25P PHARMACOKINETICS, PHARMACODYNAMICS AND PK-PD INTEGRATION OF DANOFLOXACIN IN SHEEP BIOLOGICAL FLUIDS

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The development of resistance by microorganisms to anti-microbial drugs is a major problem in human and veterinary medicine. This has stimulated research into the selection of dosage schedules to optimise efficacy and minimise opportunities for resistance development. Danofloxacin is a synthetic antimicrobial drug of the fluoroquinolone group developed for veterinary use. It is bactericidal and acts by a concentration dependent killing mechanism (Sarasola *et al.*, 2002). For fluoroquinolones it has been demonstrated in both disease model and clinical trial investigations that, of the surrogate markers Cmax/MIC, AUC/MIC and T>MIC, outcome usually correlates with AUC/MIC and Cmax/MIC and emergence of resistance correlates with Cmax/MIC (Craig, 1998). The aim of this work was to integrate pharmacodynamic and pharmacokinetic data for danofloxacin in sheep as a means of selecting dosage schedules for evaluation in clinical trials.

Danofloxacin was administered to sheep intravenously (i.v.) and intramuscularly (i.m.) at a dose rate of 1.25 mg/kg in a 2-period cross-over study. Using subcutaneously implanted tissue chambers (Higgins *et al.*, 1984), the pharmacokinetic properties of danofloxacin were established for serum, inflamed chamber fluid (exudate) and non-inflamed chamber fluid (transudate). The acute inflammation in tissue chambers was induced by intracaveal injection of 0.5 ml of a sterile 1% carrageenan solution. For serum mean pharmacokinetic values after i.v. and i.m. dosing were, respectively: elimination half-life (3.39 and 3.17 h), volume of distribution (3.37 and 3.19 l kg^{-1}) and area under curve (1.81 and 1.68 μg h ml^{-1}). For exudate corresponding half-life values were 17.60 and 17.13 h and for transudate values were 10.45 and 17.66 h

The activity of danofloxacin in serum, exudate and transudate was established *ex vivo* and *in vitro* against a pathogenic strain of *Mannheimia haemolytica*. *Ex vivo* samples were harvested at 0,1,3,6,9,12,24,30,36 and 48 h (serum) and 0,3,6,9,12,24 and 36 h (exudate and transudate) after i.m. danofloxacin dosing. Samples were incubated for 24 h at 37°C and bacterial count determined at time 0 and 24 h. The change in bacterial count (cfu ml^{-1}) and AUC_{24h}/MIC were fitted to the sigmoid Emax equation to provide values producing bacteriostasis, bactericidal activity and elimination of bacteria. Respective values were 17.8, 20.2 and 28.7 h for serum, 20.6, 25.5 and 41.6 h for exudate and 20.9, 23.2 and 33.4 h for transudate. From the *in vivo* pharmacokinetic and *in vitro* MIC data, mean Cmax/MIC ratios of 10.8, 3.0 and 1.6 were obtained for serum, exudate and transudate after i.m. danofloxacin dosing. It is proposed that these data might be used together with MIC_{90} values for *M. haemolytica* to provide a novel approach to the design of dosage schedules.

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26P CHARACTERIZATION OF MURINE ERG1a CHANNELS EXPRESSED IN HEK CELLS

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Recently ion channels encoded by *ether-a-go-go*-related genes (ERG) have been recorded in murine vascular smooth muscle cells (Ohya *et al.*, 2002) and RT-PCR experiments showed that only mERG1 isoforms were expressed. Various mERG1 splice variants exist (London *et al.*, 1997) and ERG channels are known to form hetero-multimers. Therefore, we investigated whether expression of mERG1a alone could underlie the native current observed in murine vascular myocytes by characterising ERG1a channels expressed in human embryonic kidney (HEK) cells. mERG1a was cloned into a neomycin-resistant pcDNA3.1 plasmid and HEK cells were stably transfected. Cells were bathed in a solution that contained 10 mM TEA, 5 mM 4-AP and 0.1 mM $CaCl_2$ with a K^+ concentration of either 5 mM or 140 mM. Ion currents were recorded in the conventional whole cell configuration using a pipette solution that contained 5 mM EGTA and 5 mM ATP. All data are the mean of n cells ± s.e.m

Activation of mERG1a channels was determined at a test potential of –100 mV following a 1 s test step to potentials between –120 mV and +60 mV from a holding potential of –60 mV. Depolarisation did not elicit large outward currents but significant inward currents were observed at the test potential. When cells were bathed in 140 mM K^+ to augment the amplitude of the current at –100 mV the potential for half maximal activation was –15 ± 6 mV (slope = 16 ± 2, n= 6).

The steady-state availability of mERG1a channels was determined at –120 mV in HEK cells bathed in a solution containing 140 mM K^+ following 5 s voltage steps from 0 mV to test potentials between –140 mV and +60 mV. The availability of the current at –120 mV was well fitted by a Boltzman function with a half-maximal availability at –87 ± 4 mV (slope = 9.7 ± 3, n= 5). The evoked current was inhibited markedly by 1 μM E4031 with a time to achieve half-maximal inhibition of 111 ± 5 s (n=5). Hyperpolarization of HEK cells bathed in 140 mM K^+-containing solution from a holding potential of 0 mV elicited currents with a distinctive hooked appearance at potentials negative to –40 mV due to the initial recovery of ERG channels from inactivation followed by deactivation. Recovery from inactivation could be described by a single exponential and was voltage dependent with the time constant increasing from 12.5 ± 0.8 ms at –120 mV to 47 ± 14 ms at –60 mV (n=5). Deactivation at –100 mV had a time constant of 381 ± 46 ms (n=4) that was considerably slower than the deactivation of native currents at the same potential (time constant = 68 ± 5 ms, n=16).

Stable transfection of HEK cells with mERG1a produced an inwardly rectifying voltage-dependent K^+ current that had kinetics of recovery from inactivation and deactivation slower than the native channel in murine vascular myocytes.

London *et al.*, (1997). *Circ Res*, **81**, 870-878

Ohya *et al.*, (2002). Manuscript submitted to *Circ Res*.

27P EFFECTS OF TRYPTAMINE ON THE EQUINE ISOLATED PERFUSED DIGIT PREPARATION

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Equine laminitis is a painful ischaemia and reperfusion injury of the foot. Although the vasoactive mediator(s) triggering this disease still remain unknown, increased post capillary resistance as a result of digital venoconstriction is thought to be a primary event (Allen *et al*, 1990). Previous work in our laboratory has demonstrated 5-hydroxytryptamine (5-HT) to be a potent vasoconstrictor of equine digital vessels, with no significant difference in potency between arteries and veins (Bailey & Elliott, 1998). Other amines such as tyramine, tryptamine and phenylethylamine are formed in the equine caecum and may mimic the effects endogenous amines if absorbed into the circulation. These compounds were found to have a selective effect on digital veins vs. arteries in isolated vessel preparations (Berhane *et al*, 2002). The aim of the present study was to compare the sensitivity of the arterial and venous sides of the equine digital circulation to tryptamine, with 5-HT as a reference compound, using a Krebs-perfused hoof preparation.

Hind limbs were obtained from mixed breed healthy adult horses killed at an abattoir. The digital circulation was perfused at a constant flow rate of 100 ml min^{-1} with oxygenated modified Krebs-Hensleit solution (KHS). The large vein, small vein and total perfusion pressures were recorded from the lateral digital vein, coronary band vein and digital artery, respectively, to enable the calculation of the venous, prevenous and total vascular resistances by the methods of Robinson *et al* (1975). Following a 30 min equilibration period, the peak effects of an infusion of 1 litre of KHS containing 5-HT (30 nM) or tryptamine (1 μM) on vascular resistances were obtained (n=4; randomised order), and compared by means of two-way ANOVA with Bonferroni's post-hoc test. The concentrations of 5-HT and tryptamine had been chosen to produce equivalent increases in total perfusion pressure.

The peak increases in total perfusion pressure were 128.0 ± 12.8 and 113.8 ± 12.5 mmHg for 5-HT and tryptamine, respectively. There were no significant differences in the peak increases in total resistance calculated in response to 5-HT and tryptamine. However, the increase in venous resistance caused by tryptamine (22.3 % of total resistance) was significantly greater than that caused by 5-HT (11.6 % of total).

When compared to 5-HT, tryptamine was shown to cause more pronounced venoconstriction in the equine digit. As a potent and relatively selective venoconstrictor of the equine digital vascular bed, tryptamine should be considered as a potential trigger mediator of equine laminitis.

Table 1. Effect of tryptamine and 5-HT on digital vascular resistance.

	increase in vascular resistance over baseline (mmHg /ml /min)		
compound	total	prevenous	venous
5-HT (30 nM)	1.21 ± 0.27	1.07 ± 0.26	0.14 ± 0.01
tryptamine (1 μM)	1.05 ± 0.25	0.81 ± 0.22	0.23 ± 0.03 *

*P<0.05, significant difference compared with 5-HT, 2-way ANOVA.

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Allen D, Clark ES & Moore JN (1990) *Am J vet Res* **51**, 1930-1934.
Bailey SR & Elliott J (1998) *Eur J Pharmacol*, **355**, 175-187.
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Robinson N, Dabney J & Jones G (1975) *Am J Vet Res* **36**, 1249-1253.

28P INCREASED VASOCONSTRICTION TO ENDOTHELIN-1 IN AORTA FROM APOLIPOPROTEIN E KNOCKOUT MICE DEVOID OF ATHEROSCLEROTIC LESIONS

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Tissue and plasma levels of the vasoconstrictor endothelin-1 (ET-1) are up-regulated in atherosclerosis, but it is unclear if this is a cause or a consequence of disease progression. Apolipoprotein E deficient ($ApoE^{(-/-)}$) mice spontaneously develop atherosclerotic lesions similar to human plaques and are used as a model for atherosclerosis. Our objective was to determine if responses to ET-1 are altered in the early stages of atherosclerosis.

