3μM) of NA.

All the arteries used in this investigation relaxed in a concentration-dependent manner in response to ACh and SIN-1. Vessel storage for up to 3 days resulted in no change in sensitivity to ACh or SIN-1. However, there was a significant increase in sensitivity to ACh when -$LogEC_{50}$ values for vessels analysed on day 4 were compared with those analysed on days 1 and 2. An increase in sensitivity to SIN-1 was also observed when -$LogEC_{50}$ values for vessels analysed on day 4 were compared with those analysed on day 2. There was no change in maximum relaxant response to the vasodilators during storage. The arteries produced strong contractions in response to each of the vasoconstrictors, the magnitude and sensitivity of these responses were unaffected by storage.

These results indicate that small resistance arteries remain viable if stored in PSS at 4°C for up to 4 days, with no loss in endothelial cell function. The increase in sensitivity observed with both ACh (endothelium-dependent) and SIN-1 (endothelium-independent) on day 4 suggests an increase in smooth muscle cell sensitivity to nitric oxide (NO) and not an increased production of NO from endothelial cells. This suggests that vessels may be stored for up to 3 days for analysis of functional response; after this time sensitivity may be altered.

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Table 1. Maximum response to vasoconstrictors (mN/mm) and vasodilators (% reversal of tone) and -$LogEC_{50}$. values.

	Maximum response					-$LogEC_{50}$				
	Day 0 (7-9)	Day 1 (10)	Day 2 (8-10)	Day 3 (8-10)	Day 4 (7)	Day 0 (7-9)	Day 1 (10)	Day 2 (8-10)	Day 3 (8-10)	Day 4 (7)
KCl	1.32±0.07	1.44±0.18	1.79±0.31	1.5±0.21	1.79±0.36	1.60±0.04	1.64±0.01	1.62±0.04	1.61±0.04	1.54±0.05
ET-1	2.70±0.48	3.16±0.27	2.84±0.44	2.96±0.41	2.36±0.33	8.39±0.14	8.43±0.07	8.44±0.08	8.44±0.13	8.4±0.22
ACh	92.27±3.15	94.28±1.96	96.06±2.79	90.97±4.82	100.51±0.51	7.42±0.13	7.26±0.06	7.27±0.16	7.41±0.12	7.86±0.11*
SIN-1	83.15±7.08	90.77±3.48	93.70±3.08	96.21±1.62	95.89±2.77	4.91±0.10	5.13±0.18	4.86±0.11	5.07±0.10	5.43±0.10*

Results are given as mean±s.e.mean, n numbers in brackets. *P<0.05 when compared with -logEC_{50} value for days 1&2 (ACh) and day 2 (SIN-1), using oneway analysis of variance with a Tukey multiple range test.

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319 P INDUCTION OF ANGIOTENSIN II (AII)-MEDIATED CONTRACTION OF HEPATIC ARTERIES *IN VITRO*

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Although a potent vasoconstrictor *in vivo*, AII elicits only small, variable contractions in mesenteric and hepatic arteries *in vitro* (Dunn *et al.*, 1994; Hadoke *et al.*, 1995). This may be due to the relative hyperpolarisation of vessels in isometric systems, as responses to AII can be induced in rat mesenteric arteries following sub-maximal contraction with KCl (Dunn *et al.*, 1994). This study aimed to determine whether AII-mediated contractions could be induced in hepatic arteries *in vitro* following partial constriction with KCl.

Hepatic arteries from recipients (cirrhotic; 4M, 9F, 47.2±3.8 years), and donors (non-cirrhotic; 2M, 4F, 29.8±7.9 years), were obtained at hepatic transplantation. Porcine hepatic arteries (6M, aged 6-9 months) were obtained from an abattoir. Two rings (2mm in length) from each artery were mounted in parallel organ baths containing Krebs' solution at 37°C, perfused with 95%O_2: 5% CO_2 and equilibrated at a resting force of 4g. KCl (2.5-30mM) was added cumulatively to one ring (primed) until a small, stable contraction was produced, whilst the other ring (control) was not pre-contracted. Cumulative concentration-response curves (CCRCs) were then obtained to AII (10^{-10}-$3x10^{-6}$M) in the continuing presence of KCl. The vessels were washed, equilibrated, and CCRCs to KCl (2.5-120mM) were obtained. Finally, acetylcholine (ACh;10^{-5}M) and SIN-1 (10^{-5}M; Feelisch, 1991) were added to vessels after constriction with noradrenaline (10^{-5}M).

Control porcine hepatic arteries did not contract in response to AII, whilst control human hepatic arteries gave only small, concentration-dependent contractions (Table 1). Priming concentrations of KCl produced relatively small contractions (< 40% KCl max.). In primed vessels AII produced sustained, concentration-dependent contractions significantly larger than controls. The sum of the contractions produced by AII and KCl in the primed vessels was similar to or smaller than the maximum contraction to 120mM KCl. Sensitivity and magnitude of contraction to KCl were similar in primed and control vessels. All arteries were relaxed by SIN-1 but not by ACh (Table 1).

In conclusion, priming with KCl enables AII to induce contractions in denuded hepatic arteries which otherwise respond poorly to this agonist. This suggests that the action of AII may be affected by the depolarisation state of arteries investigated *in vitro*.

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Table 1. Maximum contraction and -logEC_{50} responses to AII and KCl and maximum relaxation (% relaxation) responses to ACh and SIN-1.

		Porcine Hepatic Artery (n=6)		Donor Hepatic Artery		Recipient Hepatic Artery	
		Primed	Control	Primed	Control	Primed	Control
AII	Max. Contn. (g)	3.00±1.27	0.08±0.04*	7.28±2.28 (5)	1.36±0.57 (6)*	4.26±0.57 (13)	1.19±0.24 (13)*
	-$LogEC_{50}$	8.64±0.15	------[a]	7.58±0.21 (5)	7.14±0.34 (5)[a]	8.11±0.20 (13)	7.70±0.15 (10)[a]
KCl	Max. Contn. (g)	10.53±1.44	7.42±1.61	10.61±2.77 (5)	8.42±2.36 (6)	9.00±0.73 (13)	8.38±0.96 (13)
	-$LogEC_{50}$	1.63±0.03	1.52±0.02	1.5±0.09 (5)	1.45±0.04 (6)	1.54±0.05 (13)	1.60±0.06 (13)
	Pre-contn. (g).	3.16±0.60	------	3.89±1.22 (5)	------	3.25±0.43 (13)	------
ACh	Max.Relax. (%)	------	-47.9±12.3	24.0±18.2 (4)	13.2±15.6 (5)	7.6±6.6 (9)	-4.3±4.9 (10)
SIN-1	Max.Relax. (%)	------	88.2±5.9	105.6±8.4 (4)	94.6±7.0 (5)	91.1+2.5 (10)	79.1±7.8 (11)

Results are mean ± s.e.mean (n), negative relaxation values represent contraction. *P<0.01 compared with primed vessels using Mann-Whitney U or Student's two-sample t-test as appropriate. [a] EC_{50} values were not calculated if AII-induced contraction was < 5% that produced by 120mM KCl.

320 P MODULATION OF PHOSPHODIESTERASE ACTIVITY IN PULMONARY ARTERIES FROM CHRONIC HYPOXIC RATS

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Hypoxia-induced pulmonary vasoconstriction is a basic reflex that occurs in all mammals. Increased vascular tone associated with changes in second messenger interactions involving cyclic nucleotides is thought to be responsible for increased vasoconstriction in pulmonary hypertension (PHT). Previous studies have characterised the role of cyclic adenosine- and guanosine monophosphate (cAMP, cGMP) in regulating blood vessel tone and we have investigated the intracellular (i.c.) levels of these second messengers, as well as the activity of phosphodiesterase (PDE) enzymes which catalyse their hydrolysis in specific regions of normal and chronic hypoxic rat pulmonary circulation. Rats were maintained at 550mbar (10% O_2) for 2 weeks. Controls were maintained in room air. PDE activity was determined by a two-step procedure as described by Marchmont & Houslay (1980). Results show that cAMP levels in hypoxic rats were in general decreased in larger pulmonary vessels, yet increased in smaller intrapulmonary vessels compared with control rats. Analysis of cAMP and cGMP PDE activity showed increased activity in larger vessels from hypoxic rats, with little difference in resistance vessels.

We found that total cAMP activity was decreased in the first branch and increased in intrapulmonary branches from hypoxic rats. Underlying these changes (table 1) was an increase (1st branch-+62pmol/min/mg, intrapulmonary-+27) in cilostamide-sensitive PDE activity, whilst an increase (1st branch-+25) in cGMP stimulated cAMP PDE activity was also observed in the 1st branch. Total cGMP PDE activity was increased in main, 1st and intrapulmonary branches from hypoxic rats. Associated with this in the 1st branch was an increase in type II cGMP PDE activity (+54). In all three cases there also appeared to be an increase in cilostamide-sensitive cGMP PDE activity induced by hypoxia. The results suggest that modifications occur in i.c. cyclic nucleotide concentrations in pulmonary arteries in the chronic hypoxic rat and these changes may regulate the development of PHT. Changes in cyclic nucleotide levels largely correspond to alterations in PDE activity. Regulating these changes may ultimately prove to be of therapeutic value.

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Table 1: cAMP and (cGMP) PDE activity expressed as pmol/min/mg protein. Results are shown as mean ± se, cAMP n=8, cGMP n=4. *p<0.05, **p<0.01.

Artery	Main	1st branch	Intrapulmonary	Resistance
Control	74±4(60±2)	104±4(71±10)	60±8(42±9)	55±6(37±5)
Hypoxic	91±11(88±7)**	164±24*(113±6)*	95±11*(93±11)*	58±3(35±6)

321 P REVERSAL OF THE BENEFICIAL METABOLIC EFFECTS OF ADENOSINE IN ISOLATED WORKING RAT HEARTS: ROLE OF ENDOGENOUS CATECHOLAMINES

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Adenosine (Ado), by improving the coupling of glycolysis to glucose oxidation, inhibits proton production arising from myocardial glucose metabolism. This action enhances the recovery of mechanical function of hearts during reperfusion following severe global ischaemia (Finegan *et al.*, 1993). However, if hearts are initially stressed by transient ischaemia, the effects of Ado on glucose metabolism are reversed and Ado then increases glycolysis and proton production and worsens the recovery of mechanical function following severe ischaemia (Finegan *et al.*, 1996). This study investigated whether adrenoceptor activation contributes to the reversal of the effect of Ado on glucose metabolism.

Hearts were removed from pentobarbitone-anaesthetized rats, and perfused in the paced (5 Hz) working mode at 37°C with Krebs' solution containing 2.5 mM Ca^{2+}, 1.2 mM palmitate, 11 mM [^{3}H]glucose and [^{14}C]glucose and 100 $\mu U.ml^{-1}$ insulin. Proton production ($\mu mol.min^{-1}.g^{-1}$ dry wt) arising from glucose metabolism, determined from the directly measured rates of glycolysis and glucose oxidation, and left ventricular minute work (LV work) were measured during aerobic perfusion in control hearts and in hearts stressed by two periods of transient global ischaemia (between 15 and 25 min and 30 and 40 min of perfusion) (Finegan *et al.*, 1996).

After 45 min of aerobic perfusion, LV work (joules) was similar in control (1.20±0.10, n=9) and stressed (1.10±0.04, n=9) hearts and remained stable throughout the remainder of the 80-min perfusion period. The presence of Ado (500 μM) during the 45 to 80 min treatment period had no effect on LV work in either control or stressed hearts. In control hearts, Ado inhibited proton production from 6.6±0.7 (n=6) to 2.4±0.3 (n=9, $P < 0.05$), whereas in stressed hearts, Ado stimulated proton production from 5.1±1.2 (n=9) to 9.4±1.4 (n=7, $P < 0.05$). In stressed hearts perfused with phentolamine alone (1μM) or in combination with propranolol (0.6 μM), proton production rates (3.5±0.6, n=7 and 2.8±0.4, n=7) were similar to untreated stressed hearts. Both of these treatments prevented the Ado-induced stimulation of proton production; Ado then depressed the rate of proton production to values (1.9 ±0.4, n=7 and 1.1±0.2, n=7) similar to those measured in Ado-treated control hearts.

Thus, the Ado-induced stimulation of proton production in stressed hearts, that is associated with an impaired recovery of mechanical function during reperfusion of the post-ischaemic working heart, may depend upon α-adrenoceptor activation, possibly in response to the transient ischaemia-induced release of endogenous noradrenaline.

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322 P NON-ADRENOCEPTOR BINDING OF [^{3}H]ATIPAMEZOLE IN RAT KIDNEY

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Atipamezole [ATI;4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole] was first introduced as a potent and specific α_2-adrenoceptor antagonist (Virtanen et al., 1989), but [^{3}H]ATI also identifies a population of binding sites in rat lung distinct from both classical α_2-adrenoceptors and previously described non-adrenergic imidazoli(di)ne binding sites (Sjöholm et al., 1992). Imidazoline binding sites, or I-receptors, in the central nervous system and in the kidney may have a role in mediating the hypotensive actions of imidazoline and oxazoline drugs, such as clonidine and rilmenidine. The purpose of the present study was to characterize [^{3}H]ATI binding sites in rat kidney by receptor autoradiography and to determine whether they are pharmacologically identical with the previously described binding sites for [^{3}H]*para*-aminoclonidine (PAC) and [^{3}H]idazoxan (MacKinnon et al., 1992).

Adult male Sprague-Dawley rats were killed by decapitation and their kidneys were removed. Frozen 14 µm sections were cut and thaw-mounted onto gelatinized slides. Three kidneys from different animals were used in each experiment. The sections were incubated for 60 min at room temperature in 50 mM K^+-phosphate buffer (pH 7.4) with 0.125 to 16 nM [^{3}H]ATI, either with [^{3}H]ATI alone to determine total binding, or in the presence of unlabelled ATI to determine specific binding, or in the presence of the α_2-adrenoceptor antagonist RX821002 to preclude binding to α_2-adrenoceptors. Competition experiments were performed in the presence of 5 or 6 concentrations of the competitors.

Binding of [^{3}H]ATI to non-adrenergic binding sites was saturable and of high affinity in renal cortex and in the outer stripe of the outer medulla (K_d 2.58 and 1.40 nM, B_{max} 300 and 550 fmol/mg). No specific [^{3}H]ATI binding was detected in inner regions of the medulla. In renal cortex, [^{3}H]ATI binding was displaced by detomidine with an IC_{50} of 0.18 µM. MPV-624 (1-[2-(2,6-dimethylphenyl)ethyl] imidazole) had intermediate affinity (1.0 µM) and PAC and idazoxan had low affinity (2.6 µM and 20 µM). [^{3}H]ATI binding in the outer stripe of the outer medulla appeared to differ from the renal cortex: Detomidine and MPV-624 were less potent (IC_{50} 0.70 and 12 µM) than in cortex, and idazoxan and PAC were unable to displace the radioligand. The putative endogenous imidazoline receptor ligand, agmatine (Li et al., 1994), did not displace [^{3}H]ATI.

We conclude that [^{3}H]ATI identifies two populations of binding sites in rat kidney, both of which are distinctly different from the previously described high-affinity binding sites of [^{3}H]PAC and [^{3}H]idazoxan.

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323 P MUSCULO-ELASTIC STRUCTURE AT ORIGIN OF PULMONARY SUPERNUMERARY ARTERY RESEMBLES A BAFFLE VALVE

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The pulmonary circulation consists of two populations of arteries; conventional arteries (CA) which divide with and accompany the airway and supernumerary arteries (SA) which leave the CA at 90° unaccompanied by an airway. SA are smaller and more muscular than the CA and are also reported to have a muscular sphincter at their origin from the CA (Elliot & Reid, 1965). SA are also reported to have a dense innervation at their origin (Fillenz, 1972). Pulmonary angiograms of normal human lungs have shown that SA are unperfused under resting conditions. We have hypothesised that the SA function as recruitment vessels and play a part in the reduction in vascular resistance which accompanies increased cardiac output and that this may be achieved by regulation of the SA sphincter region. Pharmacological differences between CA and SA indicates differential regulation of these vessels (Bunton *et al.*, 1995). The present study was aimed at gleaning further understanding of the anatomical structure at the origin of SA and their innervation.

Bovine lungs were obtained from a local abattoir within 20 minutes of slaughter and transported to the laboratory in ice-cold Krebs' solution. Segments of either CA (diameter 4-5mm) or SA (diameter 0.5-1mm) or the SA incorporating the sphincter region were removed and fixed in formalin for serial sectioning. The elastin content of the tissues was assessed using van Gieson stain and the presence of smooth muscle was indicated using haematoxylin and eosin staining. The presence of nerves was indicated using the Falck-Hillarp fluorescence technique.

The results support the existence of a musculoelastic structure (MES) at the origin of the SA although they show clearly that this structure is not a true sphincter. The structure consists of a "V" shaped band of muscle on the inner surface of the CA with the open end facing the direction of blood flow. The wall of the CA between the legs of the "V"-shape structure becomes very thin and forms a distinct channel that increases in depth towards the SA and eventually becomes continuous with the wall of the SA. The structure has the appearance of a funnel for the SA except that the entrance to the SA appears to be occluded by the structure. The distal end of the structure has a dense elastin content and there is a high degree of folding at this region and immediately under this structure in the distal wall of the SA. In these unpressurised vessels this orientation of the MES appears to be due to elastic recoil and consequently active contraction of the MES would pull this structure and the distal part of the SA upwards and forwards into the direction of blood flow and would thus encourage blood flow into SA. This may suggest the MES acts as a baffle valve directing the flow of blood into the SA.

Fluorescent nerves were seen in the MES and in both the media and adventitia of CA and SA, in particular a region of the SA close to its origin is especially densely innervated. This suggests there may be active regulation of this region such as would be necessary to cause active contraction of the baffle region.

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324 P L-NAME EXPOSES A KETANSERIN-SENSITIVE 5-HT-INDUCED CONTRACTILE RESPONSE IN THE 1st BRANCH PULMONARY ARTERY OF THE RAT

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We have previously reported that 5-HT-induced contraction of the first branch pulmonary artery of the rat (RPA) is insensitive to the 5-HT_2 antagonist ritanserin but is inhibited by the α_1-adrenoceptor antagonist prazosin in a non-competitive manner (Brown & Shaw, 1995). From these initial studies we concluded that a non-5-HT_2 receptor mediates the contractile response to 5-HT. In contrast Uma *et al* (1987) found that this response was sensitive to the 5-HT_2 antagonist ketanserin. Lippton *et al* (1992), in feline pulmonary vasculature, have reported that the nitric oxide synthase inhibitor L-NAME enhances the sensitivity of the 5-HT_2 receptor mediated response suggesting a role for endogenous nitric oxide in the regulation of the 5-HT_2 receptor. We have therefore examined the effect of ketanserin on the contractile response to 5-HT in the RPA in the absence and presence of L-NAME. Since prazosin has biological activity other than blockade of α_1-adrenoceptors, we have also examined the effect of the highly selective α_1-adrenoceptor antagonist HV723 (Muramatsu *et al* 1990) on the 5-HT-induced contraction of the RPA.

Male Wistar rats (250 - 300 g) were killed by intraperitoneal injection of sodium pentobarbitone (100 mg Kg^{-1}). Artery rings (1.2 - 1.5 mg) from the first branch pulmonary artery were suspended on stainless steel hooks in Krebs' physiological salt solution (37°C) under a resting tension of 1 g and gassed with a mixture of O_2:CO_2 (95%/5% v/v). In these experiments the tissues were allowed to equilibrate for 1 hour before the addition of agonists. To examine the effect of ketanserin two concentration response curves (CRCs) to 5-HT were performed, the second in the presence of the antagonist. Untreated tissues acted as time controls. In experiments with L-NAME, 100μM was preincubated for 30 min before the addition of 5-HT. Results are expressed as the mean values ± s.e. mean. The significance of differences was determined using Student's *t*-test.

In the absence of L-NAME, 5-HT (0.1μM - 1mM) produced a concentration-dependent contractile response ($pD_2 = 5.12 \pm 0.13$, n = 12). Ketanserin produced a small rightward shift in the 5-HT concentration response curve ($pD_2 = 4.52 \pm 0.08$, $p<0.05$, n = 12). L-NAME produced a small increase in the sensitivity of the tissue to 5-HT ($pD_2 = 5.86 \pm 0.16$, n = 17, $p < 0.001$). In the presence of L-NAME, ketanserin produced a marked rightward shift in the concentration response curve for 5-HT ($pD_2 = 4.11 \pm 0.15$, n = 9, $p < 0.001$) giving a calculated pA_2 value of 8.74.
The selective α_1-adrenoceptor antagonist HV723 (10nM) did not affect the CRC to 5-HT.

These results suggest that in the absence of L-NAME the α_1-adrenoceptor does not play a significant role in the contractile response to 5-HT in the RPA. L-NAME appears to markedly increase the 5-HT_2 component of the contractile response to 5-HT in the RPA.

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325 P POTENTIATION OF ENDOTHELIN-1-EVOKED CONTRACTIONS BY ANGIOTENSIN II: THE EFFECTS OF MK886 IN BOVINE BRONCHI

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Previous work has demonstrated that contractions evoked by endothelin-1 are potentiated by angiotensin II (AII, Nally *et al.*, 1994). This potentiation was mimicked by leukotriene D_4 (LTD_4, Nally *et al.* 1996) and attenuated by nordihydroguaiaretic acid (NDGA, Nally *et al.*, 1995). This suggested a role for leukotrienes in AII-mediated potentiation of contractions evoked by endothelin-1. Although NDGA is routinely used as a non-specific inhibitor of 5-lipoxygenase, doubts have been raised regarding its specificity. For example, inhibition of cyclo-oxygenase, protein kinase C and ET_A- receptors has been proposed (Henry 1994). In this study, therefore, the effects of the selective leukotriene synthesis inhibitor, MK886, were examined. This compound specifically inhibits the 5-lipoxygenase activating protein (FLAP, Gillard *et al.*, 1989)).

Contractions of bovine bronchial rings were measured isometrically in vertical 10 ml organ baths. Cumulative concentration-response curves were constructed for contractions evoked by endothelin-1 (10^{-10}-$3x10^{-7}$M) in the presence and absence of threshold concentrations of AII (10^{-7}-10^{-6}M). Cumulative concentration-response curves were further constructed to endothelin -1 in the presence of MK886 (10^{-5}M) either alone or with AII (10^{-7}-10^{-6}M).