Aortae devoid of atherosclerotic lesions were obtained from 14-16 week $ApoE^{(-/-)}$ C57/BL6 (n = 5) and C57/BL6 (control; n = 5) mice (either sex, 25-35g; Charles River, UK). Rings (2mm) were dissected and mounted in wire myographs for the measurement of isometric tension. The segments were bathed in oxygenated Krebs' solution at 37°C and were set to 90% of the internal diameter. Prior to constructing cumulative concentration-response curves to ET-1 (0.1-300nM), the thromboxane mimetic U44619 (1-3000nM) and KCl (0.1-100mM), preparations were stimulated three times with a potassium-rich (95mM) Krebs' solution. Concentration response curves were expressed as a percentage of the maximum response to KCl (mean ± s.e.mean).

ET-1 caused a small concentration-dependent constriction in aortic rings from control mice (Figure 1; E_{MAX} 5.5 ± 1.5%; pD_2 9.2 ± 0.16, n = 5). There was a significant increase in E_{MAX} in $ApoE^{(-/-)}$ aorta (E_{MAX} 33 ± 12%; $P<0.05$, Student's *t*-test; n = 5), however there was no change in pD_2 value (pD_2 8.7 ± 0.13; $P>0.05$, Student's *t*-test; n = 5). Concentration-response curves to KCl were not significantly different between control and $ApoE^{(-/-)}$ groups (pD_2 values 1.9 ± 0.33 and 2.3 ± 0.14, n = 5 and 5, control and $ApoE^{(-/-)}$ groups respectively; $P>0.05$ Student's *t*-test). Similarly, the pD_2 values and E_{MAX} values of concentration response curves to U44619 were not significantly different between control (n = 5) and $ApoE^{(-/-)}$ (n = 3) groups (pD_2 values: 7.5 ± 0.34 and 7.2 ± 0.18, E_{MAX} values 86 ± 25% and 81 ± 11%, control and $ApoE^{(-/-)}$ respectively.

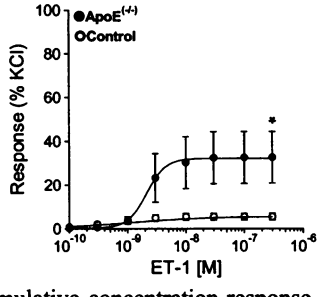

Figure 1. Cumulative concentration-response curves to ET-1 in control C57/BL6 and $ApoE^{(-/-)}$ C57/BL6 mouse aorta *in vitro* (n = 5; *$P<0.05$, Student's *t*-test).

We have demonstrated a significant increase in maximum response to ET-1 in aorta devoid of atherosclerotic lesions from young $ApoE^{(-/-)}$ mice *in vitro*. These data suggest that the ET signalling pathway may be involved in the development of atherosclerosis.

29P IDENTIFICATION OF A NOVEL VARIANT IN EXON 19 OF THE ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE

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Endothelium-derived nitric oxide (NO) regulates vascular tone, platelet aggregation, leukocyte adhesion and vascular smooth muscle proliferation. Deficiency in NO production is observed in several disease states including atherosclerosis and essential hypertension, some of which have a heritable component. Several polymorphisms have been identified in the endothelial nitric oxide synthase (eNOS) gene of which the G894T substitution in exon 7 (Hingorani *et al*, 1995) is the only polymorphism to predict an amino acid substitution in the protein. The 894T homozygote was found to be in excess in patients with ischaemic heart disease (Hingorani *et al*, 1999). Using a combination of single stranded conformational polymorphism (SSCP) analysis and a fluorescent sequencing system, we have identified the presence of a rare single nucleotide polymorphism in exon 19 of the eNOS gene.

Genomic DNA was extracted from the blood of five healthy volunteers. The polymerase chain reaction (PCR) was used to amplify a 266 bp fragment containing exon 19 of the eNOS gene using flanking intronic primers. For SSCP analysis, products were heat denatured, cooled on ice and analysed using the semi-automated Pharmacia Phastsystem. Products were visualised by silver staining. Sequencing was carried out using the same PCR primers and DyeDeoxy chain terminators on an ABI 377 DNA sequencer. This approach identified an individual heterozygous for G→A at position +2479 of the coding sequence, in exon 19 of the gene. This substitution predicts a Val→Met amino acid substitution at codon 826 (GTG→ATG) and the creation of a NcoI restriction site in the methionine-containing allele.

30 DNA samples were taken from patients with recent myocardial infarction or positive coronary angiogram. The samples were amplified by PCR for exon 19 and restriction analysis using NcoI was carried out. None of the samples was found to contain the polymorphism. This analysis was also carried out on 30 samples taken from normal healthy control subjects and again the polymorphism was not identified in any of these samples. Therefore the G5048A substitution in the NOS3 gene is a rare polymorphism.

The Valine residue at codon 826 is conserved between species in eNOS and also in human iNOS and nNOS. It is located in the flavin-binding region for which there is not yet a 3D structure. Further work is required to characterise this variant in order to establish whether the presence of this polymorphism alters the rate of synthesis of nitric oxide, or the production of superoxide by nitric oxide synthase.

Hingorani, A.D., Jia, H., Stevens, P.A. *et al* (1995) Clin. Sci. **88** 21P

Hingorani, A.D., Liang, C.F., Fatibene, J, *et al* (1999) Circulation **100** 1515-1520

30P EFFECTS OF ENDOTHELIUM AND PRE-CONSTRICTION ON β-ADRENOCEPTOR-MEDIATED RELAXATION IN RAT ISOLATED AORTA

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There are conflicting reports in the literature regarding the role of endothelium in β-adrenoceptor-mediated vasodilatation. In the present study the effects of endothelium removal and treatment with the nitric oxide (NO) synthase inhibitor, L-NAME, on the relaxant effect of isoprenaline (ISO) in rings pre-constricted with phenylephrine (PE) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were investigated in rat isolated aorta.

Male Wistar rats (200 - 250 g) were stunned and killed by cervical dislocation. Thoracic aorta rings were set up for isometric recording in Krebs gassed with 95/5 % O_2/CO_2 at 37 °C. Cumulative concentration response curves (CRCs) to PE and $PGF_{2\alpha}$ were carried out in endothelium intact (EI) rings, in endothelium denuded (ED) rings and in rings pretreated with L-NAME (L-N), 100 μM. In relaxation experiments rings were pre-constricted with either PE or $PGF_{2\alpha}$ before carrying out cumulative CRCs to ISO. The function of endothelium was checked using acetylcholine (1 μM). Experiments with $PGF_{2\alpha}$ were carried out in the presence of prazosin (0.75 μM). Values are mean±s.e.mean. Statistical analysis was carried out using ANOVA followed by post tests.

Removal of endothelium and pretreatment with L-NAME shifted the PE CRCs to the left with no change in the maximum response (R_{max}) (pEC_{50}s, n=9: EI, 6.8±0.06; ED, 8.06±0.06, $p<0.05$; L-N, 7.56±0.05, $p<0.05$. R_{max}, g tension: EI, 1.00±0.10; ED, 1.20±0.10, $P>0.05$; L-N, 1.10±0.10, $P>0.05$). Adjustment of the concentration of PE to produce a similar level of pre-constriction, 75-80% of the R_{max}, (EI, 0.5 μM; ED, 30 nM; L-N, 80 nM) resulted in identical ISO-induced concentration-dependent relaxation (pEC_{50}s, n=9: EI, 7.46±0.02; ED, 7.40±0.03; L-N, 7.39±0.02, $P>0.05$. R_{max}, % relaxation: EI, 90±2; ED, 94±1; L-N, 89±3, $P>0.05$). In contrast, acetylcholine-induced relaxation was greatly reduced by ED or L-N (% relaxation, n=9: EI, 93±3; ED, 0±0; L-N, 8±2). In experiments where the same concentration of PE was used in ED preparations as in EI rings (0.5 μM), the size of the PE constriction was significantly greater than in EI rings (g tension: EI, 0.73±0.03, n=9; ED, 1.18±0.07, n=4, $P<0.05$) and ISO failed to produce any relaxation. Similar results were obtained using $PGF_{2\alpha}$ as constrictor. Removal of endothelium shifted the $PGF_{2\alpha}$ CRCs to the left with no change in R_{max},. Adjustment of the concentration of $PGF_{2\alpha}$ to produce a similar level of preconstriction, approximately 50% of the R_{max}, (EI, 3-3.5 μM; ED, 0.7 μM; L-N, 1 μM) resulted in identical ISO-induced relaxation.

In conclusion, in rat isolated aorta endothelium removal and L-NAME shifted the CRCs to constrictors PE and $PGF_{2\alpha}$ to the left with no change in the maximum response. Adjustment of the pre-constrictor concentration to the same level of contraction revealed no effect of endothelium removal or L-NAME on isoprenaline-induced relaxation. Thus relaxation to isoprenaline in rat aorta is endothelium-independent, in agreement with a previous study (Eckly *et al.*, 1994).

Eckly, A.E., Stoclet, J-C & Lugnier, C. (1994) Eur J Pharmacol., **271**, 237-240.

31P EFFECTS OF AGING ON CYCLOOXYGENASE EXPRESSION AND THE α_1-ADRENOCEPTOR SIGNALING PATHWAY IN RAT AORTAE

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Cyclooxygenases (COX) are crucial in the production of vasoactive prostanoids for regulation of vascular tone (Mitchell & Warner, 1999).