Endothelin-1-mediated concentration-response curves were significantly ($p<0.01$) potentiated by AII with an increased response at each endothelin-1 concentration (e.g. control contraction at endothelin-1 $3x10^{-8}$M, 37.71±11.0% compared with 79.7±14.3% in the presence of AII, $p<0.05$). Endothelin-1-mediated contractions were unaltered by the presence of MK886 alone. MK886 was able however to attenuate the potentiation effected by AII such that the curve was not significantly different from either the control or the plus AII curve (contraction at endothelin-1 $3x10^{-8}$M, 61.86±20 in the presence of AII and MK886.)

In bovine bronchi, endothelin-1 does not evoke contraction via the release of leukotrienes since MK886 had no effect in the response evoked by this peptide. In contrast to this, the potentiation of endothelin-1-evoked contractions by AII does involve leukotrienes since this effect was reduced by MK886. This is in agreement with results obtained with NDGA and provides further evidence for the involvement of leukotrienes in angiotensin II-potentiated endothelin-1-evoked bronchial contractions.

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326 P EFFECT OF SB209670 ON ENDOTHELIN RESPONSES IN ISOLATED PULMONARY RESISTANCE ARTERIES FROM HEART FAILURE RABBITS

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Pulmonary hypertension (PHT) is prevalent to chronic heart failure (CHF) and plasma endothelin-1 (ET-1) levels specifically correlate with the extent of PHT in these patients (Cody *et al*, 1992). We examined the ET receptor mediated responses in pulmonary resistance arteries (PRAs) from the rabbit coronary ligation model of heart failure (Denvir *et al*, 1996). Experimental or sham-operated animals were killed 8 weeks following the procedure. Small PRAs (I.D. ~150μm) were dissected out and mounted as ring preparations (2mm length) on a wire myograph (under ~125mg tension) in Krebs bubbled with 16% O_2/6% CO_2/ balance N_2. Cumulative concentration response curves (CCRCs) were constructed to ET-1 ($ET_{A/B}$-receptor agonist) or SXS6c (ET_B-receptor agonist) (1pM-0.3μM) either in the absence or presence of the non-selective $ET_{A/B}$ receptor antagonist SB209670.

The results are summarized in table 1. Both ET-1 and SX6c were potent vasoconstrictors of the PRAs however no difference in sensitivity was seen between the two groups. SXS6c was around 10 times more potent than ET-1. This is indicative of vasoconstriction being mediated by ET_B receptors, as has previously been shown in the larger pulmonary artery (PAs) of the rabbit (Fukadora *et al.*, 1994). SB209670 failed to antagonise the ET-1-evoked response in the ligated vessels and actually potentiated the response in the lower concentration range. However a significant antagonism was observed in the sham PRAs; this reduced sensitivity occured at the higher concentration range. SB209670 had a more pronounced effect on SXS6c- induced vasoconstriction evoking a concentration-dependent shift, as has previously been demonstrated in larger calibre rabbit PAs (Ohlstein, *et al*, 1994). This inhibitory effect was markedly greater in the sham compared to the ligated preparations. This study suggests alterations in endothelin-receptor mediated control of pulmonary vascular tone.

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Table 1. pEC_{50} values for ET-1 and SXS6c in the absence and presence of SB209670. Statistical difference from control,, Students unpaired t-test, *p<0.05, **p<0.01, ***p<0.001. Statistical difference from ET-1 in the same group, $^{+}$p<0.05, $^{++}$p<0.01. n/n=number of vessels/number of animals. Values are mean ± SEM.

	SHAM		LIGATED	
	pEC50	n/n	pEC50	n/n
ET-1				
control	8.1±0.3	9/7	7.9±0.2	12/7
+1μM SB209670	7.1±0.1**	7/6	7.9±0.3	6/6
SXS6c				
control	8.6±0.3^{++}	8/7	8.6±0.2^{+}	11/7
+0.1μMSB209670	8.2±0.1	7/6	8.1±0.3	6/6
+1μMSB209670	7.0±0.1***	6/5	7.8±0.2*	6/6

327 P PROTECTION OF ENDOTHELIUM-DEPENDENT RELAXATION IN NEWBORN RABBIT PULMONARY ARTERIES BY SUPEROXIDE DISMUTASE

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Recent studies have shown that the endothelium-derived relaxing factor nitric oxide (NO) is an important mediator in the developing pulmonary circulation (Shaul *et al* 1993; Morecroft & MacLean 1995). Superoxide anions ($O_2^{\cdot-}$) can destroy the observed vasodilation to NO (Rubanyi & Vanhoutte 1986). Here we examined ACh-induced relaxation in pulmonary conduit arteries (PCAs) from rabbits at different developmental ages and investigated the effect of superoxide dismutase on this relaxation in newborn rabbit PCAs.Foetal, neonatal and adult NZW rabbits were killed with sodium pentobarbitone. PCAs were dissected out and mounted as ring preparations in 5ml organ baths in Krebs (37°C) bubbled with 3% O_2/6% CO_2/ balance N_2 for the foetal vessels and 16% O_2 for all others. The PCAs were pre-contracted with a concentration of phenylephrine (PE) which produced 70% maximum contraction. Cumulative concentration response curves (CCRCs) to ACh (1nM-1μM) were obtained. In the 0-12h rabbit PCAs, the experiment was repeated in the presence of 50 U/ml superoxide dismutase (S.O.D).

The results are shown in Table 1. Contraction to PE was observed in PCAs from all age groups. ACh induced vasorelaxation in foetus, 4 day, and adult rabbit with the most pronounced relaxation seen at 4 days. At 0-12 hours, no ACh-induced relaxation was observed. When these vessels were pre-incubated with 50 U/ml S.O.D, however, ACh induced significant vasorelaxation with the maximum relaxation not significantly different from the other age groups. These observations suggest excessive $O_2^{\cdot-}$ in PCAs at 0-12 hours may prevent NO-induced vasorelaxation. High mortality in neonates with persistent pulmonary hypertension (PPHN) is seen at 0-3 days. The above results invite the possibility that manipulating the antioxidant vs superoxide anion system may be of therapeutic value in the treatment of PPHN by protecting endothelium dependent relaxations in newborn pulmonary arteries.

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Age	A. pEC_{50} (ACh)	n/n	B. Max. relaxation (% PE-contraction)
Foetus	7.2 ± 0.1***	6/6	71.2 ± 6.3
0-12 h	——	12/12	3.0 ± 3.0
+50 U/ml S.O.D	6.4 ± 0.2***	6/6	84.2 ± 11.2†
4 days	7.4 ± 0.2	7/7	105.4 ± 3.0**
Adult	7.7 ± 0.1	8/5	67.4 ± 6.6

Table 1. Changes in endothelium dependent relaxation with developmental age, and protection by superoxide dismutase n/n=number of vessels/number of animals. Statistical difference from a) adult response, One way ANOVA, *p<0.05, **p<0.01, ***p<0.001; b) control response †p< 0.05

328 P α1A-ADRENOCEPTOR STIMULATION INCREASES $^{86}RB^+$-INFLUX RATE IN RAT CARDIOMYOCYTES WHILE BOTH α1A- AND α1B-ADRENOCEPTOR STIMULATION INCREASE INOSITOL 1,4,5-TRISPHOSPHATE

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The aim of the study was to identify the receptor subtype(s) involved in the α_1-adrenoceptor mediated increase of $^{86}Rb^+$-influx rate in isolated ventricular cardiomyocytes from adult rat heart and to elucidate a possible role for inositol-1,4,5-trisphosphate (IP_3) as a mediator of this effect. The cells were isolated by treating perfused rat hearts with a combination of trypsin and collagenase as described (Viko et al., 1995) and kept overnight in a culture medium at 37°C and 95% O_2 + 5% CO_2. The rate of $^{86}Rb^+$-influx was determined by exposing the cell samples to $^{86}Rb^+$ for 15 min. The cells were separated from the medium by filtration. The IP_3 mass content of the cells was determined by a modified receptor radioligand binding assay (Chaliss et al. 1990).

The basal $^{86}Rb^+$-influx rate was 0.22±0.01 ml/g prot×min (mean±s.e.m., n=67). After 15 min α_1-adrenoceptor stimulation by 5×10^{-5} mol/l noradrenaline in the presence of β-adrenoceptor blocade (3×10^{-5} mol/l timolol) the $^{86}Rb^+$-influx rate was increased by 33±1 % (n=67). The α_{1A}-adrenoceptor subtype selective antagonist 5-methyl-urapidil (5-MU) inhibited this response with a pK_i=8.37±0.28 (n=6). 10^{-4} mol/l 5-MU eliminated the response (-3±11%, n=6). The increased $^{86}Rb^+$-influx rate was not affected by the α_{1B}-adrenoceptor subtype selective antagonist chloro-ethyl-clonidine (CEC) up to 10^{-5} mol/l. At this concentration the response was 91±12 % (n=5) of that in the absence of the blocker. The response was also not affected by the α_{1D}-adrenoceptor subtype selective antagonist BMY 7378 up to 10^{-5} mol/l. At this concentration the response was 95±3 % (n=6) of the response in the absence of the blocker.

The basal content of IP_3 was 1.75±0.17 pmol/mg prot (n=16). Stimulation by 10^{-5} mol/l noradrenaline for 2 min in the presence of 3×10^{-5} mol/l timolol increased the IP_3 content by 65±7 % (n=16). 10^{-4} mol/l 5-MU and 10^{-5} mol/l CEC reduced the response to 27±6 % (n=9) and 18±9 % (n=9), respectively. The combination of both antagonists or 3×10^{-6} mol/l prazosin alone reduced the response to -11±7 % (n=7) and -5±11% (n=6), respectively.

The results show that the α_1-adrenoceptor mediated increase of $^{86}Rb^+$-influx rate in isolated rat cardiomyocytes is evoked solely through the α_{1A}-adrenoceptor subtype. The IP_3 increase, however, is evoked through both α_{1A}- and α_{1B} -adrenoceptor subtypes. Thus the increase of $^{86}Rb^+$-influx rate cannot easily be attributed to an increase of IP_3 content. If, however, stimulation of α_{1A}- and α_{1B}-adrenoceptor subtypes increases IP_3 in different subcellular compartments, the results may be compatible with a messenger function for IP_3 in increasing $^{86}Rb^+$-influx rate.

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329 P SIMILAR LOCATION OF MYOCARDIAL α1- AND β-ADRENOCEPTORS IN RELATION TO SYMPATHETIC NERVE ENDINGS IN RABBIT MYOCARDIUM

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When the α_1-adrenoceptors and ß-adrenoceptors in the heart are activated by the neurotransmitter noradrenaline, the relative functional importance of the two adrenoceptor populations will be influenced by their location relative to the nerve endings. In rat heart, α_1- and ß-adrenoceptors are located differently relative to sympathetic nerve endings supporting the dominant role of the ß-adrenoceptor system in the rat heart (Skomedal et al. 1988, Dybvik et al. 1995). As indirect evidence indicated a different situation in the rabbit heart (Skomedal et al. 1990), we studied the effect of blocking the neuronal uptake mechanisms upon the horizontal position of the dose-response curve for the inotropic response to separate α_1- and ß-adrenoceptor stimulation by noradrenaline also in rabbit heart.

The studies were performed in isolated, isometrically contracting rabbit papillary muscles (Skomedal et al. 1990). Inotropic responses were expressed as maximal development of tension = $dT/dt = T'_{max}$. Cumulative dose-response curves to noradrenaline were obtained either in combination with 10^{-6} mol/l of the ß-adrenoceptor blocker timolol (separate α_1-adrenoceptor stimulation) or with 10^{-7} mol/l of the α_1-adrenoceptor blocker prazosin (separate ß-adrenoceptor stimulation). The extraneuronal uptake blocker cortisol (3×10^{-5} mol/l) was present throughout all experiments. When present, 3×10^{-5} mol/l of the neuronal uptake blocker cocaine was used. All receptor and uptake blockers were added to the organ bath 45 minutes before noradrenaline.

The presence of cocaine shifted the dose-response curves to separate α_1-adrenoceptor stimulation and to separate ß-adrenoceptor stimulation to lower concentrations of noradrenaline by 1.50 ± 0.20 (from pD_2=5.59 ± 0.15 to pD_2=7.09 ± 0.13, P< 0.01, mean ± s.e.m., n=6-8) and by 1.37 ± 0.26 (from pD_2=5.19 ±0.14 to pD_2=6.56 ± 0.22, P<0.01, mean ± s.e.m., n=7-8) log units, respectively, corresponding to a potentiation of noradrenaline by about 32 and by about 23 times, respectively, compared to the absence of cocaine. Maximal inotropic response to α_1-adrenoceptor stimulation was 139.9 ± 4.9 % and 128.4 ± 1.9 % compared to control (= 100 %) in the absence and presence of cocaine, respectively (mean ± s.e.m., n=6-8). Maximal inotropic response to ß-adrenoceptor stimulation was 175.1 ± 14.7 % and 178.1 ± 11.6 % compared to control (= 100 %) in the absence and presence of cocaine, respectively (mean ± s.e.m., n=7-8).

Thus, in contrast to the rat heart, in rabbit heart both separate α_1-adrenoceptor stimulation and separate ß-adrenoceptor stimulation by noradrenaline were significantly potentiated by neuronal uptake blockade. These data thus indicated that in rabbit heart the myocardial α_1-adrenoceptor population and the ß-adrenoceptor population have a rather similar location relatively to the sympathetic nerve endings and that both adrenoceptor populations are located within the synaptic cleft (Dybvik et al. 1995).

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330 P INVOLVEMENT OF THE Na/K/Cl CO-TRANSPORTER AND K-CHANNELS IN THE α_1-ADRENOCEPTOR-MEDIATED INCREASE IN ^{86}Rb EFFLUX FROM THE RAT HEART

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The aim of the study was to study the translocation mechanisms involved in the α_1-adrenoceptor mediated increase in the efflux of the K^+ analogue $^{86}Rb^+$ from rat hearts. Hearts from male Wistar rats were isolated under ether anesthesia and perfused with a constant flow, at 31°C as previously described (Andersen *et al.* 1996). Timolol (1 µmol/l) was used to block β-adrenoceptors. After a loading period with $^{86}Rb^+$, washout started. In order to elucidate the translocation mechanisms involved, the effect of 50 µmol/l bumetanide (BUM, Na/K/2Cl cotransport inhibitor), 0.1-0.3 mmol/l 4-aminopyridin (4-AP, K^+ channel inhibitor), 10 µmol/l HOE 694 (Na^+/H^+ exchange inhibitor) and 3 µmol/l glibenclamide (K^+_{ATP} - channel inhibitor), were studied. Phenylephrine increased the $^{86}Rb^+$ efflux by 29 ± 1.6 % (mean ± SEM, n=36, p<0.0001). BUM reduced the increased efflux by 43 ± 15 % (n=10, p=0.01) and the BUM insensitive response was reduced by 55 ± 17 % (n=6, p<0.01 vs. BUM alone) when 4-AP was present in addition. The inhibitory effect of BUM was unaffected by HOE 694 (41 ± 8 % reduction, n=7, vs 36 ± 10 % reduction, n=10, in the presence of BUM without or with HOE 694, respectively, NS). The presence of HOE 694 or glibenclamide respectively, had no significant effect on the increased $^{86}Rb^+$ efflux (27 ± 2.0 % increase, n=6 in the presence of phenylephrine alone, 28 ± 2.7 %, n=6, and 25 ± 2.1 % increase, n=8, in the presence of phenylephrine and either HOE 694 or glibenclamide respectively). The BUM effect was eliminated in the presence of the selective α_{1A}-adrenoceptor antagonist 5-methylurapidil (8 ± 1.5 % increase, n=8, 8 ± 1.5 % increase, n=8, in the presence of 10 µmol/l 5-methylurapidil without or with 50 µmol/l BUM), but maintained after pretreatment with 10 µmol/l of the selective α_{1B}-adrenoceptor antagonist chloroethylclonidine (12 ± 2.3 %, n=5, and 6 ± 0.9 % increase, n=9, in chloroethylclonidine pretreated hearts without or with BUM respectively). **Conclusion:** α_1-adrenoceptor stimulation increases $^{86}Rb^+$ efflux in the rat heart via the Na/K/2Cl cotransporter and K^+ channels independently of activation of the Na^+/H^+ exchange mechanism. Activation of the cotransporter is mediated mainly through the α_{1A}-adrenoceptor subtype.

Andersen, G.Ø, Enger, M., Skomedal, T. & Osnes, J.-B. (1996) *Pharmacol. Toxicol.* 79, 169-176.

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331 P BOTH THE α_{1A}, -1B AND -1D SUB-TYPES ARE INVOLVED IN THE α_1 ADRENOCEPTOR-MEDIATED INCREASE IN ^{86}Rb EFFLUX FROM THE RAT HEART

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The aim of the study was to determine the involvement of the different receptor subtypes in the α_1-adrenoceptor mediated increase in the efflux of the K^+ analogue $^{86}Rb^+$ from rat hearts (Hanem *et al.* 1993). Hearts from male Wistar rats were isolated under ether anesthesia. The aorta was cannulated, and the hearts were spontaneously beating and perfused in a non recirculating system with a constant flow, at 31°C as previously described (Andersen *et al.* 1996). Timolol (1 µmol/l) was used to block β-adrenoceptors. After a loading period with $^{86}Rb^+$, washout started, $^{86}Rb^+$ efflux was measured during α_1-adrenoceptor stimulation (30 µmol/l phenylephrine) in the presence or absence of selective antagonists against the α_1-adrenoceptor subtypes.

Phenylephrine increased the $^{86}Rb^+$ efflux by 28 ± 1.3 % (mean ± SEM, n = 32, p < 0.0001). The increased efflux was reduced by 73 ± 12 % (n = 8), 49 ± 9 % (n = 11), and 33 ± 8 % (n = 5) in the presence of maximal concentrations of the selective α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor antagonists 5-methylurapidil (10 µmol/l), chloroethylclonidine (CEC, 10 µmol/l, 30 min pretreatment) and BMY 7378 (1 µmol/l), respectively (p < 0.001 in all three series). 5-methylurapidil inhibited the increased efflux with a pK_I = 7.3 (95 % CI: 7.7 - 7.1, n = 21) and almost eliminated the response at high concentrations (10 µmol/l, 1 ± 0.6 % increase, n=5, p < 0.0001) in hearts pretreated with CEC. The response in CEC pretreated hearts was also sensitive to the selective α_{1A}-adrenoceptor antagonists WB 4101 (1 µmol/l, 82 ± 7.5 % reduction, n=11, p<0.0001) and (+)niguldipine (1 µmol/l, 82 ± 6.5 % reduction, n=7, p<0.0001). BMY 7378 inhibited the response with a pK_I = 8.5 (95 % CI: 9.3 - 7.6, n = 31). **Conclusion:** The α_1-adrenoceptor mediated increase in $^{86}Rb^+$ efflux in the rat heart is sensitive to antagonists against all three known receptor subtypes. The relative contribution of each receptor subtype can not be determined exactly due to incomplete selectivity of the available antagonists, but an estimation of the relative contribution would be approximately 50:30:20 %, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtype, respectively.

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332 P MODIFICATION OF NITRIC OXIDE RESPONSE BY LIPOPROTEINS AND DEXAMETHASONE IN THE RAT AORTA

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Modification of basal nitric oxide (NO) release may contribute to changes in vascular reactivity (Yang *et al.*, 1996). Previous work (McPherson *et al.*, 1995) has shown that both oxidised (ox-LDL) and native (LDL) low density lipoproteins attenuate contractile responses to the NO synthase inhibitor L-NAME, suggesting a decrease in basal NO levels. Induction of inducible NO synthase (iNOS) is however, known to occur readily in the rat. The aim of this study was to ascertain how much of the observed responses to L-NAME were attributable to iNOS, and consequently whether LDL/ox-LDL act via iNOS or endothelial nitric oxide synthase (eNOS).

LDL was prepared via sequential density gradient ultracentrifugation from the freshly drawn blood of healthy, normolipidemic volunteers and oxidised with copper. Aortic rings 3-4mm wide were prepared from male Sprague Dawley rats (> 6 weeks). Rings were then immediately mounted under 1g tension for recording of isometric tension (-inc.), or incubated for 5 hours, at 37°C, 5% CO_2 95% air, in either vehicle or 500μg/ml human LDL/ox-LDL (+/- 0.5μM Dexamethasone (dex.)) (+inc.). All experiments were carried out in the presence (+E) and absence (-E) of endothelium. Concentration-response curves were constructed to phenylephrine (10^{-8} - $3x10^{-5}$M), and potassium chloride (10^{-2} - 10^{-1}M). Tissues were then contracted to EC_{20} phenylephrine and L-NAME (200μM) added at the plateau of phenylephrine contraction. The additional contraction obtained after L-NAME treatment was taken as a measurement of basal nitric oxide release. Statistical analysis was carried out using repeated measures ANOVA and Bonferroni paired multiple comparison tests.

On removal of the endothelium L-NAME responses were significantly reduced (as a % 10^{-1}M KCl; -inc.+E 77.45 +/-8.03, -inc.-E 21.09+/- 6.05*). Incubation resulted in an increase in the L-NAME response in endothelium intact tissue. This was blocked by the presence of dexamethasone (-inc.+E 77.45 +/- 8.03, +inc.+E 92.49 +/-13.42, +inc.+E+dex. 75.76 +/-9.74).
Induction was greater in tissues which were endothelium denuded (-inc.-E 21.09+/-6.05, +inc.-E 83.34+/-10.48*,+inc.-E+dex. 23.89+/-2.66). Dex/LDL reduced L-NAME responses to a similar degree as dexamethasone only (control 179.92+/-31.28, dex. 96.04+/-20.15, dex/LDL 93.28 +/-23.56). Ox-LDL showed a similar pattern with a trend towards lower responses when incubation included both ox-LDL and dex. compared with dex. alone (control 198.54 +/-27.08, dex. 133.52 +/-30.53, ox-LDL/dex. 101.72+/-22.10).

L-NAME responses observed in endothelium-intact vessels were mainly due to eNOS inhibition. On removal of the endothelium induction of iNOS increased substantially, and could be specifically inhibited by dexamethasone. LDL/ox-LDL appeared to have their main effect on iNOS (although ox-LDL may also have a small effect on eNOS).

*Highlights significant differences ($p<0.05$)

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333 P DELETION OF MOUSE GLUTATHIONE S-TRANSFERASE Pi FROM THE MOUSE GENOME BY HOMOLOGOUS RECOMBINATION

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Introduced by I.H. Stevenson

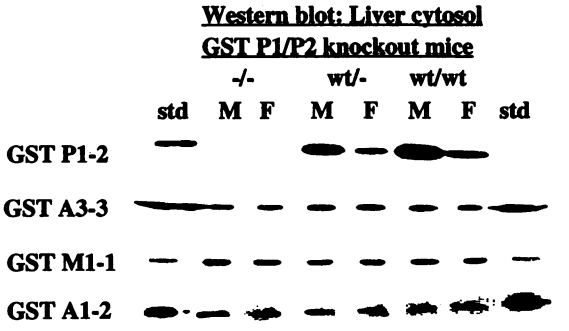
Glutathione S-transferases (GST, EC 2.5.1.18) are a family of dimeric enzymes involved in cellular detoxification (Beckett & Hayes, 1993). Cytosolic GST are grouped into five classes, alpha, mu, pi, sigma and theta, based on substrate specificity, immunological properties and amino acid sequence (Hayes & Pulford, 1995). Expression of GST Pi has been associated with malignant transformation and drug resistance (Sawaki et al., 1990; Black & Wolf, 1991). In the mouse, pi-class GST are the product of two tandem genes, GSTp-1 and GSTp-2, sharing 97% amino acid sequence identity. Both genes are expressed at higher levels in males than females; GSTp-1 is more abundant than GSTp-2 in both sexes.

We have designed targeting constructs to allow the deletion of one or both enzymes by homologous recombination in embryonic stem (ES) cells. We have employed a promotorless system, which replaces the C-terminal half of each gene with a cassette containing an internal ribosome entry site (IRES) element linked to a fusion protein, βGeo, comprising a reporter gene (*lacZ*) and a selectable marker (*neo*). This system leads to a dramatic reduction in the number of false positives, and enriches for correct targeting events. Following electroporation of the targeting constructs into ES cells, selection for neomycin resistance produced the following results on Southern blots with 3' and 5'-specific probes:

construct	total neo^r	total correct	%
p-1	25	16	64
p-1/p-2	27	11	41

Chimaeric mice were generated from each of these lines, and germline transmission confirmed by crossing into an MF1 strain. Heterozygote siblings were crossed to generate mice homozygous null for GSTp-1 or GSTp-1/p-2.

Mice nulled for GST p-1/p-2 appeared indistinguishable from their wild-type counterparts, apart from a complete lack of GST Pi mRNA or protein in all tissues tested. In addition,no detectable activity towards ethacrynic acid, a marker substrate for GST Pi, was found in hepatic cytosol from null mice, although activity toward chloro-dinitrobenzene, a general GST substrate, was unchanged in these animals as compared to their wild-type counterparts. As the figure below shows, this was not due to compensatory changes in other GST classes.



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334 P THE OPIOID ACTIVITY OF AKUAMMINE, AKUAMMICINE AND AKUAMMIDINE: ALKALOIDS EXTRACTED FROM *PICRALIMA NITIDA* (FAM. *APOCYNACEAE*)

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The seeds of *Picralima nitida* (family *Apocynaceae*) are a part of both traditional and contemporary medicine in Ghana where they are used as analgesics and antipyretics. Twelve distinct alkaloids have been isolated from *P. nitida*, and akuammine, the most abundant alkaloid, has been reported to possess analgesic activity comparable to morphine (Ansa-Asamoah & Ampofo, 1986). In this investigation, we have used isolated tissue bioassays and binding assays to determine the opioid activities of akuammine and the related alkaloids akuammidine and akuammicine (Boonchuay & Court, 1976 ; Hu *et al.*, 1989).

Hexane extracts of the dried, powdered seeds were applied to alumina columns in ethyl acetate. The alkaloids were isolated by elution in hexane:ethyl acetate (9:1); the identities of the alkaloids were confirmed by NMR. In bioassays, opioid activity was assessed by determining the effectiveness of the alkaloids in depressing the electrically-evoked, isometric contractions of the vas deferens of the mouse (μ-, δ- and κ-opioid receptors) and the guinea-pig myenteric plexus preparation (μ- and κ-opioid receptors). In the binding assays, the μ-sites were selectively labelled with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin (1 nM), the δ-sites with [^{3}H]-naltrindole (0.25 nM) and the κ-sites with [^{3}H]-CI-977 (0.25 nM); incubations were performed for 45 min at 25°C.

Akuammidine caused concentration-dependent inhibitions of the contractions in the mouse and guinea-pig preparations with IC_{50} values of 5.1 μM (range 2.8-7.0 n=6) and 6.8 μM (range 4.5-9.5 , n=6), respectively. These inhibitions were due to an action at μ-opioid receptors since they were antagonised by naloxone (pK_B = 8.9 ± 0.1, n=4) and the selective μ-opioid receptor antagonist CTOP (apparent pK_B = 7.4). Binding assays in guinea-pig brain confirmed the μ-opioid activity of akuammidine as it bound prefentially to μ-sites with a K_i value of 1.7 ± 0.5 μM (n=5). Although akuammine also showed highest affinity for μ-opioid sites (Ki = 0.48 ± 0.09 μM) it did not inhibit the contractions of either bioassay preparation. Rather, this compound was an opioid antagonist with an apparent pK_B of 5.6 against the selective μ-opioid agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin in both tissues. In contrast to the other alkaloids, akuammicine had highest affinity at κ-binding sites; the affinity for κ-sites (K_i = 0.19 ± 0.04 μM, n=5) was more than ten-fold higher than that for either μ- or δ-opioid sites. Akuammicine inhibited the contractions of the myenteric plexus preparation with an IC_{50} of 8.14 μM (range 5.5-14.5, n=6). This agonist effect of akuammicine was at κ-opioid receptors since it was antagonised by the selective κ-opioid antagonist nor-binaltorphimine (apparent pK_B = 9.6).

These data show that alkaloids from *P. nitida* possess varying degrees of agonist and antagonist activity at opioid receptors. Moreover, the reported analgesic action of akuammine would appear not to be the result of opioid agonist activity.

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335 P FUNCTIONAL CO-EXPRESSION OF CYP2D6 AND CYP3A4 WITH RAT NADPH-CYTOCHROME P450 REDUCTASE IN *S. CEREVISIAE*

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In recent years heterologous systems, like bacteria, yeasts and mammalian cell lines, have been developed for the expression of human drug metabolising enzymes. Yeasts offer the advantage of rather high expression levels, as well as inexpensive and easy growth conditions (Pompon *et al.*). CYP2D6 and CYP3A4 cDNAs were cloned into the episomal vector pMA91 (Sambrook *et al.*) and expressed in *S. cerevisiae*. The content of recombinant protein in yeast microsomes was 0.17 and 0.18 nmol P450/mg microsomal protein, respectively. The yield of spectrally active (Omura *et al.*) heterologous CYP2D6 and CYP3A4, in whole yeast cells, was 7 and 4.8 nmol P450/litre culture, respectively. However, these values were increased significantly by growing the yeast cells in rich YPD growth medium (65.1 nmol P450/l for CYP2D6 and 16.5 nmol P450/l for CYP3A4). Functional coexpression of both CYP2D6 and CYP3A4 with rat NADPH-cytochrome P450 reductase was also achieved. The turnover numbers of the recombinant CYP2D6 for bufuralol 1-hydroxylation, metoprolol α-hydroxylation and O-demethylation were 5 min^{-1}, 0.72 min^{-1} and 1.8 min^{-1} respectively. Activity of CYP3A4 towards testosterone was also measured (turnover number of 0.55 min^{-1}). It is envisaged that these cell lines will prove to be valuable for drug metabolism studies.

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336 P THE INFLUENCE OF HEPATIC METABOLISM ON THE NEUROTOXICITY OF ARTEMISININ DERIVATIVES ON CULTURED NB2a NEUROBLASTOMA CELLS

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Artemisinin and its derivatives are becomingly increasingly important as antimalarial drugs against multi-drug-resistant forms of *P. falciparum*. Despite widespread and apparently safe clinical use, there is evidence for neurotoxicity of artemether and arteether in animal studies (Brewer *et al.*, 1994). When tested for toxicity to neuronal and glial cells in culture, artemether (AEM) and arteether (AEE) show significantly less inhibition of neurite outgrowth from differentiated neuroblastoma NB2a cells than does the metabolite dihydroartemisinin (DHA) (Fishwick *et al.*, 1995). In this study we have investigated the effects of hepatic metabolism on the *in vitro* neurotoxicity of AEE and AEM.

NB2a cells were grown in 24-well plates. After 24 h, they were induced to differentiate and to generate neurites, by removal of serum from the culture medium and addition of 0.5 mM dibutyryl cyclic AMP. At the same time, artemisinin derivatives were added at a concentration approximately equivalent to their IC_{25}. In some experiments, incubations were carried out in the presence of [^{14}C]-artemether. To one half of the wells was added a micropore insert containing one of three separate preparations of male Wistar rat liver microsomes, plus NADP and an NADPH-generating system. After 24h, cells were fixed and stained. Neurite length (mean of 200 cells) was measured by microscopy linked to image analysis and expressed as a percentage of neurite length in the absence of drug. Medium was removed and the presence of [^{14}C]-artemether and metabolites was measured by reversed phase HPLC.

In the presence of active microsomes, the inhibition of neurite length produced by 68 μM AEM was significantly ($P<0.02$) increased from 16 ± 17 % to 96 ± 4 % and by 100 μM AEE from 38 ± 10 % to 92 ± 10 % (mean ± S.D., n=3). The enhanced inhibition was related to concentration of microsomal protein. Inhibition of 31 ± 6 % produced by 0.5 μM DHA was unaltered in the presence of microsomes. HPLC analysis of medium containing 68 μM AEM showed that incubation with microsomes produced an increase ($P<0.05$) in a peak of radioactivity co-eluting with DHA from 2 ± 0 % to 8 ± 1 % (n=3). This conversion of AEM to DHA was sufficient to explain the increased inhibition of neurite outgrowth.

We conclude that hepatic metabolism of AEM and AEE leads to increased toxicity due, at least for AEM, to conversion to DHA, as is known to occur *in vivo* (Leskovac & Theoharides, 1991). This may contribute to their *in vivo* neurotoxicity.

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337 P PROTECTION AGAINST KAINATE EXCITOTOXICITY BY ADENOSINE A_{2a} RECEPTOR AGONISTS IN THE RAT HIPPOCAMPUS

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Systemic injection of kainate has been widely used as an effective model for excitotoxic neurodegeneration in specific areas of the brain. Adenosine analogues protect the hippocampus in this model, highlighting the importance of determining the receptors and mechanisms involved. Systemic injections of kainate, 10mg/kg i.p., have been used to induce neuronal damage, assessed by quantifying the extent of neuronal loss on a ten-point scale after haematoxylin and eosin staining of the sectioned brain of a male Wistar rat. Results are presented as the percentage damage ± s.e.mean.

Damage from 10mg/kg kainate is highest in the CA1 and CA3a regions (56.7%±9.6 and 51.1%±9.0 respectively, n=9). The CA2 region was less affected with 35.0%±9.4 neurodegeneration, while the CA3b and CA4 areas were unaffected. CGS21680 (2-[p-(2-carboxyethyl)-phenethyl amino] -5'-N-ethylcarboxamidoadenosine) is a selective A2a adenosine receptor agonist that crosses the blood brain barrier. CGS21680 at i.p. doses of 0.1mg/kg, 0.01mg/kg and 2μg/kg in conjunction with kainate (10mg/kg), displayed significant neuronal protection of all three kainate damaged areas of the hippocampus. Protection appeared to be concentration dependant and damage was fully prevented with the highest concentration. DPMA (N^6-[2-(3,5-di methoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine), a less selective A2 agonist, displayed protection against kainate induced toxicity in both the CA1 and CA2 regions at 1mg/kg i.p. (5.0%±3.5 (n=4, $p<0.01$) and 1.3%±1.3 (n=4, $p<0.05$)), while providing only limited non-significant protection in the CA3a region (27.5%±16.4). CPX (8-cyclopentyl-1,3-dipropyl xanthine) at 50μg/kg had no significant effect on the CGS21680 (2μg/kg) protection. In the CA1 and CA3a areas, damage induced by kainate in the presence of CGS21680 (25.0%±12.0 and 19.3%±8.5 respectively, n=7), was not affected by CPX (22.5%±16.1 and 23.8%±9.7 respectively, n=4). 1mg/kg CGS15943 (9-chloro-2-(2-furyl)[1,2,4]triazole [1,5-c] quinazolin-5-amine, a non selective A1/A2 antagonist), given with CGS21680 (2μg/kg) and kainate (10mg/kg) tended to increase neuronal mortality towards the levels seen with kainate alone (n=3). 20mg/kg of the peripherally selective adenosine antagonist 8-(p-sulphophenyl) theophylline (8-PST), did not prevent the effects of CGS21680 (n=5).

The protective effects seen with CGS21680 against kainic acid excitotoxicity appear to be mediated by A2 receptors, but the mechanism of protection is not clear. A2 activation causes cerebral vasodilation and depresses glucose metabolism throughout the brain, possibly protecting cells by increasing the blood supply to the damaged brain, while decreasing the requirement for a limited resource. Platelet aggregation, astroglial effects and downregulation of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate (AMPA) receptors may also be involved.

338 P MODULATION OF EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPUS BY NUCLEOTIDES

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The perfusion of rat hippocampal slices (450μm) with a medium containing no magnesium and 4-aminopyridine (50μM) produces spontaneous bursting activity. This epileptiform activity has a frequency between 0.2 and 0.02 Hz and resembles interictal activity seen *in vivo*. It was of interest to determine whether ATP could modulate this type of epileptiform activity. Potentials were recorded using glass microelectrodes from the CA3 region of slices at 34°C.

Adenosine and ATP (2-100μM) caused a concentration dependent reduction in the rate of epileptiform activity. 50μM adenosine and ATP reduced the rate to 39.41±10.73% (s.e.mean) (n=5, P<0.05) and 46.36±4.54% (n=6, P<0.01) of the control rate respectively. This inhibitory effect was initiated within 1-2min. of the start of perfusion of adenosine or ATP. Reversal of the effect was seen within a similar time scale. 8-cyclopentyl-1,3-dimethylxanthine (CPT), an A_1receptor antagonist, increased the basal rate of activity by 43±6.74% (n=4, P<0.05) which suggests that a basal purine tone is present within the hippocampus. The perfusion of CPT (100nM) with either adenosine or ATP (50μM) caused an increase in the activity rate which was not significantly different from CPT perfused alone. At a concentration that annulled adenosine's effect, adenosine deaminase (0.2U/ml) failed to alter significantly the depression of activity caused by ATP. The non-hydrolysable ATP analogue α, β-methyleneATP (α, β-meATP) (10μM) increased the rate of epileptiform activity by 39.43±11.62% (n=6, P<0.05). This significant increase in frequency was not affected by adenosine deaminase (0.2U/ml). Another ATP analogue 2-methylthioATP (2meSATP) (10μM) and UTP (50μM) were ineffective. P_2 purinoceptor antagonists, suramin (10/50μM) and pyridoxal-phosphate-6-azophenyl-2', 4'-disulphonic acid (PPADS) (5μM), had no effect on the basal rate of activity when perfused for a period of 25 and 35 minutes respectively. Both suramin and PPADS failed to antagonise the inhibition produced by ATP (50μM). However suramin (50μM) inhibited the excitation produced by α, β-meATP, reducing the increase in burst frequency from 117.45±8.61% to 93.04±4.46% of the control rate (n=5, P<0.05).

ATP has a dual effect on spontaneous activity in the hippocampal CA3 region causing both inhibition and excitation. The excitatory effect is reproduced by α, β-meATP and inhibited by suramin which is characteristic of a P_{2x}-like purinoceptor. In most instances the inhibitory effect is predominant, inhibited by CPT and not affected by suramin, PPADS or adenosine deaminase. It is unlikely that a P_{2u} purinoceptor is involved due to the ineffectiveness of UTP at the same concentration as ATP. It is possible that another subtype of P_2 purinoceptor which is activated by both nucleosides and nucleotides and inhibited by xanthines is responsible for the inhibitory effect.

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339 P THE EFFECTS OF HEXAMETHONIUM AND METHYLLYCACONITINE ON ACETYLCHOLINE RELEASE AT THE RAT NEUROMUSCULAR JUNCTION

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Although the prejunctional effects of nicotinic antagonists such as tubocurarine have been acknowledged, the mechanisms involved in such actions remain controversial (Bowman *et al.*, 1988; Wilson *et al.*, 1982). Tian *et al.* (1994) reported that tubocurarine and vecuronium produce a decrease in acetylcholine (ACh) release from rat motor nerve terminals at high frequencies of stimulation, and that tubocurarine, but not vecuronium, produces a Ca^{2+}-dependent increase in ACh release at low frequencies of stimulation. Based on the known pharmacology of these compounds, it was suggested that two different prejunctional nicotinic ACh receptors (nAChRs) might be involved in the dual effects of tubocurarine on ACh release: neuronal-nAChRs mediating negative-feedback control and muscle-nAChRs mediating positive-feedback control. To further characterise which sub-class of nAChRs might be involved in the negative-feedback control system, we have studied the effects of two compounds selective for neuronal-nAChRs, hexamethonium (HEX) and methyllycaconitine (MLA), on ACh release. Two concentrations of extracellular Ca^{2+} ions ($[Ca^{2+}]_o$) were used in the HEX studies in order to examine the Ca^{2+}-dependence of any effects.

The methods employed were as previously described in Tian *et al.* (1994). In each cut rat hemidiaphragm preparation, about 20 - 40 miniature endplate currents (m.e.p.cs) and 60 - 100 endplate currents (e.p.cs) elicited at nine different stimulation frequencies ranging from 0.5 - 150 Hz (each block separated by a 10 s interval) were recorded, at -50 mV and 32°C, before and after a 5 minutes exposure to either 200 μM HEX, 0.4 μM MLA or 2.0 μM MLA. The $[Ca^{2+}]_o$ for the HEX studies was either 0.5 or 2.0 mM. E.p.c. quantal content (*m*) was calculated from the mean amplitude of m.e.p.cs and the amplitude of e.p.cs in plateau portion of each block nerve stimulation.

In a $[Ca^{2+}]_o$ of 0.5 mM, 200 μM HEX decreased m.e.p.c. amplitude by 22.1 ± 2.7 % (n = 8) and produced a reduction of *m* at high frequencies of stimulation. We ascribe these two effects to an action of HEX on muscle-nAChRs. In a $[Ca^{2+}]_o$ of 2.0 mM, 200 μM HEX produced a similar decrease in m.e.p.c. amplitude (18.7 ± 2.5 %, n = 8) but an increase in *m* at low frequencies. We ascribe this increase in *m* to an effect of hexamethonium on neuronal-nAChRs. With 0.4 μM MLA, a concentration that would maximally inhibit α-bungarotoxin-sensitive neuronal-nAChRs, there was no change in m.e.p.c. amplitude: control, 2.0 ± 0.1 nA; MLA, 1.9 ± 0.1 nA (n = 7, P > 0.05 vs control, paired Student's *t* test) and *m* also remained unaffected at all frequencies (Table 1). At 2.0 μM, MLA decreased m.e.p.c. amplitude by 15.8 ± 3.9 % (n = 7). This concentration of MLA also caused an increase in *m* at low frequencies of stimulation (Table 1). At this concentration MLA would inhibit ganglionic-type neuronal-nAChRs.

Table 1: Effects of HEX and MLA on e.p.c. quantal content in the rat hemidiaphragm at a high and low frequency of stimulation.

Drug:	200μM HEX	200μM HEX	0.4μM MLA	2.0μM MLA
$[Ca^{2+}]_o$:	0.5mM	2.0mM	2.0mM	2.0mM
0.5 Hz	101 ± 12	137 ± 12*	108.5 ± 8.3	135.3 ± 9.3*
150 Hz	86.4 ± 2.8*	106.6 ± 5.3	89.2 ± 6.7	107.1 ± 5.2

Values are mean and s.e.mean of data, expressed as % control, from 7 - 8 preparations in each group. *Indicates a statistically significant change from control (P < 0.05, paired Student's *t* test).

These results lend support to the notion that presynaptic neuronal-nAChRs are involved in the negative-feedback control of ACh release from rat motor nerve terminals. Additionally, the pattern of the Ca^{2+}- and frequency-dependent enhancement of ACh release seen with HEX and MLA suggests that these neuronal-nAChRs may be of the ganglionic-type.

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340 P INVESTIGATION OF DEVELOPMENTAL CHANGES IN α_1-ADRENOCEPTOR SUBTYPES IN RAT MESENTERIC RESISTANCE ARTERIES

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The aim of this study was to examine the developmental changes in the subtypes of functional α_1-adrenoceptors mediating vasoconstriction in mesenteric resistance arteries from Wistar Kyoto rats. Mixed populations (i.e., male & female) at 5, 16 and 52 week WKYs were examined. Mesenteric resistance arteries (secondary branches, internal diameter=256.70 ±6.72μm, n=44), were isolated and mounted on wire myographs and normalised to 0.9 of L100 (Mulvany & Halpern, 1977).
Cumulative concentration-response curves (CCRCs) were constructed for the α-adrenoceptor agonists; noradrenaline (NA), (R) A-61603 and phenylephrine (PE). High potency of A-61603 relative to PE has been shown to differentiate functional α_{1A} and α_{1B} from α_{1D}-adrenoceptors (Knepper et al., 1995).The effects of the competitive α_1-adrenoceptor antagonists, prazosin, WB4101, 5-Me-Urapidil, HV723 and chloroethylclonidine (CEC) were examined using noradrenaline as the agonist. All CCRCs were carried out in the presence of cocaine (3μM), propranolol (1μM) and corticosterone (10μM).