The present study was designed to determine COX-1 and COX-2 expression in rat aortae, platelets and mononuclear cells from 8 and 54 week old rats, and also to determine whether this affected the prostacyclin production and α_1-adrenoceptor signalling in rat aortae.

Experiments were conducted using aortae and blood from male Sprague-Dawley rats aged 8 and 54 weeks, approximately 250 g and 600 g, respectively. Western Blot analysis was performed on protein isolated from aortae, platelets and mononuclear cells of fresh blood by using specific antibodies of COX-1 and COX-2. Aortic rings were mounted under 2 g tension in physiological salt solution for isometric recording of vasoconstrictor responses to phenylephrine (PE). Cumulative concentration-response curves for PE (1 nM -10 μM) were constructed in the presence or absence of the selective COX-1 inhibitor, piroxicam (100 μM) or COX-2 inhibitor, NS-398 (10 μM). The production of 6-keto $PGF_{1\alpha}$ released in the incubation media was measured with and without addition of PE (10 μM) for 40 minutes using an enzyme immunoassay technique.

Western Blot analysis showed an increased expression of both COX-1 and COX-2 in aortae, platelets and mononuclear cells from 54 week rats compared to 8 week rats. This correlated with a significantly greater production of 6-keto $PGF_{1\alpha}$ from 54 week aortic rings (951 ± 98 pg/mg, n = 13) compared to 8 week rings (226 ± 40 pg/mg, n = 9, P = 0.0001). With the addition of PE (10 μM), the production of 6-keto $PGF_{1\alpha}$ was increased further to 1379 ± 141 pg/mg (n = 14) in 54 week aortae compared to 8 weeks (583 ± 80 pg/mg, n = 14, P = 0.001). In the absence of endothelium, the α_1-vasoconstriction responses to PE were significantly greater in aortic rings from 54 week rats compared to those of 8 week rats (P = 0.0001). In the absence of inhibitors the maximum responses were 1.6 ± 0.2 g (n = 17) and 2.6 ± 0.1 g (n = 14) in 8 and 54 week rats, respectively. The selective COX-1 inhibitor, piroxicam (100 μM) caused a significant reduction in the responsiveness of 54 week aortae (- log EC_{50} = 7.1 ± 0.1, n = 12, P = 0.01) to PE, whereas the selective COX-2 inhibitor, NS-398 (10 μM) had no effect (- log EC_{50}= 7.4 ± 0.1, n = 13). Neither of the inhibitors affected PE responses in the 8 week rats.

These findings suggest there is an up-regulation of COX during aging which will lead to increased production of prostacyclin and perhaps other prostanoids. This alters the α_1-adrenoceptor constriction pathways in rat aortae which is partially mediated through COX-1 in 54 week but not 8 week aortae. Increased COX expression in platelets and mononuclear cells suggests that systemic alteration occurs in aged rats, and not localised to blood vessels.

Mitchell, J.A. & Warner T.D., (1999) *Br. J. Pharmacol.*, **128,** 1121-1132

32P CHARACTERISTICS OF L-ARGININE TRANSPORT AND NITRIC OXIDE SYNTHESIS IN THE P53 MUTATED CAPAN-1 HUMAN PANCREATIC TUMOUR CELL LINE

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Inducible nitric oxide synthase (iNOS) may be critically regulated by the tumour suppressor gene p53 (Ambs *et al.*, 1998). The pathways regulated by p53 in modulating iNOS expression are, however, as yet unclear. Also unclear is the effect of p53 on cationic amino acid transporters (CATs) and the regulation of expression and function of these proteins in human tumours. In preliminary studies we have identified the nature of the CATs associated with uptake of L-arginine into CAPAN-1 cells, a human pancreatic carcinoma cell line with a mutated p53 gene. In addition, we report on the characteristics of L-arginine transport and of nitric oxide (NO) synthesis in these cells with a view to exploring the regulation of these processes by the p53 signalling pathway.

Cationic amino acid transporters in CAPAN-1 cells were identified by RT-PCR using primers specific for human CAT-1, CAT-2A, CAT-2B or CAT-3. Functional transport studies were carried out as described previously (Baydoun *et al.*, 1993). In parallel, cells were activated for 3 h - 96 h using a cocktail of cytokines consisting of human recombinant IFN-γ (1000 U ml^{-1}), TNF-α (10 ng ml^{-1}), IL-1β (0.5 ng ml^{-1}) and IL-6 (200 U ml^{-1}). NO synthesis was determined by the standard Griess (Baydoun *et al.*, 1993). Expression of iNOS and p53 were determined by Western blotting.

Sequence analysis of the p53 coding region in CAPAN-1 cells revealed a single base mutation from C to T which resulted in the substitution of ala^{159} to val. RT-PCR studies revealed transcripts for both CAT-1 and CAT-2B but not for CAT-2A or CAT-3. At the functional level, L-arginine transport was saturable with an apparent K_t of 0.25 mM and V_{max} of 2.7 pmol.μg $protein^{-1}$ min^{-1}. In addition transport was both pH and Na^+-insensitive, with characteristics reflective of the archetypical system y^+ carrier. Exposure of cells to the cytokine cocktail resulted in a time-dependent production of NO and a corresponding increase in iNOS expression, the latter reaching a peak after 24 h and declining by ~50 % after 48 h. Transport of L-arginine was also enhanced, becoming apparent at 24 h and reaching a peak at 48 h (73 % stimulation above control) but sustained at this level over 72 h. In contrast to iNOS expression, p53 expression initially decreased in a time-dependent manner following activation but returned to pre-stimulated levels 48 h post stimulation. These changes inversely mirror the changes in iNOS expression.

The events leading to the regulation of both the expression and function of the inducible L-arginine-NO pathway by p53 now remains to be elucidated.

Ambs, S., Ogunfusika, M., Merriam, W.G. et al. (1998). Proc.Natl. Acad. Sci. USA. **95**, 8823-8828.

Baydoun, A.R., Bogle, R.G., Pearson, J.D. *et. al.* (1993). Br. J. Pharmacol., **110**: 1401-1406.

33P TRANSPORT MECHANISMS FOR GW274150, A POTENT AND SELECTIVE INHIBITOR OF INDUCIBLE NITRIC OXIDE SYNTHASE

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GW274150, an acetamidine derivative of L-lysine, has been developed as a highly selective inhibitor of human inducible nitric oxide synthase (Young *et al.* 2000). This compound is effective both in intact cell systems and against the isolated enzyme. In the current studies we have examined the mechanism via which GW274150 enters cells by identifying the system(s) associated with its uptake.

All experiments were carried out on J774 macrophages cultured in Dulbecco's modified Eagle's medium supplemented with 10 % foetal bovine serum. Transport studies were carried out as described previously (Baydoun *et al.*, 1993) in Hepes-buffered Krebs solution (50µl; 37°C) containing L-[^{14}C]GW274150 (1µCiml^{-1}) and 0.1mM unlabelled compound in the absence and presence of a 10-fold excess of known substrates for different amino acid transports. Additionally, transport was monitored in controls and in cells pre-treated with bacterial lipopolysccharide (LPS; 1 µg ml^{-1}) for 24 h. 1996). Data are expressed as mean ± s.e.m. of 4 independent experiments (n=4) and analysed using a student's unpaired t test.

Kinetic studies revealed that uptake of GW274150 was saturable, showing a single affinity entry with an apparent K_t of 0.31±0.01 mM and V_{max} of 5.15±0.12 pmol. µg protein^{-1} min^{-1}. The V_{max}, but not K_t, was significantly enhanced in cells pre-treated with LPS (V_{max} = 9.8±0.21 pmol. µg protein^{-1} min^{-1}; $p< 0.01$).

Further characterisation revealed that the uptake process was largely pH insensitive and only marginally (~20%; $p<0.01$, n=3) dependent on extracellular Na^+. In addition, transport of L-[^{14}C]GW274150 was not affected by a 1mM excess of either 2-methylaminoisobutyric acid (MeAIB), L-alanine, L-valine or ß-2-amino-bicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) but was inhibited by 64±1 %, 60±2 %, 54±2 %, 43±2, 45±2 % and 50±2 % in the presence of L-arginine, L-lysine, L-leucine, L-methionine, 6-diazo-5-oxo-L-norleucine (DON) and L-glutamine respectively. These inhibitions, with the exception of that caused by L-arginine and L-lysine, were critically dependent on extracellular Na^+ and completely reversed when extracellular Na^+ was replaced with choline.

Taken together, these data suggest that GW274150 may be transported via a broad-spectrum amino acid carrier capable of transporting both cationic and neutral amino acids. The relatively high K_t, sodium insensitivity and marked inhibition caused by L-leucine, L-glutamine and L-methionine, together with the dependency of these inhibitions on extracellular Na^+, would implicate the y^+LATs as potential carriers of this compound.

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Young, R.J, Beams, R.M, Carter, K., et al. (2000). Bioorg. Med. Chem. Lett., **10**: 597-600.

34P ROLE OF PROTEIN KINASE C IN EQUINE EOSINOPHIL SUPEROXIDE PRODUCTION AND ADHERENCE

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Histamine has been implicated in the pathogenesis of the equine allergic skin disease, sweet itch and causes equine eosinophil migration, adherence and superoxide production via H_1 receptor activation (Foster & Cunningham, 1997; 1998; Foster *et al.*, 1998). The signalling events downstream of receptor activation have not been examined in these cells and in this study the effects of protein kinase C (PKC) inhibitors on histamine-induced superoxide generation and adherence have been investigated.