Table 1: agonists pD_2 ± s.e.m (n)

age (weeks)	5	16	52
NA	6.00±0.14 (5)	6.17±0.06 (4)	6.75±0.19*(5)
PE	5.44±0.17 (4)	5.45±0.16 (4)	
(R)A61603	8.07±0.01 (4)	8.33±0.21 (4)	

*p<0.05, Student's unpaired t test: 16 &52 week v/s 5 week

Table 2: antagonists:

age (weeks)	5	16	52
prazosin	9.88±0.09	9.02±0.05***	8.92±0.13**
WB4101	9.12±0.26	9.10±0.10	9.06±0.12
5Me-Urapidil	6.30±0.22	7.20±0.56	8.10±0.31**
HV723	8.89±0.21	8.28±0.29	8.58±0.22

Values are mean pK_B values ± s.e.m (-log M) from at least 4 experiments ***p<0.001, **P<0.01, Student's unpaired t test: 16 and 52week data compared to 5week data
The sensitivity of mesenteric resistance arteries to noradrenaline increases with age. The high potency of (R) A-61603 relative to PE suggests the presence of the α_{1A} subtype though it does not exclude α_{1B}.
The antagonists indicate a change in the characteristics of the α_1-adrenoceptors with age; at 5weeks the exceptionally low potency of 5Me-Urapidil suggests the presence of α_{1B} but are otherwise consistent with α_{1A}. At 16 and 52 weeks the lower potency of prazosin could indicate an α_{1L}-population (Muramatsu et al., 1990) but otherwise the increased potency of 5Me-urapidil suggests a shift towards α_{1A}. At 5 and 16 weeks, the CCRC to noradrenaline was shifted to the right and the maximum response to noradrenaline was decreased by preincubation with CEC (100μM for 60 mins, followed by 10xwash over 30mins).
Overall, in this resistance vessel, the results are consistent with α_{1A} and/or α_{1B} but against α_{1D}, in contrast to the rat aorta where α_{1D} seems dominant (Aboud et al., 1993). Ageing of the rat from 5 to 52 weeks is accompanied by a rise in sensitivity to noradrenaline, a rise in potency for 5Me-urapidil and a fall for prazosin. This does not further clarify the receptor subtypes involved but may indicate a phenotypic change in the smooth muscle cells and augurs caution in functional subtyping, particularly with subjects of differing age.

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341 P INHIBITORY EFFECTS OF SOMATOSTATIN AND RELATED PEPTIDES ON ELECTRICALLY-EVOKED CONTRACTIONS OF THE RAT ANOCOCCYGEUS MUSCLE *IN VITRO*

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Recently five different somatostatin (SRIF) receptors (named sst_1-sst_5) have been identified and cloned. In addition, several peptides have been identified which show selectivity for one or more of the recombinant SRIF receptors, for example L-363,586 (seglitide) shows high affinity for sst_2, sst_3 and sst_5.(Raynor *et al*, 1993a,b). $SRIF_{14}$ and seglitide have been shown to inhibit electrically-evoked contractions of the rat anococcygeus muscle (Priestley & Woodruff, 1988). In this study we have investigated further the receptor involved by comparing the potencies of BIM23027, BIM23056 and BIM23052, reported as selective for sst_2, sst_3 and sst_5 respectively, with $SRIF_{14}$ and seglitide in the rat anococcygeus muscle.

Anococcygeus muscles were removed from male Wistar rats (330-370g). Muscle pairs were separated, placed in 15ml organ baths and suspended under 0.5g tension for isometric recording. Preparations were bathed in a gassed ($95\%O_2/5\%CO_2$) modified Krebs solution at 37°C. Transmural field stimulation was applied for 1s every 20s (10Hz, 0.5ms). Compounds were added non-cumulatively at 60 min intervals and washed out once maximum inhibition was observed to generate concentration-effect curves for each peptide (one curve per tissue).

Maximum inhibitions are quoted as arithmetic mean ± standard error of the mean. IC_{50} values are quoted as geometric mean (95% confidence limits). Observed differences in maximum inhibition were tested for statistical significance using an unpaired Student's t-test.

SRIF14 inhibited electrically-evoked contractions of the rat anococcygeus muscle in a concentration-related manner (IC_{50}=11.2 (5.6-16.6)nM, maximal inhibition 84±4%, n=8). Seglitide was 5-fold more potent than $SRIF_{14}$ (IC_{50}=2.1 (1.5-2.6)nM, maximal inhibition 63±4%, n=8) and BIM23027 2-fold less potent than $SRIF_{14}$ (IC_{50}=24.4 (18.4-30.4)nM, maximal inhibition 72±4%, n=8). In contrast, BIM23052 was only weakly active in comparison to $SRIF_{14}$ (IC_{50}=1.7 (1.4-1.9)μM, maximal inhibition 83±15%, n=7); BIM23056 showed no activity at concentrations up to 3μM. Thus the rank order of potency of the compounds tested was seglitide > $SRIF_{14}$ ≥ BIM23027 >> BIM23052.

These data suggest that the receptor mediating inhibition of neurogenic contractions in the rat anococcygeus muscle is similar to that mediating inhibition of neurotransmission in the guinea-pig ileum (Feniuk *et al*, 1995). Furthermore, the rank order of potency of the agonists tested would suggest that this receptor is similar to the recombinant sst_2 receptor.

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342 P INHIBITION OF GABA RESPONSES OF MOUSE ILEUM BY ELECTRICAL FIELD STIMULATION: EFFECT OF INHIBITORS OF GABA UPTAKE AND METABOLISM

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Alyami et al. (1990, 1991) found that electrical field stimulation (EFS) of guinea-pig and mouse ileum was able to inhibit selectively the contractile responses to γ-aminobutyric acid (GABA) and muscimol mediated through $GABA_A$ receptors. The inhibition was reversed by washing and time. Studies by Pipelzadeh & Wood (1995) using a selective $GABA_B$ receptor antagonist suggested that EFS causes the release of GABA which in turn activates inhibitory $GABA_B$ receptors and thus reduces $GABA_A$ receptor-mediated contractions. In the present studies the effects of aminooxyacetic acid (AOAA), a GABA-transaminase inhibitor, and nipecotic acid (Nip), a GABA uptake inhibitor (Kerr & Krantis, 1983), were investigated in mouse ileum in order to establish whether they were able to enhance or prolong the EFS-induced inhibition of $GABA_A$ receptor-mediated responses.

Segments taken from the mid-ileum of adult male BKW mice (25-35 g) were mounted in organ baths containing Krebs' solution (37°C, 95%O_2/5%CO_2). The tissues were allowed to equilibrate, under 0.5 g tension, for 60 min. Changes in tension due to the application of drugs and EFS (60 s, 0.6 Hz, 30 volts, double pulses with 75 ms delay) were expressed as the mean±s.e.mean of n≥4, and statistical differences were analysed using Student's t-test ($p<0.05$ regarded as significant). Contact times were 1 min for GABA (0.25 mM) and acetylcholine (Ach, 0.5 μM) and 4 min for AOAA and Nip.

Submaximal contractile responses to GABA were elicited immediately before EFS (control) and 10-120 s after ceasing EFS (test). In the absence of inhibitors, EFS for 60 s reduced the test responses to GABA to 37.8±4.1% ($p<0.05$) of control when GABA was applied 10 s after ceasing EFS: test responses recovered with time, to 91.1±5.8% of control when GABA was applied 120 s after ceasing EFS.

Nip (0.5 μM-0.15 mM) did not affect submaximal responses to Ach or GABA directly, nor did it alter the extent of reduction of GABA responses by EFS when GABA was applied 10 s after ceasing EFS. AOAA (0.15 μM-15 μM) had no significant effect on submaximal responses to Ach or GABA directly, and only at 15 μM did it significantly ($p<0.05$) alter the responses to GABA applied 10 s after ceasing EFS, reducing them to 15.1±3.1% of control. When the time between cessation of EFS and reapplication of GABA was extended from 10 s up to 120 s in the presence of Nip (0.5 μM) or AOAA (1.5 μM), the recovery of GABA responses was significantly ($p<0.05$) attenuated at all time points. At 120 s, GABA responses were 66.5±5.4% and 66.5±7.1% of control in tissues treated with Nip and AOAA respectively, compared with 91.1±5.8% in untreated tissues.

The results are consistent with the suggestion that GABA released during EFS can inhibit responses to exogenously applied GABA, since Nip and AOAA, which are known to inhibit uptake and metabolism of GABA, have been found in the present study to prolong the inhibitory effect of EFS.

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343 P COMPARISON OF THE EFFECTS OF POTASSIUM CHANNEL OPENERS ON ELEVATED GASTROINTESTINAL TRANSIT IN THE MOUSE

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In previous experiments we have shown that the potassium channel openers (KCOs) pinacidil (PIN) and SDZ PCO400 (SDZ) relaxed, to a similar extent, isolated mucosa-free preparations of mouse distal ileum contracted by electrical field stimulation (Yeung *et al.*, 1995). In the present experiments we compare the effect of the above KCOs and cromakalim (CROM) on gastrointestinal transit *in vivo* using a modification of the mouse charcoal meal test, first described by Janssen & Jaganeau (1957), such that motility of the intestine was increased by carbachol (CARB) subsequent to the administration of the KCOs.

In initial experiments, following an overnight fast, male BKW mice (28-52g) were dosed orally with PIN (1-10mg/kg), SDZ (0.1-10mg/kg), CROM (0.1-10mg/kg), CARB (0.3-10mg/kg) as a motility enhancer, morphine (MOR, 1-10mg/kg) as an inhibitor, or appropriate vehicle (saline for CARB and MOR, 10% alcohol for PIN, SDZ and CROM). After 20, 30 or 80min of pretreatment time the charcoal meal was administered (0.1ml/10g body weight of a suspension of 10% charcoal and 5% acacia gum in distilled water). Animals were sacrificed 30min after administration of the charcoal meal and the gastrointestinal tracts removed. The total length of small intestine from pylorus to ileocaecal junction and the distance moved by the charcoal front in the small intestine were measured. In further experiments KCOs or MOR were administered, followed by CARB (10mg/kg) 30min later, and by charcoal meal 30min after CARB. After this time point the protocol proceeded as above. Results are expressed as mean percentage distance moved by the charcoal front along the small intestine ± s.e.mean of 8-10 animals. Statistical differences were tested using Students' unpaired t-test on original data ($P\leq0.05$ regarded as significant).

In initial experiments, all KCOs and MOR significantly inhibited gastrointestinal transit in a concentration-related manner. The results for each agent were not significantly different whether 20, 30 or 80min pretreatment intervals were used. From these studies, doses of PIN, SDZ, CROM and MOR were selected which gave approximately equal inhibition, to 20-30% distance moved by the charcoal at 30min; 25.6±1.2%, 24.7±2.4%, 26.5±1.6% and 32.5±1.9% respectively as compared with appropriate controls (10% alcohol vehicle, 46.8±2.7%; 0.9% saline, 55.6±1.5%). CARB (30min pretreatment) caused significant increases in the % distance moved by the charcoal front: 1mg/kg, 64.8±3.4% ($P<0.05$); 10mg/kg, 83.7±4.9% ($P<0.001$) *vs* saline: 0.3mg/kg was without effect.

CARB (10mg/kg) was used to ensure adequate increase in motility in subsequent experiments involving KCOs and MOR. The effect of CARB (10mg/kg) was significantly counteracted by 30min pretreatment with PIN (10mg/kg), SDZ (1mg/kg) and CROM (1mg/kg), PIN being the least effective: PIN+CARB 61.0±3.3% ($P<0.01$); SDZ+CARB 51.2±1.7% ($P<0.001$); CROM+CARB 56.0±2.1% ($P<0.001$). However, the stimulation of motility due to CARB was unaffected by the presence of MOR (10mg/kg): 84.1±4.6%.

The results suggest that the KCOs examined here are active on intestine when administered by the oral route and possess intestinal inhibitory action in both normal and pharmacologically stimulated conditions.

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344 P AUGMENTATION BY THE α_2-ADRENOCEPTOR ANTAGONIST, ATIPAMEZOLE, OF APOMORPHINE-INDUCED CIRCLING IN RATS WITH COMBINED DSP-4 AND UNILATERAL SUBSTANTIA NIGRA LESIONS

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The α_2-adrenoceptor antagonist, atipamezole (ATI) not only increases turnover of noradrenaline (NA) but dopamine (DA) turnover is also elevated. This study examined whether ATI can modulate DA receptor activation in a model of Parkinson's disease - the so-called Ungerstedt rat (Ungerstedt and Arbuthnott, 1970) where unilateral striatal injection of 6-hydroxydopamine (6-HD, 8 μg in 4 μl) causes asymmetric circling behaviour to challenge with DA agonists; ipsilateral circling to indirect agonists like amphetamine (AMP, 1 mg/kg, s.c.) and contralateral circling to direct receptor agonists (apomorphine, APO, 50 μg/kg, s.c.) Some rats were further treated with the NA neurotoxin, DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine, 2x 50 mg/kg i.p., 1 week between injections) to try to eliminate presynaptic α_2-adrenoceptors on NA nerves.

The experiments were designed in a cross-over manner with each rat (male Sprague-Dawley, 400 - 591 g) receiving test treatments with at least two days' wash-out. Statistics were ANOVA followed by Wilcoxon signed-rank test when comparing with rat's own response to DA agonists and 2 factor ANOVA for comparing between DSP-4 + 6-HD and rats with only 6-HD lesions.

ATI (300 μg/kg, s.c.) alone did not significantly alter the circling behaviour of the rats (ATI; ipsilateral 42 turns ± 12 s.e. mean; contralateral 3 ± 1 turns; Saline: ipsilateral 20 ± 5 turns; contralateral 2 ± 1 turns). ATI significantly augmented the ipsilateral rotation to AMP and contralateral circling to APO (table 1). This was also seen in DSP-4 treated rats. (All values of $P > 0.3$ vs 6HD alone).

Table 1: Circling behaviour for 2h after DA agonists

Ipsilateral circling to amphetamine (turns ± s.e mean)		
Treatment	6-HD alone (n =10)	6-HD+DSP-4 (n= 8)
AMP	297 ± 71	219 ± 75
AMP + ATI	833 ± 118**	697 ± 188*
Contralateral circling to apomorphine (turns ± s.e. mean)		
APO	532 ± 67	557 ± 56
APO + ATI	774 ± 78**	953 ± 90*

*P<0.05; **P < 0.01 vs DA agonist alone; Wilcoxon test

After sacrifice, DA levels in the 6-HD lesioned striata were 99.0±0.2% less than the non-lesioned side but NA (93±2% depletion) and serotonin (5HT) (19±5% depletion) were affected. DSP-4 caused a further decrease in 5HT (42±8%). In frontal cortex, 6-HD on the lesioned side caused 96±1% depletion of NA and 22±7% loss of 5HT. DSP-4 on the non-lesioned side caused a 60±18% depletion of NA but also a 34±13% loss of 5HT.

It is concluded that ATI can modulate the effects of striatal DA receptor activation, increasing motor activity. The lack of effect of DSP-4 may indicate the involvement of a post-synaptic α_2-adrenoceptor. ATI may be able to augment the effects of DA agonists in Parkinson's disease.

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345 P THE INFLUENCE OF NICOTINE PRETREATMENT ON MESOACCUMBENS DOPAMINE RESPONSES TO NICOTINE GIVEN INTO THE VENTRAL TEGMENTAL AREA OF THE RAT

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Previous studies in our laboratory have shown that pretreatment with nicotine enhances its effects on dopamine (DA) overflow in the nucleus accumbens (NAc) of the rat and that the responses seem to be mediated by nicotinic receptors located on the somatodendritic membranes of the cells (Benwell & Balfour 1992, Benwell et al 1993). The aim of this study was to investigate the effects of nicotine pretreatment on the mesoaccumbens DA responses to nicotine delivered close to the cell bodies in the ventral tegmental area (VTA) of the brain.

Male Sprague-Dawley rats (~250g) were given 5 daily injections of nicotine (0.4mg kg^{-1} sc) or saline prior to the implantation of a dialysis probe in the NAc (Benwell & Balfour 1992). A guide cannula, targeted at the ipsilateral VTA was also implanted at this time. Dialysis studies were performed on the day following surgery. Following a 60 minute equilibration period, samples of dialysate were collected every 20 minutes and analysed by HPLC with electrochemical detection. The data were analysed statistically using an analysis of variance for repeated measures; *post hoc* analyses were performed using the Student's t-test. Following the collection of 4 baseline dialysate samples, the rats were given intra-VTA injections (0.5µl in 2 min) of nicotine or vehicle (saline), different rats being used for each injection. The mean basal levels of DA and DOPAC in the 4 baseline samples collected from the saline-pretreated rats (n=18) were 9.5±1.1pg $20\mu l^{-1}$ and 4.0±0.6ng $20\mu l^{-1}$ respectively. These levels were not altered significantly by pretreatment with nicotine. The DA response to intra-VTA nicotine was influenced significantly by pretreatment with nicotine ($F(27,270)=1.6$; $P<0.05$). Subsequent analysis showed that, in saline pretreated rats, the nicotine injections had no significant effect on DA overflow whereas in the nicotine pretreated rats, nicotine evoked a significant increase ($F(27,144)=2.0$; $P<0.01$) in extracellular DA. The peak response to nicotine was measured 60 minutes after the injection. At this time the extracellular DA levels for the animals given intra-VTA injections of saline or nicotine (1.5, 6 and 12µg) were 94±7, 160±26, 192±53 and 136±20 percent of the mean baseline values respectively (n= 4-6 observations per group). The effect of intra-VTA injections of nicotine had an effect on extracellular DOPAC which was also influenced by pretreatment with nicotine ($F(27,288)=1.9$; $P<0.01$). Subsequent analysis showed that the administration of nicotine (1.5µg) reduced DOPAC overflow in nicotine-pretreated but not saline-pretreated rats, the maximal effect being observed 120 minutes after the injection when the levels were reduced ($P<0.05$) from 133±21 to 74±8 percent of baseline. Nicotine pretreatment had no significant effect on the NAc DOPAC responses to injections of saline or the higher doses of nicotine into the VTA.

The data are consistent with the hypothesis that pretreatment with nicotine causes sensitisation of the mesoaccumbens DA responses to the drug by enhancing the effects mediated by somatodendritic nicotinic receptors. This effect, however, is not invariably associated with increased DA turnover.

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346 P ROPINIROLE IS A D_3 SELECTIVE AGONIST AT CLONED HUMAN D_{2long}, D_3 AND $D_{4.4}$ RECEPTORS IN FUNCTIONAL STUDIES USING MICROPHYSIOMETRY

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Ropinirole is a dopamine agonist which is clinically effective in the treatment of Parkinson's Disease (Wheadon *et al*, 1996). Ropinirole is distinguished from some other dopamine agonists and L-DOPA by a much lower propensity to induce dyskinesias in drug naive, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated marmosets (Pearce *et al*, 1996). We have investigated the functional selectivity of ropinirole compared with dopamine and two other dopamine agonists, pergolide and talipexole, used in the treatment of Parkinson's Disease (PD).

Receptor affinities and acidification rates in a microphysiometer were determined for cloned human D_{2long}, D_3 and $D_{4.4}$ receptors expressed in CHO cells as in Boyfield *et al* (1996). In microphysiometry assays, cells were first exposed (4.5, 7.5 or 6 min for hD_2, hD_3 or hD_4 respectively) to a maximal concentration of quinpirole (1000 nM for hD2 and hD4, 100 nM for hD3), before seven increasing concentrations of test compound at half hourly intervals. The peak acidification rate to each agonist concentration was determined and concentration-response curves fitted using Robofit (Tilford *et al* 1995).

All the ligands tested were full agonists at all three receptors (82-127% quinpirole control), with the exception of talipexole at the hD_4 receptor (maximum stimulation 56 ± 15%). There was no consistent relationship between binding affinities and functional potencies, for any compound or receptor (Table 1). Ropinirole retained D_3 selectivity in functional studies (10 fold), more than dopamine (3 fold) and the other ligands tested (talipexole was 2 fold D_2 selective). Further work on the efficiency of D_3 receptor coupling in human brain, and the relative abundance of D_3 and D_2 receptors in PD tissue, is needed to determine if this translates to functional selectivity *in vivo*.

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Table 1: Functional (microphysiometer; pEC_{50}) and radioligand binding (pK_I) potencies at human dopamine receptor subtypes

Compound	hD_2		hD_3		hD_4	
	pK_I	pEC_{50}	pK_I	pEC_{50}	pK_I	pEC_{50}
Ropinirole	5.9*	7.5 ± 0.1 (9)	7.2*	8.5 ± 0.2 (8)	5.5 ± 0.1 (4)	6.8 ± 0.1 (7)
Dopamine	6.1*	6.8 ± 0.2 (5)	7.4*	7.3 ± 0.2 (7)	6.1 ± 0.1 (6)	7.0 ± 0.1 (4)
Pergolide	8.1*	9.0 ± 0.1 (5)	8.8*	8.2 ± 0.4 (6)	6.9 ± 0.1 (4)	8.2 ± 0.2 (5)
Talipexole	5.8 ± 0.2 (3)	7.9 ± 0.2 (5)	7.0 ± 0.1 (3)	8.2 ± 0.1 (5)	5.2 ± 0.1 (3)	6.5 ± 0.1 (5)

Mean ± sem from (n) experiments. * Data from Bowen *et al* (1993).

347 P LOCAL APPLICATION OF 5-HT4 ANTAGONISTS INHIBITS POTASSIUM-STIMULATED GABA EFFLUX FROM RAT SUBSTANTIA NIGRA *IN VIVO*

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Recent receptor autoradiography studies have detected particularly high levels of the 5-HT_4 receptor in parts of the basal ganglia, specifically the striatum and substantia nigra (SN) (Waeber et al, 1994). On the basis of the effects of lesions and the known neuroanatomy of the striatonigral pathway, it is thought that 5-HT_4 receptors in the SN are located on the terminals of GABAergic neurones (Patel et al, 1995). These data suggest a role for the 5-HT_4 receptor in the modulation of GABAergic neurotransmission in the SN. Here we report the effects of two selective 5-HT_4 receptor antagonists, GR 113808 and SB204070 on efflux of GABA in the SN measured *in vivo* using microdialysis.

Male Sprague-Dawley rats (250-270 g) were anaesthetised with halothane throughout the experiment. Home-made single cannula microdialysis probes were implanted stereotaxically into the right SN, (AP-5.4, L 2.2, V 8.9; Paxinos and Watson, 1986). The probes were perfused with artificial CSF (2 µl/min) and microdialysates were collected every 25 min and then analysed for GABA using o-phthaldiadehyde-sulphite derivatization and HPLC-EC (Smith & Sharp, 1994). Once basal levels of GABA had stabilized, perfusion medium was switched to one containing 30 mM potassium chloride for 25 min. The 5-HT_4 antagonists were added to the perfusion medium for at least three 25 min periods before potassium chloride, and remained throughout the experiment. Values are expressed as a percentage of mean basal levels prior to potassium chloride stimulation. Statistical analyses were performed using one way ANOVA and Dunnetts t- test.

The average basal level of GABA (± s.e.m.) in microdialysates collected from SN was 0.558±0.056 pmol/50 µl (n=20). Potassium chloride (30 mM) increased levels of GABA to 289±24% (n=11) in the sample collected during the potassium chloride infusion. Omission of calcium did not alter basal GABA levels but completely abolished the stimulatory effect of potassium chloride (n=3). In rats pretreated with 1 or 10 µM GR 113808, the potassium chloride effect on efflux of GABA in the SN was significantly reduced (to 183±14%, n=6, $p<0.02$ and 121±8%, n=7, $p<0.01$, respectively). SB 204070 (1 µM) also significantly reduced the effect of high potassium chloride (153±9% compared to basal levels, n=4, $p<0.05$).

In summary, the present study shows that two different 5-HT_4 receptor antagonists, GR 113808 and SB 204070, inhibit potassium chloride stimulated efflux of GABA in the rat SN. These findings indicate that, at least following potassium stimulation, GABA release is tonically activated by 5-HT_4 receptors.

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348 P ANALGESIC ACTIVITY OF THE 5-HT$_{1A}$ AGONISTS BUSPIRONE AND 8-HYDROXY-2-(DI-*N*-PROPYL-AMINO)TETRALIN (8-OH-DPAT) AND THEIR DIFFERING ANTAGONIST INTERACTIONS

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5-Hydroxytryptamine (5-HT) plays an important role in the modulation of nociceptive transmission (Roberts, 1984), and selective 5-HT receptor agonists either facilitate or inhibit nociception depending on the route of administration and/or the analgesic test used.

In a previous study we compared the antinociceptive activity of the anxiolytic and antidepressant 5-HT$_{1A}$ partial agonist buspirone with the prototypic agonist 8-OH-DPAT (Dobson *et al.*, 1994). Analgesia was evaluated in ICI-WSP male mice (18-23 g) using the abdominal constriction assay (Alhaider, 1991). Analgesia is expressed as % protection as compared to saline treated controls. Both 8-OH-DPAT and buspirone administered subcutaneously 20 min prior to the test produced dose related antinociceptive activity.

In order to elucidate the receptors responsible for the analgesic response we attempted to antagonise the two agonists with the following antagonists - pindolol (nonselective 5-HT$_1$), (+)WAY100135 (selective 5-HT$_{1A}$), RX821002, yohimbine, idazoxan (all α_2-adrenoceptor) and naloxone (opioid). All antagonists were administered subcutaneously 10 min prior to a 50% response dose level of each agonist (8-OH-DPAT 3.5 mgkg^{-1}, buspirone 8 mgkg^{-1}).

It was found that neither pindolol or (+)WAY100135 produced significant antagonism of the analgesic response of 8-OH-DPAT, but both significantly antagonised the response to buspirone (Table 1). Thus, although 5-HT$_{1A}$ receptors clearly have a role in mediating the analgesia produced by buspirone in this test, 8-OH-DPAT analgesia is not wholly dependent on 5-HT systems.

8-OH-DPAT antinociception has previously been shown to act through α_2-adrenoceptors in the hot plate test (Millan and Colpaert, 1991). However, none of the α_2-adrenoceptor antagonists used in this study had any effect on the analgesic response to either 8-OH-DPAT or buspirone.

The opioid antagonist naloxone did produce a significant antagonism (Table 1) of the responses to both agonists. It has been shown previously that naloxone antagonises the analgesia produced by intrathecal 5-HT (Yang *et al.*, 1994). It would therefore appear that buspirone produces analgesia which involves 5-HT$_{1A}$ receptors associated with opioid pathways, whereas visceral analgesia induced by 8-OH-DPAT probably relies on opioid pathways which are 5-HT$_{1A}$ independent.

Table 1

	Agonist alone	*+ 10 mgkg^{-1} pindolol*	*+ 3 mgkg^{-1} (+)WAY100135*	*+ 2 mgkg^{-1} naloxone*
8-OH-DPAT	52.0 ± 4.5	47.6 ± 3.5	46.8 ± 5.3	21.9 ± 8.7*
Buspirone	46.3 ± 3.7	24.1 ± 6.0*	34.3 ± 5.7*	18.6 ± 5.4*

Values represent % protection ± s.e.m., n = 8. * indicates $P < 0.01$, compared with agonist given alone (Students t-test).

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349 P EFFECT OF SCOPOLAMINE ON DELAYED NON-MATCHING-TO-POSITION PERFORMANCE FOLLOWING CENTRAL 5,7-DIHYDROXYTRYPTAMINE TREATMENT

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The operant delayed-matching- (DMTP) and non-matching-to-position (DNMTP) tasks, first developed by Dunnett (1985), have become standard tools for the assessment of memory function in rats. Briefly, this task requires a rat to respond on one randomly selected lever presented into an operant conditioning chamber. Following the response and a variable delay, two levers are presented and the rat is required to respond on the same lever (DMTP) or the opposing lever (DNMTP) to release a food reinforcement. Longer delay intervals are associated with reduced accuracy.

There is currently much interest in how the cholinergic system interacts with the serotonergic system in memory function. Research has usually been conducted using the radial arm maze or water maze. To determine whether similar performance changes can be detected in the operant technique developed by Dunnett, we have looked at the effects of combining acute scopolamine treatment with central 5,7-dihydroxytryptamine (5,7-DHT) administration. Male Wistar rats (University of Wales Cardiff strain), initially weighing between 150-170g, were trained to perform a DNMTP task to asymptote, using 1s, 4s, 7s, 15s and 30s as randomly chosen delay intervals. Once at criterion they were treated with desipramine (25mg/kg, i.p.), anaesthetized with halothane 20 min later and injected intracerebroventricularly (i.c.v.) with 10µl of either 150µg 5,7-dihydroxytryptamine (5,7-DHT; n=9) or vehicle (0.1% ascorbate in saline; n=7). Following a four week post-operative recovery period, daily training resumed until asymptotic levels of performance were restored. Drug trials (saline, 0.03mg/kg, 0.1mg/kg scopolamine, i.p., administered 15 min before the start of a 45 min session) were conducted on Tuesdays and Fridays. Normal training continued on the remaining weekdays.

Scopolamine (0.1mg/kg) induced mild deficits in performance in both sham-lesioned and 5,7-DHT treated animals at certain delays. However, this was statistically significant only with the sham group (one-way ANOVA followed by Student Newman Keul's posthoc test) at the 1s, 7s, and 15s delay intervals (15.4%, 19.2% and 18.2% decreases compared to saline treated controls in mean response accuracy respectively, $p<0.05$). Response accuracy deficits in those animals given 5,7-DHT i.c.v. were not statistically significant. We also measured the panel press rate during the delay intervals and the latency between the presentation of the two choice levers and the response. No differences were observed with respect to the latency parameter. However, scopolamine 0.1mg/kg produced some deficits in the mean panel press rate in both sham-operated and 5,7-DHT-treated groups at certain delay intervals (0.95 and 0.7 presses s^{-1} decrease at the 15s and 30s delay intervals respectively, and 1.0 and 0.8 presses s^{-1} at the 7s and 15s delay intervals respectively for the 5,7-DHT treated rats in comparison to their respective saline control groups, $p<0.05$).

These results suggest that compromised serotonergic function serves to inhibit working memory deficits induced by the cholinergic antagonist scopolamine. Moreover, the DNMTP task is sensitive to changes in memory function induced by drug treatment, but further work is required to demonstrate transference of these deficits to the DMTP task.

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350 P INTERACTIONS OF m-CHLOROPHENYLPIPERAZINE AND m-CHLOROPHENYL-BIGUANIDE WITH 5-HYDROXYTRYPTOPHAN IN THE MOUSE LIGHT/DARK TEST

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m-Chlorophenylpiperazine (MCPP) has affinity for 5-HT_2 and other 5-HT receptors and, whilst generally used as a purported 5-HT receptor agonist, it may also display antagonist or partial agonist effects (Baxter *et al.*, 1995). Its effects would therefore be hypothesised to be dependent on the degree of endogenous 5-HT tone. In the present study we compare the actions of MCPP with the relatively selective 5-HT_3 receptor agonist m-chlorophenylbiguanide (MCPBG) (Kilpatrick *et al.*, 1990) and their interactions with 5-hydroxytryptophan (5-HTP) in the mouse light dark test.

Male albino BKW mice (Bradford strain) weighing 25-30g received an intraperitoneal injection of vehicle + vehicle/MCPP/MCPGB or 5-HTP (50mg kg^{-1}) and 5-HTP + MCPP or MCPBG, with 40 min between treatments, with behavioural testing 40 min after the last treatment. Mice were placed into the centre of the light compartment of the test box and the latency of first movement from the light to the dark compartment video recorded over a 5 min period (for detailed methodology see Cheng *et al.*, 1994).

The administration of MCPP, MCPBG and 5-HTP alone decreased the latency of first movement from the light to the dark compartment. These effects were antagonised by the interaction between MCPP or MCPBG and 5-HTP. All doses of MCPP (including low doses which failed to modify behaviour in their own right), when administered in combination with 5-HTP, reversed the behavioural inhibition to one of disinhibition. Similarly, the MCPBG/5-HTP interaction antagonised or reversed the inhibitory profiles.

The studies have confirmed using MCPP and established using MCPBG that both agents exert a behavioural inhibitory or anxiogenic profile in the mouse light/dark test. However, the important finding of an antagonism or reversal of the inhibitory profiles to one of disinhibition by co-administration of 5-HTP indicates a complex action of MCPP and MCPBG to modify mouse behaviour in the light dark test that may be dependent on the degree of 5-HT tone.

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Table 1. The effect of MCPP and MCPBG and their interaction with 5-HTP in the mouse light/dark test box

MCPP mg kg^{-1} i.p.	plus Vehicle	or 5-HTP	MCPBG mg kg^{-1} i.p.	plus Vehicle	or 5-HTP
Vehicle	12.1 ± 1.6	2.1 ± 0.4*	Vehicle	11.3 ± 1.5	2.0 ± 0.6*
0.001	10.6 ± 1.9	18 ± 1.3^{+}*	0.001	12.7 ± 1.5	3.2 ± 0.6*
0.005	11.4 ± 2.2	23 ± 2.5^{+}*	0.005	16 ± 3.5	12 ± 1.4^{+}
0.01	9.8 ± 1.0	24 ± 2.0^{+}*	0.01	1.8 ± 0.7*	14 ± 1.9^{+}
0.05	4.3 ± 1.8*	26 ± 1.8^{+}*	0.05	2.3 ± 0.4*	22 ± 2.7^{+}*
0.1	2.0 ± 0.3*	28 ± 2.4+*	0.1	2.6 ± 0.5*	19 ± 2.6^{+}*
0.5	2.1 ± 0.6*	24 ± 2.8^{+}*	0.5	3.8 ± 1.1*	26 ± 2.5^{+}*

Values (mean ± s.e. mean) indicate the latency (s) of first movement from the light to the dark compartment (n = 10). *P<0.01 compared to vehicle/vehicle controls $^{+}$P<0.01 compared to 5-HTP + vehicle (one way ANOVA followed by Dunnett's t test).

351 P INTERACTION OF FLUOXETINE WITH 5-HYDROXYTRYPTOPHAN TO MODIFY MOUSE BEHAVIOUR IN THE LIGHT/DARK TEST

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The administration of 5-hydroxytryptophan (5-HTP) to the mouse inhibits behavioural responding to the aversive situation of the light/dark test. The 5-HT_2 receptor antagonists reverse the 5-HTP induced inhibition to disinhibition, which in turn is blocked by 5-HT_4 receptor antagonists (Cheng *et al.*, 1994; Costall & Naylor, 1995). The present study investigates whether fluoxetine, by enhancing synaptic 5-hydroxytryptamine function (Stanford, 1996), may increase the efficacy of 5-HTP to disinhibit behaviour in the mouse light/dark test.

Male albino BKW mice (Bradford strain) weighing 25-30g received an intraperitoneal injection of vehicle + vehicle, vehicle + 5-HTP (0.01-50.0 mg kg^{-1}), vehicle + fluoxetine (10 mg kg^{-1}), vehicle + ritanserin (1.0 mg kg^{-1}), fluoxetine + 5-HTP, ritanserin + 5-HTP and fluoxetine + ritanserin + 5-HTP, fluoxetine + ritanserin + GR113808 + 5-HTP with 40 min between treatments and testing 40 min after the last treatment. Mice were placed into the centre of the light compartment of the test box and the latency of first movement from the light (L) to dark (D) compartment and line crossings were video recorded over a 5 min period (for detailed methodology see Cheng *et al.*, 1994). 15 animals were used for each treatment (in groups of 5) and the results analysed using one way ANOVA followed by Dunnetts t-test.

The first movement of vehicle treated control animals from the light to the dark compartment occurred within 10.4 ± 1.1s. The ED_{50} of 5-HTP to reduce this latency to 5.0s was 22.8 ± 4.9 mg kg^{-1}. A comparable value was attained in fluoxetine treated mice, 5-HTP again reducing the latency value with an ED_{50} of 25.1 ± 3.3 mg kg^{-1}. However, in the presence of ritanserin, the inhibitory effect of 5-HTP was abolished, and ritanserin reversed the effect to one of disinhibition; 5-HTP (50.0 mg kg^{-1}) increased the latency to a maximum of 28.0 ± 3.2 (s) with an ED_{50} value of 21.6 ± 2.5 mg kg^{-1}. In the presence of ritanserin + fluoxetine, 5-HTP again increased the latency value but with an ED_{50} dose of 5-HTP as low as 0.3 ± 0.05 mg kg^{-1}. Using a dose of 3.13 mg kg^{-1} 5-HTP to maximally increase the latency value (27.4 ± 2.4s) in the presence of ritanserin and fluoxetine, GR113808 (0.01 mg kg^{-1}) antagonised the disinhibitory effect, returning the latency value to control levels (11.2 ± 1.4s). All the described drug induced changes in latency measurements were obtained in the absence of non-specific changes in line crossings. The administration of vehicle + ritanserin or GR113808 or fluoxetine failed to modify mouse behaviour (latency values in the range 10.9 ± 1.3 to 11.5 ± 0.8s.

In the presence of ritanserin, fluoxetine enhanced the disinhibitory potency of 5-HTP 72-fold, the behavioural disinhibitory effects being mediated via a 5-HT_4 receptor. The failure of fluoxetine alone to enhance the inhibitory potency of 5-HTP may reflect an action of 5-HTP on both the 5-HT_2 ("inhibitory") and the 5-HT_4 ("disinhibitory") receptor mechanisms. Compensatory changes in the interaction between these two mechanisms and/or a low endogenous 5-HT tone may explain the failure of ritanserin, GR113808 or fluoxetine when administered alone to modify mouse behaviour in the light dark test box.

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Costall, B., Naylor, R.J. (1995) *Br. J. Pharmacol.* 116, 2989-2999.
Stanford, C. (1996) *TIPS* 17, 150-154.

352 P 5-HT4 RECEPTOR ANTAGONISTS ATTENUATE THE DISINHIBITORY EFFECTS OF DIAZEPAM IN THE MOUSE LIGHT/DARK TEST

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The 5-HT_3/5-HT_4 receptor antagonists SDZ205-557 and tropisetron attenuate the disinhibitory profile of diazepam and other putative anxiolytic agents in rodent models of anxiety (Costall & Naylor, 1993). In the present study we investigate the effect of the selective 5-HT_4 receptor antagonists RS23597-190 (Eglen *et al.*, 1993), GR113808 (Grossman *et al.*, 1993) and SB204070 (Wardle *et al.*, 1993) to modify the disinhibitory effects of diazepam in the mouse.

Male albino BKW mice (Bradford strain) weighing 25-30g received an intraperitoneal injection of vehicle or diazepam plus vehicle/RS23507-190/GR113808/SB204070 with 40 min between treatments and behavioural testing 40 min after the last treatment. Mice were placed into the centre of the light compartment of the test box and the latency of first movement from the light (L) to the dark (D) compartment and line crossings were video recorded over a 5 min period (for detailed methodology see Cheng *et al.*, 1994).

Diazepam increased the latency of first movement from the light to the dark compartment and this was antagonised by treatment with the three 5-HT receptor antagonists (which failed to modify behaviour in their own right), values returning to those shown in vehicle treated controls. The drug-induced changes in behaviour occurred in the absence of non-specific changes in line crossings, i.e. locomotor activity. The studies indicate a 5-HT_4 receptor involvement in diazepam induced behavioural disinhibition in the mouse test.

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Table 1. Effect of diazepam, 5-HT_4 receptor antagonists and their interaction in the mouse light/dark test

Treatment	Latency L>D (s)	Treatment	Latency L>D (s)
Vehicle + vehicle	12.4 ± 1.9	Diazepam (0.5 mg kg^{-1}) + vehicle	27.3 ± 2.8^{+}
Vehicle + RS23957-190 (0.001 mg kg^{-1})	11.6 ± 2.0	Diazepam + RS23957-190 (0.001 mg kg^{-1})	10.7 ± 1.4*
Vehicle + RS23957-190 (0.01 mg kg^{-1})	11.9 ± 1.4	Diazepam + RS23957-190 (0.01 mg kg^{-1})	11.2 ± 1.3*
Vehicle + GR113808 (0.001 mg kg^{-1})	10.3 ± 1.8	Diazepam + GR113808 (0.001 mg kg^{-1})	12.8 ± 1.9*
Vehicle + GR113808 (0.01 mg kg^{-1})	12.5 ± 1.5	Diazepam + GR113808 (0.01 mg kg^{-1})	10.2 ± 2.0*
Vehicle + SB204070 (0.001 mg kg^{-1})	11.0 ± 1.3	Diazepam + SB204070 (0.001 mg kg^{-1})	11.5 ± 1.2*
Vehicle + SB204070 (0.01 mg kg^{-1})	10.8 ± 1.6	Diazepam + SB204070 (0.01 mg kg^{-1})	11.7 ± 1.7*

Values (mean ± s.e. mean) (n = 10). *P<0.01 compared to diazepam + vehicle control; $^{+}$P<0.01 compared to vehicle + vehicle (one way ANOVA followed by Dunnett's t test).

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353 P MINERALOCORTICOID RECEPTOR BLOCKADE AMELIORATES RETENTION DEFICITS IN A LEARNING TASK INDUCED BY CHOLINERGIC BLOCKADE

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Central cholinergic (ACh) blockade with scopolamine (SCOP) produces profound cognitive impairments in human and animal subjects (Fibiger, 1991). Our previous research demonstrated that hippocampal ACh blockade increased corticosterone (CORT) secretion in rats (Bhatnagar et al., 1994). In the present study we investigated whether or not intrahippocampal infusions of the mineralocorticoid receptor (MR) antagonist spironolactone (SPIRO) would affect SCOP-induced cognitive impairments in a water maze task.

Adult male, Lister hooded rats (350-500 g) were implanted bilaterally with hippocampal cannulae (A-P -3.3; M-L ±2.5; D-V -2.3 mm) under pentobarbital anaesthesia (60 mg/kg IP), 3 weeks prior to testing. On the first test day, rats were infused with 3µl vehicle (VEH; artificial CSF with 5% ethanol), or 150 ng of SPIRO, 20 min before being injected with either VEH or 2.0 mg/kg SCOP IP and tested 10 min later in a water maze (n=6/group). Rats were given 8 trials of training to locate the platform, with each trial limited to a 60 sec maximum. On the next day, rats were tested for their retention of the task. Data (latencies to locate the platform, aggregates of 2 trials blocked together) were assessed by ANOVA (applying a Greenhouse-Geiser correction) and *post hoc* testing was performed using Bonferroni corrected t-tests.

ANOVA on the latency data revealed a significant effect of SCOP treatment $F(1,20)=13.02$, $P<.002$. As shown in Fig. 1A, SCOP treatment significantly impaired acquisition of the task compared to the VEH group ($P<.05$). Analysis of retention data revealed a significant 3-way pretreatment x test drug x trial block interaction $F(3,20)=3.33$, $P<.05$. As illustrated in Fig. 1B, rats previously treated with SCOP still displayed impaired performance over trial blocks 3-4 compared to all other groups (P's<.01). The most significant result was that rats infused with SPIRO prior to SCOP were not different from controls. Thus, while SCOP-induced acquisition deficits were unaffected by SPIRO administration, SPIRO very effectively ameliorated the long-term retention deficit normally observed following SCOP treatment.

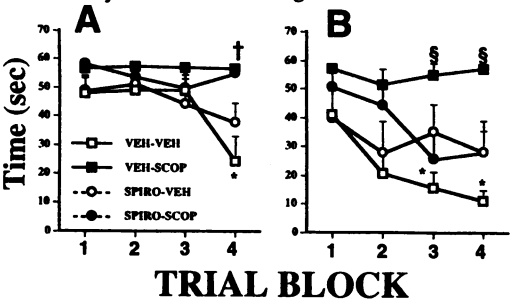

Fig. 1. Effects of SCOP, SPIRO or SPIRO+SCOP on latencies to locate the platform during the acquisition (A) or retention (B) phases of a water maze task.
*significantly different from VEH, trial block 1 (P<.01)
†significantly different from VEH, trial block 4 (P<.01)
§significantly different from all other groups (P's<.01-.05)

The fact that the MR antagonist SPIRO can prevent retention deficits produced by SCOP suggests that CORT modifies cognitive function via hippocampal MR. The increase in CORT secretion elicited by SCOP may contribute to its learning impairments. In conclusion, SPIRO ameliorates retention deficits produced by cholinergic blockade.

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354 P 7-OH-DPAT INDUCES BIPHASIC EFFECTS ON LOCOMOTOR BEHAVIOUR IN THE COMMON MARMOSET

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The putative D_3 receptor agonist 7-OH-DPAT exhibits a biphasic effect on locomotor activity in the rat (Daly & Waddington, 1993) and induces emesis in the dog (McDermed *et al.*, 1975). The studies presented here investigate the effects of 7-OH-DPAT on locomotor behaviour and emesis in the marmoset and examine the ability of selective dopamine D_2 receptor antagonists to attenuate these responses to 7-OH-DPAT.