The effects of the non-selective PKC inhibitors, staurosporine (0.1nM-10µM) and Ro31-8220 (0.1nM-1µM), on histamine (100µM)-induced superoxide production were first examined using eosinophils from 3 normal and 3 sweet itch ponies. In a subsequent study using cells from 6 ponies in each group the effects of Gö6976 (20nM-2µM) and rottlerin (1-100µM), which selectively inhibit classical PKCs and novel PKCδ, respectively, were determined. Finally the effects of staurosporine (0.1nM-10µM) and Ro31-8220 (0.1nM-1µM) on histamine (100µM)-induced adherence of eosinophils from 6 normal ponies to autologous serum coated plastic was examined. Superoxide production and adherence were measured as previously described (Foster & Cunningham, 1997; 1998). Results are expressed as means±SEM. The effects of PKC inhibitors were examined by 1 way-ANOVA and Dunnett's test. The effects on cells from normal and sweet ponies itch ponies were compared by analysis of co-variance.

An initial study showed that 100µM histamine stimulated production of similar amounts of superoxide by eosinophils from normal and sweet itch ponies (30±3 and 27±4 nmol reduced cytochrome C/10^6 cells, respectively; n=3). Staurosporine, Ro31-8220 and Gö 6976, but not rottlerin, inhibited superoxide production. At the highest concentrations tested Ro31-8220 (1µM) completely inhibited the response in eosinophils from both groups of ponies, staurosporine (10µM) completely inhibited the response of cells from normal ponies, reducing that of cells from sweet itch ponies by 83±6% and Gö 6976 (2µM) caused 68±3% and 75±2% inhibition in cells from normal and sweet itch ponies, respectively. There was no significant difference between the effects of staurosporine or Ro31-8220 in cells from the two groups of animals. However Gö 6976 caused significantly greater inhibition in cells from sweet itch ponies ($p <0.05$). Neither Ro31-8220 nor staurosporine significantly affected histamine-induced adherence (10±2% of cells added initially versus 13±2% and 14±2% versus 21±3% in the absence and presence of 1µM Ro31-8220 or 10µM staurosporine, respectively).

These results suggest that PKC in equine eosinophils is involved in the regulation of histamine-induced superoxide production but not adherence.

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35P EFFECT OF PHOSPHODIESTERASE INHIBITORS ON EQUINE PLATELET AGGREGATION

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The non-selective phosphodiesterase (PDE) inhibitor, theophylline, has been reported to inhibit platelet activating factor (PAF)- and adenosine diphosphate (ADP)-induced aggregation of human platelets (Misso & Thompson, 1992). Activation of equine platelets in conditions such as endotoxaemia is thought to involve adenine nucleotides and platelet activating factor (Jarvis & Evans, 1994). In the present study the effects of theophylline and selective inhibitors of PDE3 and PDE5 on equine platelet aggregation induced by PAF and ADP have been examined.

Theophylline (10^{-4}-10^{-3}M), the PDE3 inhibitors, trequinsin ($3x10^{-10}$-$3x10^{-7}$M) and quazinone ($6x10^{-8}$-$6x10^{-5}$M), zaprinast, a PDE5 inhibitor (10^{-5}M) or vehicle control were added to platelet rich plasma prepared from normal ponies 1min prior to the agonist (Ablett *et al.*, 1997). PAF was used at a concentration of 10^{-9}M that produced a sub-maximal response. ADP was used at a concentration that caused a response of similar magnitude in each horse. This varied between 5 x 10^{-6}M and 10^{-4}M in the individual ponies studied.

Theophylline and the two PDE3 inhibitors caused concentration dependent inhibition of both PAF- and ADP-induced aggregation and the response to PAF appeared to be more sensitive to inhibition (Table 1). Zaprinast did not affect the response to either agonist (PAF: 18±5mV with versus 18±4mV without zaprinast. ADP: 16±2 mV with versus 17±3 mV without zaprinast (means ± SD (n = 5).

Table 1: Effect of theophylline, trequinsin and quazinone on PAF- and ADP-induced equine platelet aggregation

	% inhibition of aggregation			
(a) Theophylline	10^{-4}M	$3.3x10^{-4}$M	$6.6x10^{-4}$M	10^{-3}M
PAF	2±3	*23 ±11	36±10	57± 8
ADP	1±2	0.5 ±1	17±16	42±26
Control responses 19±3mV and 21±3mV for PAF and ADP				
(b) Trequinsin	$3x10^{-10}$M	$3x10^{-9}$M	$3x10^{-8}$M	$3x10^{-7}$
PAF	10±5	14±11	**50±14	100±0
ADP	2±3	5±7	21±20	85±18
Control responses 18±4mV and 17±3mV for PAF and ADP				
(c) Quazinone	$6x10^{-8}$M	$6x10^{-7}$M	$6x10^{-6}$M	$6x10^{-5}$
PAF	5±7	13±12	***77±20	97±4
ADP	3±4	8±5	44±20	94±6
Control responses 18 ±3mV and 18±5 mV for PAF and ADP				

Values are means±SD; n = 5 or 6; * = $p < 0.05$, ** = $p < 0.01$, ***$p < 0.001$ versus effect on ADP; 2-way ANOVA followed by Bonferroni's test

These data suggest that PDE3 is present in equine platelets and inhibition of this isoenzyme attenuates PAF- and ADP-induced platelet aggregation. PDE3 inhibitors may be of benefit in clinical conditions where platelet activation occurs.

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36P DETECTION OF MULTIPLE FORMS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ENDOSTATIN IN LEG ULCER FLUID.

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Vascular endothelial growth factor (VEGF) is a well-documented stimulator of angiogenesis, and endostatin, a 20 kD fragment of the C-terminal domain (NC1) of collagen XVIII, has anti-angiogenic activity (O'Reilly *et al*, 1997). These agents have been shown to modulate the activities of each other in *in vitro* models of angiogenesis (Yamaguchi *et al*, 1999). VEGF is elevated in some venous leg ulcers (Lauer *et al*, 2000) but the role of endogenous inhibitors of angiogenesis in leg ulcers is unclear. In this study we have started to characterise VEGF and endostatin in fluid from venous leg ulcers in order to understand how modulators of angiogenesis may affect the pathology of this disorder.

Bandages from patients with chronic leg ulcers were immersed in saline, the wound fluid was expressed from the bandages, centrifuged and frozen at –70°C. Mastectomy fluid was collected from drainage bottles. Levels of VEGF and endostatin in the fluids were measured by ELISA (Oncogene Research Products) and multiple forms of endostatin and VEGF were characterised by western blot analysis using an ECL detection system.

Levels of VEGF for three wounds were in the range 2.0 – 7.4 ng ml^{-1} (n=10), with a mean of 3.7 ng ml^{-1}. These are elevated compared to reported values for human serum which are approx. 0.3 ng ml^{-1} (Kraft *et al*, 1999). As determined by western blot analysis, two leg ulcer fluids contained a band corresponding to recombinant human $VEGF_{165}$, although a band of slightly higher mol wt than the $VEGF_{165}$ standard was the predominant form in each case. A number of higher mol wt bands were also present for each wound fluid. Endostatin levels were in the range 10.0 – 65.5 ng ml^{-1} (n=4), with a mean of 25.6 ng ml^{-1}. Endostatin levels in serum and mastectomy fluid were 7.7 and 79.5 ng ml^{-1} respectively. Endostatin (mol wt 20 kd) was detected by western blot analysis in all three leg ulcer fluids. In addition higher mol wt bands between 30-50 kd were present in all three fluids.

This study indicates that levels of both endostatin and VEGF are elevated in some leg ulcer fluids relative to levels normally found in serum. Multiple forms of both endostatin and VEGF were present in the leg ulcer fluids. This may be due to the production of several isoforms, in the case of VEGF, and the generation of multiple proteolytic degradation products for both VEGF and endostatin.

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37P EFFECTS OF SELECTIVE INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) INHIBITION ON ALLERGEN-INDUCED AIRWAY RESPONSES IN CONSCIOUS GUINEA-PIGS

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Allergen inhalation in atopic asthma may result in an early asthmatic reaction (EAR), late asthmatic reaction (LAR), airway hyperresponsiveness (AHR) and increased leukocyte influx (Cockcroft, 1983; Robinson *et al*, 1993). These four parameters of asthma are also found in our animal model of asthma (Spruntulis & Broadley, 1999). This study investigated the involvement of iNOS-derived NO in this model of asthma.

Male Dunkin-Hartley guinea-pigs (250-300g) were sensitised with ovalbumin (OA) (1ml i.p. of a suspension containing 10μg OA and 100mg $Al(OH)_3$ in normal saline). 14-21 days later they received inhaled OA (100μg.ml^{-1} in saline) or saline for 1h. Airway function was measured as changes in specific airway conductance (sGaw) at intervals up to12h and at 24h by whole body plethysmography. Each group (n=7) was exposed to inhaled histamine (1mM nose only for 20s) both 24h before and 24h after OA or saline challenge (Spruntulis & Broadley, 1999). 1h before OA challenge and 4.5h following OA challenge each group received either the selective iNOS inhibitor, aminoguanidine (10mg.kg^{-1} in 1ml saline, i.p.) (Misko *et al*, 1993; Imasaki *et al*, 2001) or its vehicle (1ml saline, i.p.). 24h following OA or saline challenge, guinea-pigs were overdosed (pentobarbitone sodium 200mg.kg^{-1}) and lungs lavaged (saline 1ml.100g^{-1}, twice) to determine leukocyte numbers (Spruntulis & Broadley, 1999). Statistical analysis was by analysis of variance followed by paired or unpaired Student's *t*-test.