Subjects were four adult (290-380g) male common marmosets (*Callithrix jacchus*). All drugs were given by subcutaneous (s.c.) injection (1 ml/kg) except domperidone which was given orally (2 ml/kg). Animals received 7-OH-DPAT (0.01-0.4 mg/kg) or vehicle (0.9% saline) and were placed immediately in individual observation cages (dimensions 76 x 50 x 61 cm fitted with two 50 cm perches); nausea-specific behaviours and emesis were recorded over a 15 min treatment period using a 7-point scoring protocol (Costall *et al.*, 1986). Locomotor behaviours were analysed 15 min after 7-OH-DPAT or vehicle treatment by assessment of the total number of jumps, rears and perch crossings over a 15 min test period. Antagonists were given 45 min prior to 7-OH-DPAT or vehicle treatment; behaviour was assessed as above. Data are shown as mean ±s.e.m total locomotor behaviours/15 min (LA/15 min) and mean± s.e.m total nausea score (E/15 min). Data were analysed by one-way ANOVA with Dunnett's t-test.
7-OH-DPAT (0.01-0.4 mg/kg) produced a significant effect on locomotor behaviours in the marmoset ($F(3,23)=13.2$, $p<0.001$). At 0.025 mg/kg 7-OH-DPAT significantly reduced locomotor behaviours (2.0±1 LA/15 min; $p<0.001$) compared to vehicle (25±2 LA/15 min) and this response was not antagonised by raclopride 0.1 mg/kg (5±3 LA/15 min) or sulpiride 10 mg/kg (4±2 LA/15 min). In contrast, higher doses of 0.1-0.4 mg/kg 07-OH-DPAT significantly increased locomotor behaviours (352±68-168±40 LA/15 min; $p<0.001$) compared to vehicle (25±2 LA/15 min). The response to 7-OH-DPAT (0.1 mg/kg) (352±68 LA/15 min; $p<0.001$) was significantly ($p<0.001$) antagonised by raclopride 0.1 mg/kg (5±5 LA/15 min) and sulpiride 10 mg/kg (31±19 LA/15 min) but not domperidone 2 mg/kg (195±30 LA/15 min). Antagonist treatment alone did not significantly alter locomotor behaviours. Nausea and emesis were observed following 7-OH-DPAT 0.05-0.4 mg/kg (4±0.3-7±0 E/15 min). The nausea response to 7-OH-DPAT 0.1 mg/kg (6±0.3 E/15 min) was significantly ($p<0.001$) reduced by raclopride 0.1 mg/kg (0.5±0.3 E/15 min) and sulpiride 10 mg/kg (0.3±0.3 E/15 min); domperidone 2 mg/kg failed to reduce nausea (6±0.5 E/15 min). All antagonists abolished the emetic response to 7-OH-DPAT.

These studies have demonstrated a biphasic effect of 7-OH-DPAT on locomotor behaviour in the marmoset. Selective centrally active D_2 receptor antagonists attenuated the stimulatory action but failed to reverse the inhibitory action of 7-OH-DPAT on locomotion. This data supports previous work in the rat (Storey *et al.*, 1995) and may indicate a differential action of low and high doses of 7-OH-DPAT atD_3 and D_2 receptors respectively. In addition, reversal of the emesis and nausea responses to 7-OH-DPAT by D_2 receptor antagonists suggests a non-discriminatory agonist action of 7-OH-DPAT at D_2 receptors involved in emesis.

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McDermed, J.D., McKenzie, G.M., Phillips, A.P. (1975) *J. Med. Chem.* 18, 362-367

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355 P GLUTAMIC ACID DECARBOXYLASE 65 AND 67 mRNA IN THE PALLIDUM OF NORMAL MONKEYS EXHIBITING L-DOPA-INDUCED DYSKINESIAS

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It has been reported that gene expression of glutamic acid decarboxylase (GAD) 67 is elevated in the internal pallidum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys; an effect abolished by L-DOPA treatment (Herrro et al., 1996). We now report on the expression of GAD 65 and 67 mRNA in the pallidum of normal monkeys chronically treated with L-DOPA some of which displayed severe dyskinesias.

Six normal macaque monkeys (*Macaca fascicularis*) aged from 18 to 36 months, 5 males and 5 females, weighing 3 to 4 kg, were treated with L-DOPA (80 mg/kg) plus carbidopa (20 mg/kg) by gavage once daily for 13 weeks while a control group (n=4) received vehicle treatment. The animals were observed by a clinical neurologist for the appearance of dyskinesias which were graded as mild, moderate or severe. At the end of drug treatment, the animals were given a lethal dose of barbiturate and the brains were sectioned (12µm) coronally and frozen to -70°C. Using GAD 65 and 65 cRNA probes (generously provided by N.J.K. Tillikaratne et al., UCLA), nonradioactive *in situ* hybridization study of brain striatal sections for GAD65 and 67 was performed (Augood & Emson, 1994). The number of GAD65 and 67 mRNA-containing cells in thepallidum were counted manually using an eye-piece counting grid by an investigator blind to the treatment. Results were analysed by paired Student's *t* test.

In control animals, the number of GAD 65 and 67 mRNA-containing cells tended to be greater in the external globus pallidus (Gpe) than the internal globus pallidus (Gpi), but this did not reach statistical significance (Table 1). The number of GAD 65 and 67 mRNA-containing cells in the Gpe and Gpi of L-DOPA-treated monkeys tended to be increased, but again they did not reach statistical significance. GAD65 mRNA-containing cells were increased in Gpe, but decreased in the Gpi, although no significant, in L-DOPA-treated monkeys exhibiting dyskinesias when compared to the monkeys without dyskinesias after L-DOPA treatment. However, no such alteration was observed in the number of GAD67 mRNA-containing cells.

The increase in GAD 65, but not GAD67 mRNA in Gpe and, decrease in Gpi of dyskinetic monkeys, suggest that L-DOPA may differentially regulate pallidal GAD65 and 67 mRNA expression. This may indicate that the elevation of GABA activity in Gpe inhibits the activity of subthalamic neurons which is known to correlate with the occurrence of the dyskinesia. (Crossman, 1990).

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Table 1. Effect of L-DOPA on pallidal GAD65 and 67 mRNAs-containing cells in the monkeys with or without dyskinesias (mean±sem)

	Normal monkeys (n=4)		L-DOPA treated monkeys (n=6)					
			All animals (n=6)		without dyskinesias (n=3)		with severe dyskinesias (n=3)	
	GPi	GPe	GPi	GPe	GPi	GPe	GPi	GPe
GAD65	262±16	398±51	339±41	465±31	399±66	412±21	279±26	518±41
GAD67	307±13	390.±38	341±36	418±34	378±63	477±42	304±34	360±27

356 P PROLONGATION OF QTc INTERVAL BY KETOCONAZOLE IN CONSCIOUS GUINEA-PIGS IMPLANTED WITH ECG TELEMETRY TRANSDUCERS

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Concern has been expressed in recent years over the incidence of torsades de pointes in patients receiving certain 'second generation' antihistamines, such as terfenadine. Co-therapy with the antifungal agent, ketoconazole, has been contraindicated, as ketoconazole inhibits their metabolism (Botstein, 1993). A recent study (Hey *et al.*, 1996) examined interactions between ketoconazole (400 mg/kg, p.o.) and antihistamines (terfenadine and ebastine) in guinea-pigs, and reported that neither ketoconazole nor the antihistamines had direct effects on QTc interval or heart rate, but when administered 2 h after ketoconazole, both antihistamines caused prolongation of QTc and a bradycardia. Since this dose of ketoconazole is more than twice the LD_{50} in this species (Heel, 1982), we have re-examined the direct effects of this large dose of ketoconazole in conscious, telemetered guinea-pigs.

Six male Dunkin-Hartley guinea-pigs were anaesthetized with halothane and implanted with lead II ECG telemetry transducers (Data Sciences, type TA10CA-F40), using strict aseptic techniques. 12-18 Days later, the guinea-pigs were dosed with vehicle (0.5% w/v methylcellulose/0.1% v/v Tween 80; 5 ml/kg, p.o.). ECG samples (10 s every 5 min; sampling frequency 1000 Hz, filter cut-off 30 Hz) were taken for 1 h before and 4 h after dosing; QTc was derived by Bazett's formula ($QTc = QT/\sqrt{RR}$). Two days later the experiment was repeated with ketoconazole (400 mg/kg, p.o.). At the time of dosing the animals weighed 432-606 g.

Ketoconazole (400 mg/kg, p.o.) caused a bradycardia (e.g. at 4 h post-dose: vehicle = 249 ± 3 b.p.m.; ketoconazole = 162 ± 9 b.p.m.; $P < 0.001$, 2-tailed paired *t*-test), together with a progressive prolongation of QTc (Fig 1):

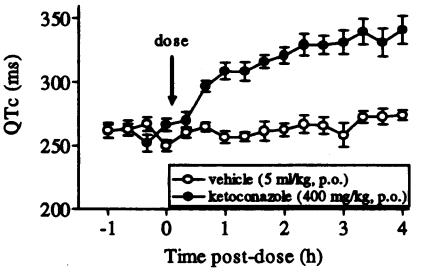

Figure 1: Increase in QTc after ketoconazole (400 mg/kg, p.o.). P < 0.001; 2-tailed paired t-test on AUCs 0-240 min post-dose.

Our demonstration of a direct effect of ketoconazole on heart rate and QTc contrasts with the report of Hey *et al.* (1996). The magnitude of the ketoconazole effect in the present study is similar to their reported effect of ketoconazole + terfenadine. Our results suggest that a direct effect of the large dose of ketoconazole used could account for their findings. Future studies should examine the interaction of lower doses of ketoconazole with antihistamines.

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357 P EFFECTS OF CHRONIC ANTIDEPRESSANT ADMINISTRATION ON GENE EXPRESSION OF THE VOLTAGE-DEPENDENT POTASSIUM CHANNEL SUBUNIT Kv 4.2 IN THE RAT HIPPOCAMPUS

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Recent behavioural and neurochemical work has raised the possibility that regulation of potassium channels may be among the many long-term targets of antidepressant treatments (Wang & Grahame-Smith, 1992). More specifically, in a previous study we have shown that hippocampal 5-HT release, when stimulated by 4-aminopyridine, a potent blocking agent of voltage dependent potassium channels, was significantly enhanced in rats which had received long term lithium diet (Pei et al., 1995). The aim of the present study was to investigate the effects of various antidepressant treatments on mRNA levels of the voltage sensitive potassium channel subunit Kv 4.2, which normally occurs in high abundance in hippocampal cell layers (Sheng et al., 1992). For this purpose we have used *in situ* hybridization histochemistry (ISHH) to study the effect of electroconvulsive shock (ECS), long term lithium diet or chronic fluoxetine injections on Kv 4.2 gene expression in rat hippocampus.

Male Sprague-Dawley rats (250-270g) were used in all experiments. In the ECS study rats were anaesthetised with halothane and received either chronic ECS (150V, 50Hz, 1s, via an ear clip electrode, 5 shocks over 10 days) or a single shock. Control rats were anaesthetised with halothane and had electrodes placed but no current delivered. For the lithium experiments rats were fed pellets containing 0.1% Li_2CO_3 for 21 days. In the fluoxetine group rats were daily injected with the drug (5 mg/kg, ip) for 21 days. Rats in all three administration groups were sacrificed 24 h after the last procedure. Hippocampal sections (12 µm) were mounted on slides and pre-treated for ISHH using a standard procedure. Following pre-treatment, an oligonucleotide complementary to the the rat voltage dependent potassium-channel subunit Kv4.2 gene were 3'-tail labelled with [^{35}S]-dATP and added to the hippocampal sections. The relative abundance of Kv 4.2 mRNA in hippocampus was determined by densitometric quantification of autoradiograms. Values are expressed as a percentage of mean optical densities from the matched control groups. Statistical analyses of mRNA changes were performed using one way ANOVA and Dunnetts t-test.
Following chronic ECS, the relative abundance of Kv 4.2 mRNA in the dentate gyrus was increased (145±7.2%, mean±s.e.m., n=6, $p<0.01$) compared to the control group, an effect not seen in any of the other subfields of the hippocampus. In comparison, mRNA levels for Kv 4.2 in the dentate gyrus from rats which had received a single ECS 24 h previously were unaltered. Long term lithium diet did not affect mRNA levels for Kv4.2 (95±8.5%, n=5) compared to rats receiving the normal food pellets. In addition chronic fluoxetine administration did not alter the mRNA levels for Kv 4.2 (108±6.9%, n=5) compared to saline injected control rats.In summary these data show that chronic ECS, but not a single shock, increases hippocampal mRNA levels of the voltage dependent potassium channel unit Kv 4.2. This appears to be restricted to the dentate gyrus and may indicate an adaptive response. Neither chronic lithium diet or fluoxethine injections produced this change, suggesting that the alteration of Kv 4.2 gene expression may be unique to ECS administration.

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358 P BINDING TO THE HIGH- AND LOW-AFFINITY STATES OF RAT RECOMBINANT α2-ADRENOCEPTOR SUBTYPES

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Three α_2-adrenoceptor genes termed RG20, RNG and RG10 have been identified in rat. These correspond to the pharmacologically defined α_{2A}, α_{2B} and α_{2C} receptors (Bylund, 1992). The binding properties of each receptor were investigated using murine erythroleukaemia (MEL) cell lines stably transfected to express each receptor. Binding was conducted in 50mM Tris buffer (pH 7.4) containing 10mM $MgCl_2$ and 1mM EDTA using membranes prepared from each cell line. Incubations were conducted at 25°C for 60 min. Two different radioligands were studied: 2nM [^{3}H]UK14304 (Kd = 5.3, 22.0 and 2.0 nM for α_{2A}, $_B$ and $_C$ respectively) or 2nM [^{3}H]Rx821002 in the presence of 0.1mM GppNHp (Kd = 1.4, 5.4 and 1.6 nM). Incubations were conducted in 96-well plates and were terminated by filtering through GF/C filters using a Packard Filtermate cell harvester.

α_2-Adrenoceptor agonists such as noradrenaline and UK14304 displaced [^{3}H]UK14304 with higher affinity than when [^{3}H]Rx821002 was used as the radioligand. The antagonists prazosin and rauwolscine exhibited the opposite selectivity. This supports the hypothesis that the ratio of affinities of compounds for the high- and low-affinity conformations of a receptor predict intrinsic efficacy (Harley et al., 1995). The results also show that the rank order of high/low affinity ratios for the compounds tested were similar for the α_{2A} and α_{2C} receptors. In contrast, the compounds showed a different rank order at the α_{2B} receptor and the high/low affinity ratios were considerably lower.

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Table 1: Ki values for the displacement of [^{3}H]UK14304 and [^{3}H]Rx821002.

	[^{3}H] UK14304 Ki (nM)			[^{3}H] Rx821002 Ki (nM)			Ki Rx / Ki UK		
	α_{2A}	α_{2B}	α_{2C}	α_{2A}	α_{2B}	α_{2C}	α_{2A}	α_{2B}	α_{2C}
Noradrenaline	13.1 ± 3.3	13.1 ± 2.9	2.5 ± 0.5	5280 ± 706	413.0 ± 60.4	1095 ± 125	403.5	31.5	439.8
UK 14304	1.9 ± 0.03	13.4 ± 1.3	1.3 ± 0.1	439.6 ± 48.3	585.0 ± 8.8	343.2 ± 21.1	226.7	43.8	267.5
ST91	24.6 ± 3.9	48.5 ± 12.0	9.3 ± 1.1	3265 ± 319	666.7 ± 32.3	616.3 ± 35.5	132.5	13.8	66.4
Xylazine	74.6 ± 1.3	269.5 ± 31.3	57.9 ± 8.2	8614 ± 548	1748 ± 343	4259 ± 596	115.5	6.5	73.5
Clonidine	1.9 ± 0.2	18.9 ± 0.5	6.1 ± 0.2	150.1 ± 16.5	100.6 ± 3.3	137.8 ± 4.5	80.9	5.3	22.7
Oxymetazoline	7.6 ± 1.7	22.1 ± 8.1	9.2 ± 2.6	92.9 ± 9.4	929.3 ± 123.7	218.2 ± 14.6	12.3	42.0	23.7
Prazosin	2837 ± 123	109.4 ± 8.8	154.4 ± 16.2	1741 ± 123	53.4 ± 4.9	82.9 ± 7.7	0.6	0.5	0.5
Rauwolscine	534.0 ± 98.1	48.9 ± 2.4	7.2 ± 0.9	157.1 ± 17.9	9.3 ± 2.0	2.7 ± 0.5	0.3	0.2	0.4

Values are means ± s.e. mean from at least 3 experiments each performed in triplicate.

359 P WHOLE-CELL RADIOLIGAND BINDING ASSAY REVEALS α1L-ADRENOCEPTOR (AR) ANTAGONIST PROFILE FOR THE HUMAN CLONED α1A-AR IN CHINESE HAMSTER OVARY (CHO-K1) CELLS

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Several α_1-AR antagonists display varying affinities for the human cloned α_{1A}-AR expressed in CHO-K1 cells, dependent upon the nature of the investigation. For example, prazosin and RS-17053, but not tamsulosin and indoramin, antagonize functional responses (inositol phosphates accumulation) with affinity estimates significantly below those estimated from radioligand binding studies (Ford *et al.*, 1996). This observation appears unique to the α_{1A}-AR, as functional and binding studies of α_{1B}- or α_{1D}-AR expressed in CHO-K1 cells reveal equivalent affinity estimates. In this study, the effect of binding assay conditions on antagonist affinity estimates has been investigated.

Binding affinities (pK_i) were estimated from competition studies using [^{3}H]prazosin (20°C) or [^{3}H]tamsulosin (37°C) in CHO-K1 cells expressing the human α_{1A}-AR, as membrane homogenates or whole cells. The assay buffer was either 50 mM Tris-HCl / 1 mM EDTA or Ham's culture medium (both pH 7.4). Incubations were performed at 20°C for 70min or 37°C for 30min.

As previously reported (Ford *et al.*, 1996) binding studies in Tris buffer at 20°C revealed a pharmacological profile typical of the classically-defined α_{1A}-AR (Table 1) with the antagonists RS-17053, WB 4101, 5-methylurapidil, REC 15/2739 and *S*-niguldipine all showing high affinity (pK_i>9). This profile differs markedly from that obtained in functional studies (inositol phosphates accumulation, Ford *et al.*, 1996; see Table1).

When binding was conducted in Ham's medium at 37°C, the correlation between binding and functional affinities was improved (sum of squares of differences in affinity [ssq] = 1.9 compared with 7.6 for Tris buffer at 20°C). Additional modification of the binding assay by the use of whole cells further improved the correlation with functional data (ssq=0.9), pK_i values for some antagonists (e.g. RS-17053, *S*-niguldipine) being greatly affected, whereas others (tamsulosin, indoramin) were essentially unchanged (Table 1).

Table 1. Affinity estimates at cloned α_{1A}-AR

ANTAGONIST	α_{1A}-CHO binding (pK_i) Tris/EDTA 20°C[a] Membranes	Ham's 37°C Membranes	Ham's 37°C Cells	pK_b α_{1A}-CHO InsPs[a]
Prazosin	9.9 ±.1	9.2 ±.1	8.4 ±.1	8.7 ±.1
RS-17053[a]	9.2 ±.1	8.9 ±.1	7.9 ±.1	8.3 ±.1
WB 4101[a]	9.8 ±.1	9.3 ±.1	8.9 ±.1	8.8 ±.1
5-Me-urapidil	9.2 ±.1	8.3 ±.1	8.1 ±.1	8.1 ±.1
S-Niguldipine	9.9 ±.2	9.1 ±.1	7.9 ±.1	8.2 ±.3
Tamsulosin	10.4 ±.2	10.0 ±.1	10.0 ±.1	10.2 ±.1
REC 15/2739[a]	9.7 ±.2	9.2 ±.1	8.6 ±.2	9.3 ±.1
Indoramin	8.4 ±.1	8.1 ±.1	8.3 ±.1	8.4 ±.1
ssq vs. InsPs	7.6	1.9	0.9	

Values shown are means ± s.e. mean, n≥3; [a]Ford *et al.*, 1996

The whole cell binding data also show good agreement with antagonist affinities estimated from *in vitro* studies of human prostatic tissue (Ford *et al.*, 1996) (ssq=1.2 compared to 12.9 using standard membrane homogenate binding assay).

Thus, in binding studies of the human cloned α_{1A}-AR, temperature and cellular integrity appear to influence affinity estimates for several key α_{1A}-AR antagonists, and α_{1L}-AR pharmacology is revealed. These data emphasise the importance of conducting pharmacological studies under optimal physiological conditions whenever possible.

Ford, APDW, Daniels, DV, Chang, DJ *et al.* (1996) *Br. J. Pharmacol.*, **118**, 29P.

360 P FUNCTIONAL PHARMACOLOGICAL CHARACTERISTICS OF HUMAN, RAT AND RABBIT CLONED α1A-ADRENOCEPTORS EXPRESSED IN CHINESE HAMSTER OVARY (CHO-K1) CELLS

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Recent data from stably transfected CHO-K1 cells have indicated that a different profile of antagonist affinities is obtained upon functional (second messenger) analysis from that observed in radioligand binding for the human cloned α_{1A}-adrenoceptor, but not the α_{1B} or α_{1D}-adrenoceptors (Ford *et al.*, 1996a). Furthermore, this distinct functional pharmacological profile of the α_{1A}-adrenoceptor resembles closely that of the so-called α_{1L}-adrenoceptor, proposed to mediate, for example, contraction of lower urinary tract tissues of rabbit and man (Ford *et al.*, 1996b). In fact, a functional pharmacological profile consistent with the classically defined α_{1A}-adrenoceptor has been well characterized solely in isolated tissues from rat (Ford *et al.*, 1996b).

In the present study, the functional antagonist affinity profiles of the cloned α_{1A}-adrenoceptors from rat, rabbit and man have been compared, by studying inhibition of noradrenaline-stimulated [^{3}H]inositol phosphates accumulation in stably transfected CHO-K1 cells, as described previously (Ford *et al.*, 1996a).

Noradrenaline concentration-dependently stimulated [^{3}H]inositol phosphates accumulation in CHO-K1 cells expressing α_{1A}-adrenoceptors. As shown in Table 1, functional estimates of affinity (pK_b) for six key antagonists for rat and rabbit α_{1A}-adrenoceptors equated well with those observed for the human α_{1A}-adrenoceptor. Accordingly, prazosin (PRA), RS-17053 (RS), WB 4101 (WB) and 5-me-urapidil (5MU) all displayed lower affinity than observed in radioligand binding studies of the human cloned α_{1A}-adrenoceptor. Tamsulosin (TAM) and indoramin (IND), by contrast, displayed functional affinity estimates for α_{1A}-adrenoceptors from the 3 species which were consistent with those obtained in radioligand binding studies.

Table 1. Antagonist affinity estimates and noradrenaline potency for cloned α_{1A}-adrenoceptors from man, rat and rabbit.

	[a] pK_i	α_{1A} [^{3}H]InsPs (pK_b)		
ANTAGONIST	HUMAN (binding)	HUMAN	RAT	RABBIT
Prazosin	9.9 ±.0	8.7 ±.1	8.9 ±.1	8.7 ±.1
RS-17053[a]	9.3 ±.1	8.2 ±.1	8.2 ±.1	7.9 ±.1
WB 4101[a]	9.8 ±.1	8.9 ±.1	9.1 ±.1	8.6 ±.2
5-Me-urapidil	9.2 ±.1	8.2 ±.1	8.6 ±.1	8.2 ±.1
Tamsulosin	10.4 ±.2	10.5±.1	10.6 ±.1	10.3 ±.2
Indoramin	8.4 ±.1	8.4 ±.1	8.1 ±.1	8.3 ±.1
Noradrenaline	($p[A]_{50}$)	6.6 ±.1	6.7 ±.1	6.7 ±.1

means ± s.e. mean shown; n≥3; [a]Ford *et al.*, 1996b

These data indicate that the cloned α_{1A}-adrenoceptors from rat and rabbit, like the cloned α_{1A}-adrenoceptor from man, display a pharmacological profile typical of the α_{1L}-adrenoceptor upon functional analysis in CHO-K1 cells.

Although studies in isolated tissues from rat have successfully generated α_{1A}-adrenoceptor pharmacological profiles using these antagonists, clear α_{1A}-adrenoceptor characterizations in tissues from rabbit and man have been less evident (see Ford *et al.*, 1996b). Data from the present study suggest that the primary sequence of the rat α_{1A}-adrenoceptor does not in itself confer a tendency to yield classical α_{1A}-adrenoceptor pharmacology in functional studies.

Ford, APDW, Daniels, DV, Chang, DJ *et al.* (1996a) *Br. J. Pharmacol.* **118**, 29P.
Ford, APDW, Arredondo, NF, Blue, DR *et al.* (1996b) *Mol. Pharmacol.* **49**, 209-215.

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361 P ROLIPRAM-INHIBITABLE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) IN RAT ADENOHYPOPHYSIS: POTENTIAL FUNCTIONAL ROLE IN CORTICOTROPHS AND SOMATOTROPHS

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Phosphodiesterase activity in anterior pituitary corticotroph cells is not well characterised. Rolipram is a highly selective blocker of Type IV (cAMP specific) PDE, and has been used as a therapeutic agent in mood disorders and experimental models of autoimmune diseases (Müller et al,1996). Both of these conditions may be ameliorated by hypercortisolaemia, and hence we have analysed the effects of rolipram on corticotrophin releasing-factor (CRF41) induced cAMP responses and PDE activity in rat anterior pituitary gland as well as the mouse corticotroph tumour cell line AtT20.

In acutely dispersed anterior pituitary as well as AtT20 cells rolipram enhanced the cAMP response evoked by 0.3nM CRF41 in a concentration dependent manner. The minimal effective concentration was 3μM. Concentrations higher than 100μM were not used as rolipram is unlikely to be selective above this concentration. The cAMP response to growth hormone releasing hormone was also enhanced to 200% of control by 30 μM rolipram suggesting that corticotrophs are not the only pituitary target of this compound.

PDE activity in cell and tissue homogenates was measured by a radiometric assay based on $BaSO_4$ precipitation. The PDE activity of AtT20 cells (0.35±0.05 nmol/min/mg protein, mean±SEM, n=3, 1μM cAMP substrate) could be differentiated into Ca^{2+}/calmodulin (CaM)-independent and Ca^{2+}/CaM-stimulated (100μM Ca^{2+}/100nM CaM) (PDE1) activities, which in unstimulated cells constituted 30% and 70% of the total PDE activity, respectively. Kinetic studies showed that the PDE1 and PDE4 activities have a Km of 1.6μM and 1.1μM, respectively. Up to 70% of the Ca^{2+}/CaM-independent activity (i.e. c. 20% of total) could be inhibited by 10μM rolipram (IC_{50}= 200nM or 10 nM depending on the techniques of homogenisation) and is defined as PDE4 activity. Study of the subcellular distribution showed that more than 90% of PDE1 activity is in the soluble fraction, while PDE4 activity is equally distributed in the soluble and particulate fractions. Subsequent analysis was carried out in the soluble fractions as well as whole homogenate using 1μM cAMP substrate. Treatment with 1mM 8-(4-Chloro-phenylthio)-cAMP (CPT-cAMP) resulted in the activation of PDE4 to 225±35% and the inhibition of PDE1 to 30±9% (mean±SEM, n=3, 1μM cAMP substrate) of control, so that PDE4 accounted for over 50% of total activity. Incubation of cell homogenate with the catalytic subunit of protein kinase A (PKA) gave similar changes in PDE activity. The reduction of PDE1 activity was partly due a decrease in V_{max} from 0.5 to 0.2nmol/min/mg protein as well as a 10-fold reduction in EC_{50} of CaM activation. Both the effect of CPT-cAMP in intact cells and the action of PKA in homogenates were blocked by 10μM H-89. The PDE activity in homogenates of rat anterior pituitary tissue was also mainly Ca^{2+}/CAM-dependent (75%) and 67% of the Ca^{2+} independent activity (20% of total) could be blocked by rolipram (IC_{50}=200 nM). Over 90% of PDE1 was in the soluble fraction, while rolipram inhibitable activity was roughly equally distributed between the soluble and the particulate fractions.

These studies are the first direct demonstration of rolipram inhibitable PDE activity in the adenohypophysis and lend support to previous reports showing rolipram-induced enhancements of plasma corticotrophin and adrenal corticosteroid *in vivo*, and the stimulation of corticotrophin secretion *in vitro* (Kumari et al,1993; Pettipher et al.,1996). Furthermore observations in AtT20 cells show dynamic changes in PDE activities upon activation by cAMP generating agents.

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362 P POSITIVE ALLOSTERIC MODULATION OF RECOMBINANT GLYCINE AND $GABA_A$ RECEPTORS BY GENERAL ANAESTHETICS: A COMPARATIVE STUDY

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The behavioural effects of many general anaesthetics may be mediated, at least in part, through an enhancement of inhibitory GABAergic activity (Franks & Lieb, 1994). Propofol, depressant barbiturates, etomidate and neurosteroids, share the property of enhancing GABA-evoked currents recorded from *Xenopus laevis* oocytes expressing recombinant $GABA_A$ receptors (Lambert *et al.*, 1995; Belelli *et al.*, 1996). However, inhibitory neurotransmission in the central nervous system is also mediated via strychnine-sensitive glycine receptors, predominantly in brainstem and spinal cord, where glycine receptors are prevalent, but in higher regions also (Betz, 1991). Given the close structural and functional relationship between the two classes of receptor, potentiation of glycinergic activity may potentially contribute to the mechanism(s) of action of general anaesthetics (Harris *et al.*, 1995). Here, we have determined the effects of the structurally diverse anaesthetics propofol, pentobarbitone, etomidate, 5α-pregnan-3α-ol-20-one (5α3α) and δ-hexachlorocyclohexane (δ-HCH) on human recombinant $\alpha_3\beta_1\gamma_2$ $GABA_A$ receptors in comparison with their actions on glycine receptors (human α_1, rat β) by utilizing the *Xenopus laevis* oocyte expression system and two electrode voltage-clamp techniques. All results reported below are the mean ± s.e.m. of 3-5 experiments conducted at room temperature (18-23°C). For cRNA ($GABA_A$) or cDNA (glycine) injected oocytes, voltage-clamped at a holding potential of -60mV, bath applied GABA or glycine evoked concentration-dependent inward current responses with EC_{50} values of 102±18μM and of 70±3μM respectively. Utilizing the appropriate agonist EC_{10} (Belelli *et al.*, 1996), the modulatory effects of anaesthetics on GABA- or glycine-evoked currents were determined as a percent of the current evoked by saturating concentrations of the agonist (I_{max}; see Table 1). Our results demonstrate that although some compounds are relatively selective for $GABA_A$ receptors (pentobarbitone, etomidate, 5α3α), a modulation of glycine receptor activity may contribute to the behavioural actions of propofol and δ-HCH.

Table 1. Modulation of $GABA_A$ and glycine receptors by general anaesthetics

Compound	$GABA_A$ receptor (EC_{50} and (I_{max}))	Glycine receptor (EC_{50} and (I_{max}))
Propofol	3.4±0.1μM (108±18%)	28±2μM (98±6%)
Pentobarbitone	44±5μM (110±17%)	757±39μM (50±9%)
Etomidate	8.1±0.9μM (75±8%)	10±1μM (29±4%)
5α3α	73±1nM (67±7%)	inactive (≤10μM)
δ-HCH	3.5±0.4μM (43±4%)	26±3μM (80±7%)

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363 P BICUCULLINE-SENSITIVE AND -INSENSITIVE EFFECTS OF PREGNANOLONE AND ALPHAXOLANE ON [^{35}S]-TBPS BINDING TO THE PICROTOXIN SITE ON THE $GABA_A$ RECEPTOR

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Neuroactive steroids, that include 5β-pregnan-3α-ol-20-one (pregnanolone) and 5α-pregnan-3α-ol-11,20-dione (alphaxalone), a synthesized analogue, are known to act at the $GABA_A$ receptor complex and modulate GABA responses. GABA-evoked currents and [^{3}H]-muscimol binding are potentiated (Harrison & Simmonds 1984, Peters *et al* 1988). Binding experiments have shown that the same steroids inhibit specific binding of [^{35}S]-TBPS, a ligand that labels the convulsant picrotoxinin site (Majewska *et al* 1986). GABA also inhibits [^{35}S]-TBPS binding and it is not clear to what extent the neurosteroid effect is dependent upon the inhibition by GABA.

We have, therefore, investigated the influence of the competitive GABA antagonist bicuculline (100 μM) on the modulation of the picrotoxinin site by pregnanolone and alphaxalone. Membranes were prepared from cerebral cortex of male Wistar rats (200-250 g). The 'buffy' coat fraction (Zukin *et al* 1974) was obtained following exposure to distilled water and washed three times with a 5 mM Tris-HCl buffer containing 1mM EDTA at pH 7.4 prior to storage at -20^0C until required. On the day of assay, the membranes were washed twice more with distilled water followed by two washes using assay buffer, 50 mM Tris-HCl with 150 mM NaCl. This repeated washing was intended to remove as much endogenous GABA as possible. Saturation studies were performed using 0.3-150 nM [^{35}S]-TBPS for 180 min at room temperature. Non-specific binding was determined using 100μM picrotoxinin. The membranes were harvested by filtration and the bound radioactivity determined by liquid scintillation counting. Steroids were initially dissolved in DMSO which was present at a final concentration of 0.45% in all cases.

Scatchard plots of [^{35}S]-TBPS binding yielded a best fit to a single component with a K_D of 24.5±2.5 nM and a Bmax of 507.3±37.8 fmol.mg^{-1} protein (n = 7). Pregnanolone 10μM reduced the affinity for [^{35}S]-TBPS (K_D = 37.1±6.1 nM) and at the same time increased the maximum binding (Bmax = 842.4±133.6 fmol.mg^{-1} protein) (n = 4, P = 0.049 and 0.014, respectively, by Student's *t*-test). In the presence of bicuculline 100μM, the K_D was 43.8±1.4 nM and the Bmax 697.3 ±58.1 fmol.mg^{-1} protein (n = 6), both values being significantly different from the corresponding control values (P < 0.05). Bicuculline altered the effect of pregnanolone which now caused an increase in affinity for [^{35}S]-TBPS (K_D = 22.7±3.4 nM) (n = 4, P < 0.001) rather than a decrease. The increase in maximum binding due to pregnanolone was still obtained in the presence of bicuculline (Bmax = 972.3±62.4 fmol.mg^{-1} protein) (n = 4, P = 0.010). Alphaxalone 10μM, like pregnanolone, reduced the affinity for [^{35}S]-TBPS (K_D = 59.5±11.7 nM) (n = 2, P = 0.002) but did not alter the maximum binding (Bmax = 497.9±51.9 fmol.mg^{-1} protein). In the presence of bicuculline, alphaxalone, again like pregnanolone, increased the affinity for [^{35}S]-TBPS (K_D = 17.3±3.9 nM) (n = 2, P < 0.001) but did not alter the maximum binding (Bmax = 576.6±70.6 fmol.mg^{-1} protein).

If it is assumed that bicuculline simply blocked the actions of any residual GABA present in the membranes, it would appear that the GABA-dependent component of the steroid action on [^{35}S]-TBPS binding was a reduction in affinity. The direct effects of the steroids on the picrotoxinin site were an increase in the affinity for [^{35}S]-TBPS, with an increase in maximum binding shown by pregnanolone only.

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364 P THE INTERACTION OF INTRAVENOUS ANAESTHETICS WITH RAT INHIBITORY AND EXCITATORY AMINO ACID RECEPTORS EXPRESSED IN *XENOPUS LAEVIS* OOCYTES

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The molecular actions of general anaesthetics relevant to anaesthesia are uncertain, although ionotropic inhibitory and excitatory amino acid receptors present plausible targets (Franks & Lieb, 1994). In an attempt to distinguish between some potential candidates, we have examined the influence of the structurally dissimilar intravenous anaesthetics, pentobarbitone, minaxolone and etomidate, upon responses mediated by rat $GABA_A$, non-NMDA glutamate and glycine receptors expressed in *Xenopus laevis* oocytes.

Cerebella and spinal cords were dissected from male Sprague Dawley rats (100-200g) killed by cervical dislocation and poly(A)$^+$-RNA was extracted using the FastTrack kit (InVitrogen). *Xenopus* oocytes were used to study cerebellar non-NMDA glutamate and $GABA_A$ receptors or spinal glycine receptors subsequent to the injection of 50-100nl of poly(A)$^+$-RNA (1μg μl^{-1}) prepared from the appropriate tissue. Recordings were made using the two electrode voltage-clamp technique, 2-15 days after injection, at a holding potential of -60mV and ambient temperature (18-23°C). Data are reported as the mean ± s.e. mean.

Preinjected oocytes displayed concentration-dependent inward currents to bath applied GABA (1μM-3mM), glycine (30μM-3mM) or kainate (3μM-3mM) with EC_{50} values of 60±3μM (n=4), 255±5μM (n=4) and 74±3μM (n=4) respectively. Anaesthetic effects were examined against the EC_{10} for GABA or glycine and the EC_{50} for kainate. Peak inward currents evoked by GABA or glycine were potentiated by pentobarbitone with EC_{50} values of 51±5μM and 0.8±0.1mM respectively. The degree of enhancement by maximally effective concentrations of pentobarbitone (expressed as a percentage of the current elicited by a saturating concentration of agonist in the absence of anaesthetic; parenthesis) at $GABA_A$ receptors (101±5%) was larger than that observed at glycine receptors (33±2%) and occurred at a 10-fold lower concentration (200μM). By contrast, pentobarbitone inhibited kainate evoked currents with an IC_{50} value of 173±20μM (n=4). In comparison to pentobarbitone, minaxolone was more potent in potentiating GABA- (EC_{50}=0.5±0.1μM) and glycine- (EC_{50}=11±1μM) evoked currents. The maximal degree of enhancement (to ≈ 90%) was similar at both receptor classes, but occurred at a 30-fold lower concentration (2μM) for GABA. Minaxolone (100nM-100μM) had no effect upon currents evoked by kainate (n=4). Etomidate potently enhanced currents evoked by GABA (EC_{50}=1.8±0.2μM) with a maximal enhancement of 91±4% at 10μM. Currents evoked by glycine were only marginally enhanced by etomidate (<15% at 300μM) precluding calculation of an EC_{50}. Etomidate (300nM-100μM) had little effect on kainate-evoked currents, but produced a modest depression (to 70±5% of control) at 1mM.

In conclusion, the data confirm the sensitivity of $GABA_A$ receptor mediated currents to modulation by etomidate, minaxolone, and pentobarbitone (Franks & Lieb, 1994; Lambert *et al.*, 1996; Yang & Uchida, 1996). For minaxolone and pentobarbitone, this action extends, albeit with reduced potency, to the strychnine sensitive glycine receptor. Minaxolone and etomidate, but not pentobarbitone, display selectivity towards inhibitory amino acid receptors. The data reinforce the notion that the $GABA_A$ receptor is a plausible site of action of many general anaesthetic agents (Franks & Lieb, 1994).

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365 P THE EFFECTS OF PROPOFOL ON NEURONAL $GABA_A$ AND GLYCINE RECEPTORS IN THE RAT OPTIC NERVE: AN *IN VITRO* ELECTROPHYSIOLOGICAL STUDY

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Propofol (2,6 di-isopropyl phenol) has a variety of clinical effects including antiemetic, antiepileptic and anaesthetic properties (Smith *et al.*, 1994). At low micromolar concentrations (achieved during anaesthesia, Vuyk *et al.*, 1992), propofol potentiates the effects of GABA at the $GABA_A$ receptor, an action that probably contributes to its central depressant actions (Franks & Leib, 1994), but has little or no effect at 5-HT_3, nACh or P_{2x} purinoceptors (Patten *et al.*, 1995). The effects of propofol at the strychnine-sensitive glycine receptor, however, are unclear. In the present study, therefore, we have determined the effects of propofol on $GABA_A$ and glycine receptor-mediated responses in the rat isolated optic nerve.

Agonist-evoked responses were recorded from optic nerves, dissected from male Sprague-Dawley rats (200-250g), using a standard extracellular (*grease-gap*) recording technique. Agonists and antagonists were perfused onto the nerve in a physiologically balanced salt solution. All recordings were made at ambient room temperature (20-23°C). Agonist-evoked responses were measured at their peak amplitude. The contact time for GABA and glycine was between 1.0 and 1.5 minutes. In antagonist/propofol experiments, agonists were applied in the continued presence of such drugs. Responses in the presence of antagonists/propofol are expressed as a percentage of those in their absence (control).

Application of GABA (0.1-10mM) or glycine (0.1-10mM) to the optic nerve evoked concentration-dependent depolarisations. The EC_{50} values (geometric mean and 95% C.I.) for GABA and glycine were 1.6mM (0.9-2.5mM, n=9) and 2.6mM (2.0-3.5mM, n=8), respectively. Submaximal (1mM) GABA-evoked responses were inhibited by picrotoxin (3μM) to (mean±s.e.m) 50±9%, (n=3) of control. Submaximal (1mM) glycine-evoked responses were inhibited by strychnine at 0.5μM to 41±12% of control (n=4) and abolished at 1μM (n=4). In contrast, propofol (10μM) potentiated submaximal (EC_{20}) GABA (0.3mM) and glycine (1mM) -evoked responses to 307±12%, (n=3) and 124±6%, (n=7) of control, respectively.

In conclusion, these data demonstrate that propofol, at concentrations which markedly potentiate $GABA_A$-mediated responses, produces a more modest enhancement of glycine responses. Our findings are consistent with the effects of propofol at spinal neurone $GABA_A$ and glycine receptors (Hales & Lambert, 1991), but contrast with findings using rat hippocampal neurones, where propofol was found to potentiate $GABA_A$-, but not glycine-mediated, responses (Hara *et al.*, 1993). These discrepancies may be the result of the use of different preparations and/or techniques. Alternatively, these data could suggest that there may be differences in the pharmacological properties of glycine receptors in different regions of the central nervous system.

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366 P FURTHER PHARMACOLOGICAL CHARACTERISATION OF [^{3}H]5-CT BINDING IN RAT BRAIN TISSUES

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The 5-HT_7 receptor has been cloned from rat, mouse, guinea pig and human tissue. 5-HT_7 receptor mRNA is localised in cortical and limbic brain regions which together with a high affinity for many antipsychotic agents suggests a role for the receptor in control of affective behaviour (e.g. Ruat et al; 1993). Competition assays using [^{3}H]5-CT to label the 5-HT_7 receptor in rat brain have demonstrated shallow curves with methiothepin (Boyland et al; 1996). This study attempted to use [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT) to further characterise 5-HT_7 receptor binding in rat brain tissue.

Whole brains (minus cerebellum and striatum) or hypothalamus from female Wistar rats (180-220g) were frozen at -80°C prior to preparation of binding homogenate. To prepare the radioligand binding homogenate, brain tissue was defrosted and homogenised in ice-cold Tris buffer (50 mM; pH 7.4 using a Polytron blender (full power; 10 s). The homogenate was washed three times by centrifugation/resuspension in Tris buffer (50 mM; pH7.4) incubated at 37°C for 30 mins and finally resuspended in Tris buffer (50 mM; pH 7.4 containing 0.1% ascorbic acid, 10 µM pargyline and 4 mM $CaCl_2$) at a concentration of 75mg wet weight /ml. For competition radioligand binding experiments, [^{3}H]5-CT (0.5 nM) was incubated with brain homogenate for 2 hours at 37°C in the absence (total binding) and presence of the competing drug. Non-specific binding was determined in the presence of 10 µM 5-HT. Binding was terminated by rapid filtration under vacuum through 0.3% PEI- pre-treated Whatman GF/B glass fibre filters followed by immediate washing with ice-cold Tris buffer. Radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy.

(±)-Pindolol (up to 100 µM) competed for around 80% of the specific [^{3}H]5-CT binding (defined by 5-HT 10 µM) with half maximal pindolol competition occurring at 0.14 ± 0.05 µM (mean ± SEM, n = 6). Subsequently, the pindolol (10 µM)-insensitive binding was subject to further characterisation.