Vehicle- and aminoguanidine-treated guinea-pigs gave a similar immediate EAR (-38.2±6.23%, -37.1±5.5% reduction in sGaw) which recovered by 5h. In comparison to vehicle (-18.0±1.8%), the aminoguanidine-treated group did not give a LAR between 6 and 12h (-6.2±1.4%, P<0.001). In the vehicle treated-group, 24h following OA challenge, histamine caused a significant (P<0.01) bronchoconstriction (–22.7±6.2%) compared with no response before OA, indicating AHR. No increase in airway reactivity to histamine was noted in the aminoguanidine-treated group. In comparison to vehicle, the aminoguanidine-treated group gave significantly reduced total cell (8.9±1.2, 3.6±0.7×10^6.ml^{-1}, P<0.01), macrophage (4.3± 0.5, 2.0±0.4×10^6.ml^{-1}, P<0.01) and eosinophil numbers (4.4± 0.7, 1.5±0.3×10^6.ml^{-1}, P<0.01) in bronchoalveolar lavage (BAL), 24h following OA challenge. Leukocyte numbers after aminoguanidine approached levels in the saline challenged group (2.7±0.3, 2.4±0.2 and 0.3±0.07×10^6.ml^{-1})

These results indicate the involvement of iNOS-derived NO in the development of the LAR, AHR and leukocyte influx in our animal model of asthma.

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38P CHARACTERISATION OF TRANSFECTED HUMAN ADENOSINE A_3 RECEPTORS

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To date very few studies have investigated binding and functional characterisation of A_3 adenosine receptors (A_3AR) in native tissues and/or cells. Such studies are essential to further our understanding of A_3 receptor function. Previous studies have characterised human A_3AR binding using a transfected cell line (Klotz *et al.*, 1999). The aim of this study was to validate and extend binding and functional assays (inhibition of cAMP production) for the human A_3AR in a transfected cell line to enable future studies to be conducted in native tissues such as heart, lung, tumour and immune cells.

Human A_3AR-transfected Chinese Hamster Ovary (CHO) cells were maintained in DMEM/F12 with 10% foetal calf serum, 0.666mg/ml G418, 500μg/ml hygromycin and 1U/ml adenosine deaminase. Radioligand binding assays were performed using the A_3AR agonist [^{125}I]-AB-MECA and a modified method of Olah *et al.* (1994). Assays were conducted at room temperature for 60min using a 50mM Tris, 10mM $MgCl_2$ pH 7.4 buffer, 20μg membranes and 0.3nM [^{125}I]-AB-MECA. The ability of adenosine analogues to inhibit binding was investigated. Non-specific binding was determined using the respective analogue at its highest concentration. Intracellular cAMP production was measured directly using a non-acetylation cAMP enzyme immunoassay (Amersham). Cells were incubated at 37°C with adenosine analogues (5mins) followed by incubation with forskolin (1x10^{-5}M, 10mins). Dose-response curves were constructed and IC_{50} values calculated. In studies with the A_3AR antagonist, MRS1220, cells were initially incubated in MRS1220 (5mins), followed by IB-MECA (5mins) then forskolin (10mins). Dose-response curves were constructed and IC_{50} values in the presence and absence of MRS1220 and concentration-ratios (CR) calculated. Kd values were determined from a Schild plot of log (CR-1) against MRS1220 concentration.

Table 1. IC_{50} or Kd values of adenosine ligands from radioligand binding and functional assay.

Agonist	Binding IC_{50} nM	Functional IC_{50} nM
IB-MECA	30.4 (12.3-64.5)	0.7 (0.15-2.33)
NECA	57 (35.3-89)	18.3 (4-64)
R-PIA	81.5 (45.8-138)	48.7 (30-74)
AB-MECA	218.7 (99.7-438)	—
CPA	315 (66.3-1043)	309.7 (37-1798)
Antagonist	**Binding IC_{50} μM**	**Functional Kd (nM)**
DPCPX	3.5 (1.8-6.3)	—
MRS1220	0.0033 (0.0023-0.0046)	0.63
8-SPT	40.5 (29.9-53.1)	—
8-PT	6.9 (2.1-18.6)	—

Numbers in brackets are 95% confidence limits, n = 3

Both assays displayed a similar rank order of agonist potency with IB-MECA>NECA>R-PIA>AB-MECA>CPA. This confirmed the findings of Klotz *et al.* (1999) and is characteristic of A_3AR. However, the agonists were more potent in the functional assay which may be due to binding to the high affinity state of the receptor while in the binding assay the low affinity state of the receptor predominated. The binding assay also displayed a rank order of antagonist potency characteristic of A_3AR. The Kd value for MRS1220 in the functional assay is consistent with the Ki value from binding assays (Kim *et al.*, 1996). This study validates and characterises methods for the identification of A_3AR in native tissues.

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39P INDUCTION OF APOPTOSIS IN THE EPITHELIAL CELL LINE HT29 BY NICOTINE AND TOBACCO SMOKE CONDENSATE

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We have applied a technique whereby apoptotic cells are identified by staining with the fluorescent stain 4',6-diamino-2-phenylindole (DAPI; Ruiz *et al.*, 1999) to the measurement of apoptosis in the epithelial cell line HT29 induced by nicotine and tobacco smoke condensate (TSC; BAT industries; 8% nicotine w/w).

Jurkat cells were cultured as described by Summers *et al.* (2002). Apoptosis was induced in Jurkat cells by incubation at room temperature for 48h (Shimura *et al.*, 1998). Aliquots (100µl) of cultures were taken placed on a microscope slide by cytocentrifugation and stained with DAPI as described by Ruiz *et al.* (1999). Slides were examined under UV light and the number of apoptotoic and non-apoptotic cells counted. In some experiments DNA was extracted from Jurkat cells and subjected to electrophoresis on 2% agarose gels. Gels were stained with ethidium bromide (5µgml^{-1}) and DNA visualised under UV light. HT-29 cells were cultured in 6 well plates as described by Ihenetu *et al.* (2001). Once cells had reached confluence, the medium was replaced with FCS-free medium containing vehicle (0.1% DMSO), nicotine (0.08-8 ngml^{-1}) or TSC (1-100ngml^{-1}). After 24h incubation at 37°C, the medium was removed and the cells fixed and stained with DAPI. Plates were examined by UV microscopy and the number of apoptotic and non-apoptotic cells counted. The number of apoptotic cells were expressed as a percentage of the total number of cells counted.

When Jurkat cells were incubated at room temperature an increase in apoptosis was seen such that after 48h 11.3±3% (n=6) of the cells appeared apoptotic. When DNA was extracted from these cells and subjected to electrophoresis, DNA laddering, consistent with the development of apoptosis, was seen.

When incubated with HT-29 cells, TSC (1-100ngml^{-1}) caused a concentration-related increase in the percentage of apoptotic cells as measured by DAPI. The highest concentration of TSC tested (100ngml^{-1}) caused a significant ($P<0.05$; ANOVA) increase in apoptosis from 6.3±1.1 (vehicle treated) to 22.8±1.9 (n=6). Similarly, nicotine (0.08-8ngml^{-1}) caused a concentration-related increase in apoptosis of HT-29 cells in culture. The highest concentration of nicotine tested (8ngml^{-1}) also significantly ($P<0.05$; unpaired t test) increased the percentage of apoptotic cells from 8.7±2.9 to 26.7±7.0 (n=6).

We conclude that TSC and nicotine induce apoptosis in the human epithelial cell line HT-29 *in vitro* as measured by cell morphology. However, further studies are required to determine the significance of these effects.

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40P PROTEIN EXPRESSION IN RAT LIVER: RESPONSE TO SHORT TERM TREATMENT WITH PHENOBARBITAL USING TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Phenobarbital (PB) is a non-genotoxic carcinogen in rodent liver. It has been suggested that PB may prevent the detection and repair of DNA damage and indirectly increase the frequency of genotoxic events (Heller & Kronke, 1994). However, the mechanism of tumorigenesis is not fully elucidated. Changes in protein expression in rat liver in response to short-term exposure to PB may highlight early mechanisms of tumorigenesis and identify early tumour markers. This study investigated protein expression in rat liver using both mini- and large format- two-dimensional gel electrophoresis following short-term treatment with PB.

Groups of five male Sprague-Dawley rats were treated with PB (50 mg/kg, i.p.) or saline for up to 3 days. Livers were collected 12h, or 24 h after a single dose, 24h after 2 daily doses and 24h or 48h after three daily doses. First dimensional separation employed isoelectric focusing, followed by SDS-polyacrylamide gel electrophoresis for the second dimension. Proteins were visualised by silver staining and gel images were analysed for changes in protein expression using Phoretix software. Proteins were defined as up- or down-regulated when the treated sample showed at least 50% change in spot density compared to control. Using mini-2D gel electrophoresis, PB treatment caused a suppression of 5 proteins (M.W.: 60, 65, 66, 75 and 80 kD) 12 h after a single dose and all cumulative dosing schedules.

The greatest suppression was observed in liver harvested 48h after the three daily dose regime. Further work to identify these proteins is on-going. Clear identification of proteins up-regulated in response to PB was more difficult and it was decided to run the samples on large format gels. Large-format gel electrophoresis followed by mass spectrometry and mass fingerprint analysis identified four up-regulated proteins; 6-phosphofructo-2-kinase, Cysteine rich protein 2 (CRP2), Protein kinase C (PKC), and Tubulin α. Products of 6-phosphofructo-2-kinase activity affect phosphofructokinase (Uyeda et al, 1982), a key regulatory enzyme of glycolysis.

Gene expression of glycolytic enzymes including phospho-fructokinase has been reportedly increased in tumour cells (Dang & Semenza, 1999). There is no direct evidence linking CRP2 to tumorigenesis, but CRP2 is thought to be involved in the processes of cell proliferation, development and differentiation (Weiskirchen & Gressner, 2000). PKC has been implicated in various biological processes including neoplastic transformation (Goodnight et al, 1994). Tubulin α is a substrate for tubulin tyrosine ligase (TTL), an enzyme involved in apoptosis. TTL is commonly suppressed during tumour growth (Idriss, 2000).

Western blot will be used to confirm and give a quantitative assessment of the changes in expression of these proteins.

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41P PREVIOUS EXPOSURE TO CHOLECYSTOKININ SENSITISES THE GUINEA PIG ISOLATED ILEUM TO THE CONTRACTILE EFFECTS OF ACETYLCHOLINE

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We have recently demonstrated that previous exposure to cholecystokinin (CCK) sensitises the guinea pig isolated pyloric sphincter muscle to the contractile effects of acetylcholine (ACh). The present study was undertaken to investigate whether exposure to CCK would sensitise the guinea pig isolated ileum to the effects of ACh.

Adult male guinea-pigs (in bred strain) were killed by exposure to CO_2 and cervical dislocation. 3 cm lengths of the ileum were set up in a 10 ml isolated organ bath and attached to isometric force transducers. Experiment 1. Concentration-response curves were constructed for ACh (n= 26, ACh concentration range: $1x10^{-11}$ to $1x10^{-5}$M) and CCK (n=8; CCK concentration range $1x10^{-9}$ to $1x10^{-5}$M). In all cases the tissue was exposed to a particular concentration of the drug for 30s before it was washed out. The tissue was allowed to recover for 60s before it was exposed to the next concentration of the drug. Experiment 2. The ileum was exposed to CCK ($1x10^{-6}$M) for (a) 2 min (n=3) or (b) 3 min (n=4) before it was washed out. 60s later the tissue was exposed to ACh $1x10^{-6}$M (n=4). This procedure was repeated 5 times for each tissue. The contractile response of the muscle to ACh ($1x10^{-6}$M) measured prior to exposure to CCK was compared with those recorded following exposure to CCK. Experiment 3. A similar procedure to that used for Experiment 2a was used except that 30s prior to exposure to ACh, atropine was added to the bath. The results obtained in this study were analysed by repeated measures ANOVA followed by *post-hoc* tests.

Both ACh and CCK produced concentration-dependent increases in the contractile responses of the guinea pig ileum (EC50 = $2.38x10^{-7}$ M and $1.1x10^{-7}$M respectively). The results obtained in Experiment 2 show that the contractile responses to ACh ($1x10^{-7}$M) were significantly increased in tissue that had been previously exposed to CCK ($1x10^{-6}$M) for 2 min ($F=4.122$, $P<0.01$) or 3 min ($F=3.027$, $P<0.05$) [see Fig. 1a & B]. The increases in the response to ACh after incubation with CCK was greater after 2 min exposure compared with 3 min exposure. In control experiments we also established that the responses to ACh did not alter with successive trials when the tissue was incubated with saline solution instead of CCK (data not shown).

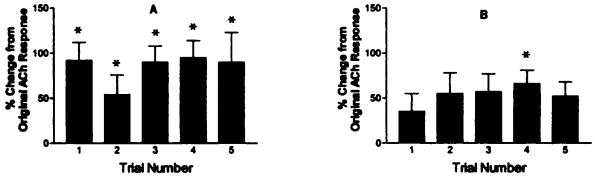

Figure 1. Effects of ACh ($1x10^{-6}$M) ($1x10^{-7}$M) on contractile responses of the guinea pig ileum that has been previously exposed to CCK ($1x10^{-6}$M) for (A) 2 min, or (B) 3 min. Vertical lines are + s.e.mean, *$P<0.05$ compared with the original response to ACh.

The results from Experiment 3 show that the response to ACh ($1x10^{-7}$M) after previous exposure to CCK ($1x10^{-6}$M) was reduced in a dose-related manner by atropine. For example, atropine 10^{-8}M reduced the response by 40.7%, and atropine 10^{-6}M totally abolished the contractile response of the tissue to ACh.

The results of this study indicate that CCK sensitises the guinea pig ileum to the contractile effects of ACh. This enhancement appears to be mediated by post-synaptic ACh muscarinic receptors, as it can be totally abolished with atropine. The present results extend previous results obtained with the isolated pyloric sphincter muscle (Patel and Ebenezer, 2002)

Patel, J.D. and Ebenezer, I.S. (2002) *Br. J. Pharmacol.*, P in press.

42P ALTERATIONS IN BASAL AND EVOKED CONTRACTILE RESPONSES OF INTESTINAL TISSUE FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Gastrointestinal disturbances are common in diabetic patients (Mathison & Davison, 1988) and altered gut smooth muscle responsiveness to neurotransmitters in tissues taken from diabetic animals has been reported (Carrier & Aronstam, 1990). The purpose of the present study is to examine alterations in basal and evoked contractile activity of small and large intestinal tissue taken from diabetic rats.

Male Wistar rats (200-350g) were rendered diabetic by a single injection of streptozotocin (STZ) 65mgkg^{-1} i.p. Age-matched controls were injected with vehicle alone. After eight weeks, animals were killed and 2cm lengths of distal ileum and 1cm lengths of proximal colon were removed and mounted in organ baths containing oxygenated (95%O_2/5%CO_2) Krebs solution at 37°C. Tissues were placed under 1g resting tension for isometric recording. On the ileum, cumulative concentration-response curves were established to agonists and electrical field stimulation (EFS 30V, 10Hz, 0.25-5ms for 10s every 5min) was applied via parallel platinum electrodes. Values are expressed as tension in g / g wet weight with the mean ± s.e.m. Student's unpaired t-test was used to determine significant differences between control and STZ tissues.

Eight weeks after STZ administration, blood sugar levels were significantly ($p<0.05$) elevated in the treated rats (396±16.17mgdl^{-1}) compared to controls (92.57±5.43mgdl^{-1}) (n=30 in each case). Both the ileum and the colon showed basal contractile activity which was atropine (1μM) and tetrodotoxin (1μM) insensitive, but abolished by nifedipine (10μM). This basal activity was significantly greater in tissues from diabetic animals than controls ($p<0.05$).

Table 1: The contractile responses (gram/gram wet tissue weight) of the rat ileum to applied stimulants.

Agonist	Concentration	n	Control	STZ
Carbachol	10^{-8}M	10	0.34±0.04	1.01±0.23
	10^{-7}M		2.74±0.49	7.89±1.23*
	10^{-6}M		9.87±1.13	11.9±11.50*
	10^{-5}M		8.17±0.86	10.98±1.19*
Prostaglandin	10^{-8}M	6	0.48±0.18	1.21±0.36
	10^{-7}M		1.45±0.39	3.64±0.79*
	10^{-6}M		1.99±0.53	4.47±0.92*
	10^{-5}M		2.43±0.71	5.23±1.26*
Calcium Ionophore	10^{-7}M	7	0.67±0.21	0.64±0.11
	10^{-6}M		3.81±0.45	4.48±0.41
	10^{-5}M		6.37±0.46	9.49±0.77*
EFS	0.25ms	7	2.01±0.87	5.96±1.47*
	0.50ms		2.93±1.00	8.43±1.54*
	1.00ms		3.57±0.94	9.48±1.61*

Carbachol, prostaglandin $F_{2\alpha}$ and the calcium ionophore A23187 produced concentration-related contractile responses of the ileum, which were greater in tissues from STZ-treated animals than controls (table 1). EFS produced a pulse-duration related atropine sensitive contractile response and again, the responses of STZ tissues were greater than controls (*$p<0.05$).

These results suggest that prolonged hyperglycaemia may lead to hyperexcitability of intestinal smooth muscle and possibly enhanced acetylcholine release to EFS. The changes may underlie the motility disturbances seen in diabetic patients.

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A.F. was supported by Diabetes UK.

43P INVESTIGATION OF THE EXCITATORY EFFECT OF 5-HT ON CONTRACTILE RESPONSES OF HUMAN COLON SMOOTH MUSCLE

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We have previously shown that exogenous application of 5-HT to human colon smooth muscle strips *in vitro* causes potentiation of electrically-induced, cholinergic nerve-mediated contractions. By the use of selective antagonists, we have shown that, in contrast to animal species, the receptor mediating this effect of 5-HT corresponds to a 5-HT_{2B} receptor (Borman et al., 2002). However, in the same study we have shown that within the human colon, the 5-HT_{2B} receptor is localised on both longitudinal smooth muscle and on myenteric neurones. It is, therefore, unclear whether this 5-HT_{2B}-receptor-mediated potentiation is a direct effect on the smooth muscle itself, an effect on the release of excitatory neurotransmitters, such as acetylcholine, or on muscarinic receptors. In the present study, we have investigated the effects of 5-HT on contractile responses resulting from both receptor-mediated and receptor-independent mechanisms.

All tissue samples were obtained through medically qualified intermediaries with the informed consent of the donor, and with the approval of the local ethics committee. Smooth muscle strips of human colon longitudinal muscle were mounted in organ baths, bathed by gassed (95% O_2:5% CO_2) Krebs solution at 37°C, for the measurement of isometric changes in muscle tension. After 60min equilibration, with washes every 15 min, at an initial resting tension of 15mN, concentration–effect curves were generated to either acetylcholine (ACh) or potassium chloride (KCl). After a maximum response had been obtained, the tissues were washed until tension had returned to baseline. From the resulting concentration-effect curve to each spasmogen, a concentration was chosen that produced approximately 50% of the maximum contractile response to that spasmogen (sub-maximal concentration). After a further 30min equilibration, the muscle strips were subjected to a challenge- wash cycle, which consisted of a sub-maximal concentration of spasmogen, 5 min equilibration to allow a maximum contractile response to be obtained, followed by three washes over a 10 min period. After a further 5 min equilibration, this cycle was repeated until adjacent contractile responses to each spasmogen differed by no more than 10%. At this time, the challenges to each spasmogen were repeated in the absence and presence of ascending concentrations of 5-HT (10^{-9} to 10^{-5}M), whereby each concentration of 5-HT was applied three minutes prior to the spasmogen challenge, and washed out afterwards as before. In this way, it was possible to generate a non-cumulative concentration-effect curve to 5-HT, to investigate its effect on contractile responses to both KCl and ACh. Data are given as mean±s.e.mean, statistical analyses used the students t test, with $p<0.05$ taken to indicate statistical significance.