[^{3}H]5-CT competition experiments with whole rat brain (minus cerebellum and striatum), resulted in inhibition curves for 5-CT, 8-OHDPAT, 5-HT, dihydroergotamine and RU 24969 with Hill slopes close to unity (1.02 ± 0.08, 1.12 ± 0.02, 0.90 ± 0.05, 0.96 ± 0.02, 1.04 ± 0.14, respectively, mean ± SEM, n=3), with pIC_{50} values of 8.68 ± 0.11, 6.49 ± 0.08, 7.41 ± 0.26, 7.97 ± 0.02 and 6.48 ± 0.17, respectively (mean ± SEM; n=3). Methiothepin and ritanserin competed for [^{3}H]5-CT with relatively high affinity (pIC_{50} = 7.75 ± 0.13 and 6.59 ± 0.13, respectively, mean ± SEM, n = 7-9) however consistently produced shallow competition curves; 0.86 ± 0.05 and 0.86 ± 0.05 (mean ± SEM, n=7-9, respectively). In contrast, in homogenates of rat hypothalamus, methiothepin and ritanserin competed in a monophasic manner whilst retaining relatively high affinity (Hill slopes = 1.34 ± 0.13 and 1.00 ± 0.15; pIC_{50} = 7.91 ± 0.14 and 6.71 ± 0.14, respectively, mean ± SEM, n = 5).

These results indicate that whilst the overall pharmacological profile of the [^{3}H]5-CT binding site in whole rat brain (minus cerebellum and striatum) homogenate is similar to the 5-HT_7 receptor, under the present binding conditions methiothepin and ritanserin recognise more than one population of [^{3}H]5-CT binding site. In contrast, in the hypothalamus preparation, [^{3}H]5-CT may label a homogenous population of sites.

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367 P THE EFFECT OF THE ENDOPEPTIDASE INHIBITOR RB38A ON HIPPOCAMPAL EXPRESSION OF APP mRNA ISOFORMS

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Neuropeptides have been implicated in learning and memory. The concentrations of such peptides are largely maintained through the action of peptidases, some of whose activity is altered in certain diseases (Gottfries *et al.*, 1995).

The endogenous amyloid precursor protein (APP) exists in four main isoforms in the rat brain and undergoes metabolism to soluble and insoluble amyloid peptide products. Insoluble β–amyloid is thought to contribute to the development of Alzheimer's disease pathology. It has been suggested that amyloid metabolites are formed as a consequence of 'secretase' activity, through their proposed metalloprotease, endopeptidase 24.11 and endopeptidase 24.15, activity (McDermott *et al.*, 1992, Marks *et al.*, 1994, Papastoitsis *et al.*, 1994). Thus, endopeptidases and their inhibitors may regulate processing and expression of proteins such as APP. The endopeptidase inhibitor, RB38A, has shown inhibitory activity on neutral endopeptidase, dipeptidylaminopeptidase, and aminopeptidase N, and thus may modify amyloid processing.

This study examined the effect of RB38A on hippocampal mRNA expression of the isoforms APP695, 714, 751, and 770. Halothane-anaesthetised Wistar rats received unilateral intrahippocampal injections of 6.2nmol/µL RB38A (n=3) or vehicle (n=3). Further animals were injected similarly with 50nmol/µL ibotenic acid (IBO) three weeks prior to injection with RB38A (n=3) or vehicle (n=3). IBO has previously been shown to alter the expression of the APP isoforms (Hutchings *et al.*, 1994). Four hours later, rats were perfusion-fixed and brain sections taken. These were incubated with ^{35}S-oligonucleotide probes and exposed to photographic film.

Densitometric analysis showed RB38A alone did not appear to alter the hybridization signal of APP695, 714, 751, and 770 mRNAs in the hippocampus or dentate gyrus at four hours (Table 1). Similarly, the ibotenic acid-induced reduction in APP695 and APP714 mRNA signals and elevation of APP751 and APP770 mRNA signals were not affected by exposure of brain tissue to this inhibitor.

Modification of the expression of APP mRNA isoforms by RB38A, either directly or indirectly, could result in altered APP processing. However, we have shown no evidence to support this hypothesis at the dose level employed. It remains to be confirmed as to whether inhibition of 'secretase' activity by a metalloprotease inhibitor, such as RB38A, may alter the regulation of amyloid metabolite formation.

Table 1

	APP695	APP714	APP751	APP770
VEHICLE	0.126±0.009	0.056±0.002	0.026±0.008	0.018±0.003
RB38A	[a]0.112±0.009	[a]0.047±0.009	[a]0.026±0.003	[a]0. 016±0.001
IBO/VEH	0 .062±0.006	0.036±0.006	0.027±0.004	0.048±0.007
IBO/RB38A	[b]0.071±0.005	[b] 0.034±0.003	[b]0.033±0.004	[b]0.044±0.004

Mean optical density values±s.e.m.
a-P>0.05 vs vehicle, b-P>0.05 vs ibo/vehicle (t-test)

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368 P EXPRESSION OF THE NMDAR1 RECEPTOR SUBUNIT IN HUMAN BRAIN STUDIED WITH *IN SITU* HYBRIDIZATION ON HUMAN WHOLE-BRAIN SECTIONS

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The NMDA subtype of glutamate ionotropic receptors appears to be important in physiological and pathological processes such as synaptic plasticity, memory formation, epilepsy and excitotoxic cell damage. The genes encoding for the subunits of the rat and human NMDA receptor have been cloned, and classified as two related families, NR1 and NR2. Distribution studies in rat brain have shown that the NR1 mRNA is abundantly expressed in every examined neuronal type in the adult rat CNS (Moriyoshi et al., 1991), and in-situ hybridization (ISH) studies on pieces of human brain showed marked differences between different regions of the hippocampus and of the cerebral cortex (Böckers et al., 1994). Rigby et al. (1996) in a recent paper showed heterogeneity in the distribution of NR1-1 to NR1-4 splice variants in selected areas of the human brain but did not correlate them with the distribution of the NR1-pan. In the present study, we have employed ISH with radiolabelled oligonucleotides directed against the human NR1-pan subunit mRNA in human whole brain sections to assess in detail the anatomical localization of this family of subunits.

Human whole brain sections (40 μm), obtained from post-mortem material (postmortem delay 30-71 hrs, 2 males, 1 female age between 76 and 82), were mounted onto Hybond-N DNA transfer membrane (Amersham) pre-incubated with 1% blocking reagent (Boehringer Mannheim) at 42°C overnight. Sections were subsequently fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) pH 7.0 for 15 min and washed in PBS for 30 min before being dried and stored at -20°C for up to 2 weeks. [^{35}S]-labelled oligonucleotides were diluted at a concentration of 10-20 ng /ml (corresponding to 6.7 10^6 cpm/ml) in hybridization buffer (with modifications from Dagerlind et al., 1992). Sections were hybridized overnight at 42°C, and then washed 5 min at 20°C in standard saline citrate (SSC, 2 times normal concentration), 60 min at 55°C and 60 min at 20°C in SSC. Washed sections were dehydrated, air-dried and apposed to ^{3}H-Hyperfilm (Amersham) for 3-7 days. Specificity of hybridization was assessed by the use of three different oligonucleotides directed against three different parts of the NR1 mRNA sequence and by incubation in the presence of 100-fold excess of unlabelled oligo.

Autoradiographical hybridization signals were semi-quantified microdensitometrically as optical density and expressed as percentage of the highest signal present in the dentate gyrus of the hippocampus. Percentage values were ranked from very weak (<60%),weak (60-70%), medium (70-80%), strong (80-90%), intense (>90%).

The signal from hybridized sections was weak in the pulvinar, medium in dorsomedial thalamic nucleus, medial geniculate and body of the caudate, strong in the cortex (entorhinal, temporal and cingulate sulcus) and subiculum, and intense in the superior frontal and lateral sulcus of the cortex. In the hippocampus, the signal was intense in the dentate gyrus, CA3 and CA2 field, but only medium in CA1 and CA4. Preliminary results showed an intense signal in the granule cell layer of the cerebellum, with no signal in the molecular layer, medullary layer and dentate nucleus.

These results confirm and extend previously published data (Böckers et al., 1994), and show that the mRNA for the NR1 subunit is widely expressed throughout the human brain with a characteristic pattern of distribution. The distribution of this subunit in the human brain correlates well with the distribution in rat reported by Laurie et al., (1995), with comparatively higher densities in the molecular layer of the cerebellum, CA2 and CA3 field of the hippocampus and superior frontal cortex. Further comparative studies into the distribution of splice variants of the NR1 subunit might prove useful in clarifying the changes in receptor composition accompanying pathological and physiological processes in wich the NMDA receptor is involved.

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369 P CHARACTERISATION OF P2X-PURINOCEPTORS IN NEURONES OF THE NEONATAL RAT DORSAL ROOT GANGLIA

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P_{2X}-purinoceptors are ligand-gated cation channels which mediate many of the physiological and pharmacological actions of adenosine 5'-triphosphate (ATP). Unlike other P_{2X}-subtypes the P_{2X3}-purinoceptor has a highly restricted distribution, being selectively expressed at high levels in sensory C-fibres, many of which are nociceptive (see Kennedy & Leff, 1995). We have recently characterised the biophysical and pharmacological properties of the P_{2X3}-purinoceptor present in neonatal rat dorsal root ganglia (Robertson *et al.*, 1996). We found that ATP, 2-methylthioATP and α,β-methyleneATP (α,β-meATP) evoked rapidly developing and desensitising inward currents with EC_{50} values of around 1 μM. The currents were inhibited by the P_{2X}-purinoceptor antagonist suramin. Here we have extended the pharmacological characterisation of this receptor.

Neurones of rat pup dorsal root ganglia were isolated by acute enzymatic dissociation. The whole-cell patch-clamp technique was used to record currents from cells held at -60 mV. Agonists were applied for 500 ms at 10 min intervals, via a solenoid valve-controlled U-tube system (equilibration time <10 ms) to minimise desensitisation. Antagonists, applied in the superfusate were allowed to equilibrate with the cells for at least 5 min.

Adenosine 5'-diphosphate (300 nM - 3 mM) (ADP), D-β,γ-methyleneATP (0.3 - 300 μM) and uridine 5'-triphosphate (UTP) (1 μM - 3 mM) evoked inward currents. The maximum current elicited by ADP and D-β,γ-methyleneATP was 2262 ± 452 pA (n=8) and 1486 ± 180 pA (n=28) respectively. In contrast, the concentration response curve to UTP did not reach a maximum, even at 3 mM. L-β,γ-methyleneATP is thought to display selectivity for smooth muscle P_{2X}-purinoceptors over neuronal P_{2X}-purinoceptors. Consistent with this idea, L-β,γ-methylene ATP evoked an inward current only at concentrations of 100 μM and above (132 ± 22 pA at 100 μM, n=14, 167 ± 27 pA at 300 μM, n=15). For all agonists the time to onset of the response was in the order of a few ms, consistent with activation of a ligand-gated channel. The P_{2X}-purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (10 μM) applied in the superfusate abolished the currents evoked by ATP (800 nM) (n=3), α,β-meATP (2 μM) (n=4), ADP (150 μM) (n=4), D-β,γ-methyleneATP (30 μM) (n=3) and UTP (50 μM) (n=5).

The results of this study show that several structural analogues of ATP rapidly activate an inward current in neurones of the rat dorsal root ganglia, which can be inhibited by PPADS. UTP activated a similar inward current, which was also PPADS-sensitive, suggesting that UTP can act as an agonist at the P_{2X3}-purinoceptor in these cells.

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370 P CHARACTERISATION OF THE SITES AT WHICH ATP ACTS TO EVOKE CONTRACTION OF THE RAT TAIL ARTERY

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When released as a cotransmitter with noradrenaline from sympathetic nerves, adenosine 5'-triphosphate (ATP) acts at P_{2X1}-purinoceptors to evoke contraction of visceral and vascular smooth muscle. Exogenous ATP is also assumed to act at these receptors, but recent reports suggest that it can act at additional sites to contract smooth muscle (Bailey & Hourani, 1995; Reilly & Hirst, 1996). Our aim was to investigate the site of action of ATP in smooth muscle of the rat tail artery, by comparing the effect of the P_2-antagonists suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) on contractions evoked by ATP, the P_{2X}-agonist α,β-methyleneATP (α,β-meATP) and the P_{2U}-agonist uridine 5'-triphosphate (UTP).

Rat tail artery smooth muscle rings from which the endothelium had been removed by gentle mechanical rubbing, were mounted under isometric conditions in Krebs' solution at 37°C and bubbled with 95% O_2, 5% CO_2. Antagonists were allowed to equilibrate with the tissue for at least 30 min and were tested against equi-effective concentrations of each agonist. Data were analysed by Student's t-test and considered significant at $P<0.05$.

ATP (1 μM-30 mM) and α,β-meATP (0.1 μM-100 μM) elicited concentration-dependent contractions which reached a peak within 1 min and decayed rapidly in the continued presence of agonist. Contractions evoked by UTP (1 μM-30 mM) developed more slowly and were more maintained. As there were no clear maxima to the concentration-response curves EC_{50} values could not be calculated, but at equi-effective concentrations α,β-meATP was about 500 times more potent than ATP and UTP, which were approximately equipotent. Suramin (1 μM-1 mM) and PPADS (100 nM-300 μM) inhibited responses to α,β-meATP (5 μM) in a concentration-dependent manner, with IC_{50} values of 29.9 ± 2.7 μM and 8.1 ± 0.3 μM respectively, and abolished the contractions at the highest concentrations used. Suramin and PPADS also inhibited contractions to ATP (1 mM) (IC_{50} = 3.8 ± 0.4 μM and 42.0 ± 5.0 μM respectively), but did not abolish the contractions. Approximately 30% of the response to ATP was resistant to concentrations of antagonist which abolished contractions to α,β-meATP. Suramin and PPADS had no significant effect on contractions evoked by UTP (1 mM).

These results show that exogenous ATP acts at more than one site to evoke contraction of the rat tail artery. The similarity in the IC_{50} values for suramin and PPADS against ATP and α,β-meATP suggest that P_{2X1}-purinoceptors mediate up to 70% of the response to ATP. It is not clear if the remainder of the response is mediated via suramin- and PPADS-resistant P_{2X}-purinoceptor subtypes, the P_{2U}-purinoceptor or another site.

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371 P ACTIVATION OF A NAD+-ACTIVATED NON-SELECTIVE CATION CHANNEL BY ATP AND ITS NON-HYDROLYZABLE ANALOGUES IN CRI-G1 INSULIN-SECRETING CELLS

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A β-NAD^+-activated non-selective cation channel which requires both internal Ca^{2+} and Mg^{2+} for activity, is present in the insulinoma cell line, CRI-G1 (Reale et al., 1994; Herson et al., 1996). Previous studies indicate that ATP and other adenine nucleotides modulate calcium activated non-selective cation channels in the same cell line (Sturgess et al., 1986). In the present study, the actions of ATP and the non-hydrolyzable analogues of ATP, AMP-PNP and AMP-PCP, were investigated using inside out patch-clamp recordings from CRI-G1 cells. Results are expressed as mean ± s.e.m., all groups compared using Student's *t*-test.

The patch pipettes contained (mM): 140 NaCl, 1 $MgCl_2$, 1 $CaCl_2$ and 10 HEPES (pH 7.2). The bath solution contained (mM): 140 NaCl, 0.6 $MgCl_2$, 5 EGTA, 10 HEPES (pH7.2) with 50 μM free Ca^{2+} and 200 μM β-NAD^+. Upon patch excision, a channel was observed characterized by a conductance of 74 pS, cation selectivity and which displayed long open and closed states. The addition of 1 mM ATP to the bathing solution increased channel open-state probability (P_o) from 0.29 ± 0.05 to 0.49 ± 0.06 which returned to 0.17 ± 0.05 upon removal of ATP (n=20; $P<0.05$). Furthermore, in separate experiments, application of 100 μM ATP (Fig 1) increased P_o from 0.28 ± 0.07 to 0.43 ± 0.08 which returned to 0.15 ± 0.05 upon washout (n=14; $P<0.05$). This increase in P_o is not due to phosphorylation or hydrolysis of ATP as identical results were obtained using non-hydrolyzable analogues of ATP. 100 μM AMP-PNP increased P_o from 0.15 ± 0.07 to 0.47 ± 0.06 returning to 0.13 ± 0.07 (n=8; $P<0.05$) and AMP-PCP caused P_o to change from 0.15 ± 0.06 to 0.53 ± 0.16 returning to 0.05 ± 0.04 (n=4; $P<0.05$). The single channel conductance was not significantly altered by the presence of the nucleotides. The P_o after washout is consistently less than the initial level due to rundown of channel activity with time. Further investigation is required to test the effects of these nucleotides on the rate of rundown.

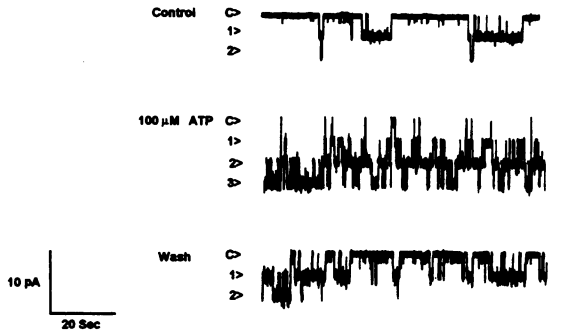

Fig.1 Effect of 100 μM ATP on single channel currents recorded from an inside-out patch at a membrane potential of -40 mV. 200μM ß-NAD^+ present throughout entire trace.

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372 P AN INVESTIGATION OF THE ANTIDEPRESSANT PROPERTIES OF 8-OH-DPAT AND IPSAPIRONE IN RATS

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Evidence is accumulating that 5-HT_{1A} receptor ligands, such as ipsapirone may represent a new class of mixed anxiolytics/antidepressants (Stahl *et al.*, 1992). The purpose of this study was to compare the properties of ipsapirone with those of the full 5-HT_{1A} agonist 8-OH-DPAT, in animal models indicative of antidepressant potential (Kelly & Leonard, 1994).

Male Sprague Dawley rats (250-280g, n = 8-10 per group) were housed 4 per cage, under a 12 h light cycle, (lights on; 0800 h), with free access to food and water. Bilateral olfactory bulbectomy (OB) was performed under tribromoethanol anaesthesia (2.5% w/v; 10 ml/kg, i.p.) (Cairncross *et al.*, 1977). Animals were allowed 14 days to recover after surgery. Both 8-OH-DPAT (hydrobromide; 1 mg/kg, i.p. twice daily) and ipsapirone (3 and 10 mg/kg, i.p. once daily) were administered for a period of 16 days. Controls received injections of saline vehicle alone. Immobility time in the forced swim test was recorded over 5 min following the third injection. Hyperactivity of the OB rat was assessed on the morning of the 15th day of the study and prior to drug treatment on that day. The effect of a challenge dose of 8-OH-DPAT (0.15 mg/kg, s.c.) on rectal temperature was determined on day 16, 2 h following treatment with the test substance. Results were expressed as group means ± s.e.means and a two-way analysis of variance was performed, followed by a posteriori least significant difference test.

In conclusion, 8-OH-DPAT and ipsapirone display antidepressant-like activity in all 3 tests and these changes could be due to 5-HT_{1A} autoreceptor desensitization, which might implicate 5-HT_{1A} receptor modulation in the mode of action of antidepressants (Goodwin, 1989).

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Table 1. The effect of 8-OH-DPAT and ipsapirone in animal models indicative of antidepressant potential

	8-OH-DPAT				Ipsapirone		
	Forced Swim (Immobility s)	'Open field' (Ambulation)	8-OH-DPAT challenge (°C at 30 min)		Forced Swim (Immobility s)	'Open field' (Ambulation)	8-OH-DPAT challenge (°C at 30 min)
Sham				Sham			
Vehicle	238 ± 8	69 ± 4	35.5 ± 0.3	Vehicle	213 ± 11	59 ± 6	36.3 ± 0.1
1 mg/kg	115 ± 14*	59 ± 7	36.9 ± 0.1*	3 mg/kg	150 ± 15*	59 ± 5	36.8 ± 0.1*
				10 mg/kg	121 ± 8*	56 ± 6	37.1 ± 0.1*
OB				OB			
Vehicle	241 ± 7	107 ± 8*	34.9 ± 0.1	Vehicle	244 ± 8	98 ± 7*	36.1 ± 0.2
1 mg/kg	123 ± 24+	79 ± 8+	37.2 ± 0.2+	3 mg/kg	180 ± 12+	84 ± 5	36.9 ± 0.3+
				10 mg/kg	117 ± 18+	72 ± 4+	36.6 ± 0.1

* $p<0.05$ versus vehicle-treated sham animals. + $p<0.05$ versus vehicle-treated OB animals.

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373 P A CAL SYSTEM FOR TEACHING ELEMENTARY DRUG DISPOSITION AND PHARMACOKINETICS: VERSION 2

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This program is the first part of a second version of a previous CAL package on Drug Disposition and Pharmacokinetics (Ogg and Stevenson, 1993). Although this package is incomplete according to the original remit, it does cover a large section of basic drug disposition and pharmacokinetics and may be considered as a stand-alone package. The revision was thought desirable due to the perceived design criteria adopted by the PharmaCALogy and the PCCAL consortia. A consistent, well evaluated, user interface should eliminate the students' need to learn how to navigate and use the material presented. The original package did not allow any interaction and animations had to be produced in a different package. Animations are very important in order to stimulate student learning and to allow them to conceptualise the more difficult material. In many ways this is the most important point of any CAL system, to avoid the "text-book on screen". The objective here therefore was to change student perception and use of the material to one of "use" rather than "avoid" This new version has been re-written in Authorware (Macromedia) which allowed an integrated development of the original material with the specific inclusion of student-machine interaction. The program was produced as an Honours B.Sc. Student project in 10 weeks. This was possible since many of the original text and diagrams from the earlier program were retained. Due to time constraints, however, it was not possible to include as much interactive material as desired. Over the next year this package will be completed to cover the rest of version one.

The PCCAL consortium has produced some good CAL software in pharmacokinetics, especially the pharmacokinetics workshop package. The main pharmacokinetics package we felt was rather weak in some areas, in comparison to our original coverage in version one. This version two of our package integrates well with the PCCAL software and therefore complements it to a great extent. The software therefore keeps true to version one's premise of not just telling, "why" but also the "how".

Student evaluation of this version revealed a strong preference for the new design but indicated that there was not enough interaction (based on other pharmaCALogy & PCCAL packages). There is therefore a strong case for modifying this program in light of these comments in order that a richer more fulfilling experience of CAL can be achieved.

Ogg G.D.and I.H. Stevenson (1993) A CAL System for Teaching Elementary Drug Disposition and Pharmacokinetics. Br. J. Pharmacol. 108 311P.