In the absence of 5-HT, contractile responses to both KCl and ACh showed a high degree of reproducibility over time. While application of 5-HT had no direct contractile effect at any concentration, it caused a significant, concentration-dependent augmentation of contractile responses to both ACh and KCl. The maximum 5-HT-induced enhancement was 5.0±1.4mN (89.5±30.0% of pre-5-HT contraction) and 11.3±4.6mN (98.9±47.3% of pre-5-HT contraction) for KCl and ACh respectively (both n=4). The pEC_{50} values for 5-HT were 7.9±0.4 and 8.0±0.7 respectively.

In summary, we have shown that application of 5-HT to isolated longitudinal smooth muscle strips of human colon caused potentiation of responses to both ACh and KCl. We conclude that 5-HT is capable of causing augmentation of both receptor-mediated and receptor-independent contractile responses in human colon smooth muscle, in addition to any putative effects on neurotransmitter release.

Borman, R.A., Tilford, N.S., Harmer, D.W. *et al.*, Br. J. Pharmacol., 135, 1144-51.

44P THE EFFECT OF MYOSIN LIGHT CHAIN KINASE INHIBITION ON PHARMALOGICALLY INDUCED CHANGES IN PARACELLULAR INTESTINAL EPITHELIAL PERMEABILITY

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Tight junctions (TJ) between intestinal epithelial cells act as a dynamic barrier to the paracellular passage of hydrophilic molecules. Epithelial paracellular permeability is dependent on structural changes within the TJ involving junctional proteins and peri-junctional actin and myosin. The precise mechanisms involved in TJ regulation remain poorly understood but include activated protein kinase C phosphorylation of myosin light chain kinase (MLCK) which can lead to opening of TJs. Disruption of perijunctional actin filaments with cytochalasins causes an increase in TJ permeability which appears to be regulated by MLCK activation [Ma *et al.*, 2000]. Bile salts can cause reversible increases in TJ permeability but the mechanism is unknown. In this study we investigated whether ML-9 (a MLCK-inhibitor) inhibited cytochalasin D (CD) and bile salt induced increases in epithelial permeability.

Caco-2 intestinal epithelial cell monolayers were used for all experiments. Transepithelial electrical resistance (TEER) was recorded using an Endohm voltmeter (WPI). Permeability to ^{14}C mannitol was determined and expressed as Papp (apparent permeability coefficient). TEER and Papp were measured for each monolayer before and after the addition of either CD ($2\,\mu g.ml^{-1}$) or taurodeoxycholic acid (2mM) to the apical compartment in separate experiments. Similar experiments were performed on monolayers which were treated with ML-9 ($50\,\mu M$) apically and basolaterally prior to the addition of either CD or the bile salt. Results are presented as mean ±SEM of n=4-7 observations. Statistical analysis was carried out using the Students unpaired two tailed t-test.

TEER was high, $1035\pm22\,\Omega cm^2$, and Papp low, $2.4\pm0.1\times10^{-7}$ $cm.sec^{-1}$, (n=24) at the start of each experiment representing mature monolayers with intact junctions. TEER and Papp of control monolayers remained constant throughout the experimental period. CD caused a decrease of 24±2% in TEER after 20 minutes which was significantly less in monolayers pre-treated with ML-9, 12±3% ($p<0.05$). In addition CD caused an increase in Papp, $4.5\pm0.4\times10^{-7}$ $cm.sec^{-1}$ after 60 minutes, which was inhibited by pre-treatment with ML-9, $2.9\pm0.3\times10^{-7}$cm/sec ($p=0.01$). Taurodeoxycholic acid resulted in a significant increase in Papp compared with controls at 60 minutes: $13.1\pm2.8\times10^{-7}$ vs. $2.2\pm0.1\times10^{-7}cm.sec^{-1}$ ($p<0.05$). Pre-treatment of monolayers with ML-9 resulted in an even greater increase in Papp when taurodeoxycholic acid was added compared with monolayers exposed to bile salt alone $62.7\pm9.2\times10^{-7}$ vs. $13.1\pm2.8\times10^{-7}$ ($p<0.001$). Lower concentrations of the bile salt had no significant effect on TEER and mannitol flux.

We have confirmed previous findings that cytochalasin induced increase in TJ permeability is inhibited by ML-9 suggesting that it is regulated by MLCK activation. MLCK inhibition by ML-9 however causes a paradoxical rise in bile salt induced increase in mannitol flux. This suggests that bile salts affect epithelial paracellular permeability via an alternative mechanism.

Ma, T., Hoa, N., Tran, D., et al. 2000. Am J Physiol 279: G875-G885

45P MEGACOLON IN THE STREPTOZOTOCIN-TREATED DIABETIC RAT

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Idiopathic megacolon has been reported as a feature of uncontrolled diabetes in experimental models of diabetes. Although the mechanism(s) which account for colonic distension are not known, such dilation may influence susceptibility to colitis or exacerbation of low levels of chronic inflammation of the gut. This study was designed to confirm that megacolon is a feature of diabetic rats and secondly to ask whether altered epithelial ion transport may contribute to these pathological events.

Male Wistar rats (200-250g) were kept in paired cages. Diabetes was induced in one of each pair by an intraperitoneal injection of streptozotocin (STZ; 42.5mg/kg). Control animals received a vehicle injection. Eight weeks later the animals were killed. Blood sugar levels were measured and colonic mass determined gravimetrically. Sheets of distal colonic mucosa were stripped of their underlying smooth muscle and mounted in Ussing chambers (window area = 0.63cm^2), bathed on each surface by identical Krebs-bicarbonate solution, gassed with 95%O_2/5%CO_2 and maintained at 37°C. Tissues were voltage clamped to zero transepithelial potential difference by applying a short circuit current (SCC). SCC was recorded continuously with imposed breaks every 5 minutes in order to calculate transepithelial electrical resistance (TER) by applying the Ohmic relationship.

Statistical analysis was carried out using Mann-Whitney tests. Results are presented as mean ± s.e.m (n=6-10). STZ produced a clear increase in blood glucose (24.8±1.2 mmol/l) over that of untreated controls (6.3±0.2mmol/l). Although body weight in the STZ group was less than that of matched controls (382±14g vs 424±8g respectively; $p<0.05$) colon tissue weights were higher ($p<0.05$) in the STZ group (3.7±0.3g) than in controls (2.4±0.2g).

Notwithstanding the differences induced by STZ treatment, basal electrophysiological parameters were not different in voltage-clamped mucosae when the control and experimental groups were compared. For example, SCC values were 48±6 μA.cm^{-2} and 54±8μA.cm^{-2} and TER values were 37±11Ω.cm^2 and 35±9Ω.cm^2 for control and STZ-treated groups respectively. Similarly, ion transport responses to agents which stimulate electrogenic chloride secretion in colonic epithelia (carbachol; 10^{-7}-10^{-6}M and prostaglandin E_2; (10^{-7}-10^{-6}M) were not significantly different in tissues from animals pre-treated with STZ when compared with their controls.

These data suggest that the distended colon of animals with uncontrolled diabetes is not a consequence of altered epithelial function or secretory capacity. Thus the consequences of STZ treatment on the lower bowel may be due to an influence on vasculature, smooth muscle or autonomic function rather than on epithelial cells.

46P CAPACITATIVE CALCIUM ENTRY IN GUINEA-PIG GALLBLADDER SMOOTH MUSCLE

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There is increasing evidence to suggest that capacitative Ca^{2+} entry through store-operated channels (SOCs) may be important for the maintenance of sustained tone in some smooth muscle preparations (Gibson *et al.*, 1998). Recently, we reported that Ca^{2+} entry through SOCs may provide a source of activator Ca^{2+} for bradykinin induced contractions of guinea-pig gallbladder smooth muscle (O'Riordan *et al.*, 2001). This study was undertaken (a) to confirm the involvement of capacitative Ca^{2+} entry in excitation-contraction coupling in guinea-pig gallbladder smooth muscle and (b) to investigate the effects of agents known to inhibit capacitative Ca^{2+} entry in other cell types (Parekh and Penner, 1997) namely, the general Ca^{2+} entry blocker, SK&F96365 and the tyrosine kinase inhibitor, genistein, on capacitative Ca^{2+} entry in this tissue.

Strips of gallbladder from adult Dunkin-Hartley guinea-pigs were suspended under 0.5 g of tension in Krebs solution at 37°C gassed with 95% O_2/5% CO_2. Mechanical activity was recorded isometrically. Following a 1 hr equilibration period, tissues were exposed to carbachol (10 μM) to determine the maximal contractile capacity. Statistical analysis was carried out using the Mann-Whitney two tailed test. Results are presented as mean ± s.e.m. of n=5-7 observations, except where indicated.

Carbachol produced 2.13 ± 0.8 g of tension (n=62) in guinea-pig gallbladder smooth muscle strips. Thapsigargin (1 μM, a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor) produced a slowly developing tonic contraction. Peak tension occurred approximately 35 min after the addition of thapsigargin (55 ± 11% of the maximal carbachol response). The contractile response to thapsigargin was abolished in a nominally Ca^{2+}-free medium. Subsequent re-addition of Ca^{2+} (2.5 mM) caused a tonic contraction (99 ± 6% of the maximal response to carbachol). The contractile response to Ca^{2+} re-addition was attenuated by (a) the L-type voltage-dependent Ca^{2+} channel antagonist, nifedipine (10 μM) (54 ± 7% of the maximal carbachol response; $p<0.005$) and (b) SK&F96365 (50 and 100 μM) (19 ± 3 and 9 ± 3% of the maximal carbachol response; $p<0.005$) and it was essentially abolished by (c) genistein (100 μM) (0.8 ± 0.7% of the maximal carbachol response; $P<0.005$).

We conclude that the contractile response to Ca^{2+} re-addition in guinea-pig gallbladder smooth muscle following depletion of sarcoplasmic reticulum Ca^{2+} stores with thapsigargin, is mediated in part by Ca^{2+} entry through voltage-operated Ca^{2+} channels and by capacitative Ca^{2+} entry through SOCs which can be blocked by SK&F96365. Furthermore, capacitative Ca^{2+} entry in this tissue is modulated by tyrosine kinase.

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47P THE ROLE OF NON-CONSERVED PROLINE RESIDUES IN THE EXTRACELLULAR DOMAIN OF THE MOUSE 5-HT$_{3A}$ RECEPTOR

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The 5-HT$_3$ receptor is a member of the Cys-loop family of ligand gated ion channels. Receptor subunits of this family are composed of a large N-terminal extracellular domain responsible for ligand binding, and four transmembrane domains, one of which (M2) lines the channel pore. We have been examining the role of proline residues in the N-terminal domain; as these residues impose considerable restraint on protein folding, they are often critical for the structure and/or function of the molecule. Previous studies (Deane & Lummis, 2001) have concentrated on proline residues conserved throughout this protein family. Here we examine the role of non-conserved proline residues.

Proline (P) residues in the mouse 5-HT$_{3A(b)}$ receptor subunit were converted to alanine (A) using the Kunkel method (Kunkel, 1985) in the expression vector pcDNA3 (Clontech); this was then transiently transfected into HEK 293 cells using calcium phosphate precipitation (Chen & Okayama, 1988). Receptor characteristics were examined using radioligand binding assays with the 5-HT$_3$ receptor antagonist [^{3}H]granisetron on transfected HEK293 cell membranes (Spier & Lummis, 2000), and with immunocytochemistry using a 5-HT$_3$ receptor specific antiserum (pAb120; Spier et al.,1998) on permeabilised and non-permeabilised HEK293 cells.

All mutant receptors except one (P155A) had specific binding; K_d values are shown in Table 1. HEK293 cells transfected with this non-binding mutant also showed no labelling with pAb120 in either permeabilised or non-permeabilised cells (n=3).

Table 1.
[^{3}H]granisetron binding affinities for 5-HT$_3$ receptor mutants

Receptor	K_d	Receptor	K_d
Wild type	0.25 ± 0.05	P140A	0.20 ± 0.08
P37A	0.09 ± 0.02*	P155A	no binding
P63A	0.14 ± 0.11	P198A	0.25 ± 0.07
P116A	0.14 ± 0.02*	P221A	0.20 ± 0.08
P137A	0.24 ± 0.07	P247A	0.12 ± 0.01*

Values are mean ± s.e.m., n=3-7. Data were analysed using PRISM (Graphpad) software. * indicates significantly different to wild type ($P<0.05$, Student's t test).K_d = nM

The data suggest that, except for P155, the non-conserved proline residues in the extracellular domain do not play a major structural role. Changing P37, P116 and P247 slightly modifies antagonist affinity, indicating a possible minor role in binding site structure or access. Conversely P155 appears critical for receptor expression, suggesting a role in assembly and/or structure.

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48P IDENTIFICATION OF THE CRITICAL RESIDUES IN MELANIN CONCENTRATING HORMONE (MCH) INVOLVED IN HUMAN MCH$_1$ (SLC-1) RECEPTOR ACTIVATION

N. Cumberbatch, G. Calvert, K.S.J. Thompson, S.C. Cheetham, J.P. Watts & V. Cockcroft. Knoll Ltd., Nottingham, NG1 1GF, UK.

Melanin concentrating hormone (MCH), a cyclic Cys^7-Cys^{16} bridged 19 amino acid peptide, is involved in the central control of body weight. Of particular note is the finding that MCH knock-out mice are lean due to hypophagia and increased metabolic activity (Shimada *et al.*, 1998). Two human MCH receptors have been identified, MCH$_1$ (SLC-1) and MCH$_2$ (Chambers *et al.*, 1999; Saito *et al.*, 1999; Hill *et al.*, 2001). In order to determine the key residues in MCH involved in functional activity at the MCH$_1$ receptor systematic amino acid substitutions in the native peptide have been made and agonist activity determined in CHO cells expressing the human cloned receptor.

Cells (CHO-hMCH; Euroscreen) were grown in 3 layer flasks (450cm^2 growth area; Nunc) in Nutrient Mix (Hams) F12 (+glutamax) with 10% dialysed foetal calf serum and 500µgml^{-1} G418 in a 5%CO_2/95% air atmosphere to ~60% confluence. Cells were harvested in 0.02% EDTA in DPBS, washed and resuspended in assay buffer at 4x10^6 cells.ml^{-1}. Peptides (human MCH, 1pM to 10nM, Phoenix; substituted analogues, 10nM, Sigma Genosys) were incubated with 2x10^5 cells in the presence of forskolin (30µM) in a final volume of 500µl, for 6 minutes at 37°C. The reaction was terminated by heating to 100°C for 5 minutes. Cyclic AMP was measured using cAMP-SPA kits (Amersham).

Human MCH (cyclo[C^7-C^{16}]-DFDMLRCMLGRVYRPCWQV-amide) potently and concentration-dependently inhibited forskolin-stimulated cAMP accumulation (EC_{50} = 94±5pM, maximal inhibition = 90±1%; means±s.e.mean; n=3). Substitution of d- for l-amino acids at positions 7, 8, 11, 12, 13, 14 and 15 in MCH caused marked reductions in agonist activity (in most cases completely abolishing it), versus the native peptide (Figure 1). Similarly, [l-Cit^{11}] substitution abolished activity, whereas [l-Phe^{13}], [l-Ala^{14}] and [l-Gln^{14}] substitutions were tolerated. Linear (non-Cys^7-Cys^{16} bridged) MCH was inactive (Figure 1).

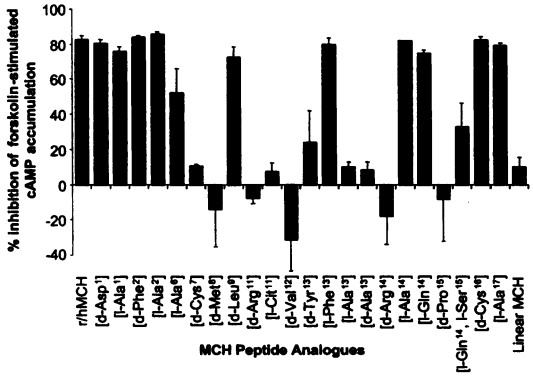

Figure 1. Percentage inhibition of forskolin–stimulated cAMP levels by MCH and substituted peptides at human MCH$_1$ receptors. Basal levels of cAMP 0.52±0.04pmoles; forskolin-stimulated levels of cAMP 8.8±0.7pmoles; values are means±s.e.mean; n=3.

These data support the finding that Arg^{11} in MCH is a key residue for agonist mediated MCH$_1$ receptor activation (MacDonald *et al.*, 2000). Furthermore, they demonstrate that the cluster of amino acid residues from 6-15, immediately adjacent to and within the Cys^7-Cys^{16} bridged loop of the MCH peptide, play a significant role in agonist activity.

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49P RESIDUES IN MELANIN CONCENTRATING HORMONE (MCH) INVOLVED IN HIGH AFFINITY BINDING TO MCH_1 (SLC-1) RECEPTORS

R. Liddle, J. Aitken, K. Oldfield, J.P. Watts, V. Cockcroft & S.C. Cheetham. Knoll Ltd., Nottingham, NG1 1GF, UK.

Melanin concentrating hormone (MCH; a cyclic Cys^7-Cys^{16} bridged 19 amino acid peptide) has long been implicated in the central control of body weight. The most compelling evidence is that MCH knock-out mice are lean due to hypophagia and increased metabolic rate (Shimada *et al.*, 1998). To date, two human MCH receptors have been identified, MCH_1 (SLC-1) and MCH_2 (Chambers *et al.*, 1999; Saito *et al.*, 1999; Hill *et al.*, 2001). In order to determine the residues in MCH involved in high affinity binding to the MCH_1 receptor systematic amino acid substitutions in the native peptide have been made and affinity assessed by radioligand receptor binding using CHO cells expressing the human cloned receptor.

Membranes (400µl; equivalent to 8.92µg protein $tube^{-1}$) prepared from CHO cells expressing the human cloned MCH_1 receptor (Batch 1138; Euroscreen) were incubated 50µl [^{3}H]Phe^{13}, Tyr^{19}-MCH (0.23nM; Amersham 96Ci/mmol) and 50µl of incubation buffer (total binding) or peptides (10 concentrations; human MCH, Phoenix; substituted analogues, Sigma Genosys) or MCH (1.5µM; non-specific binding) at 25°C for 60 minutes. Binding was terminated by rapid filtration through GF/C filters, pre-soaked in 0.5% polyethylenimine, using a Skatron cell harvester and radioactivity determined by scintillation counting. Data were analysed using a programme based on Ligand.

Human MCH potently inhibited [^{3}H]Phe^{13},Tyr^{19}-MCH binding (K_i = 1.01±0.05nM, pK_i = 9.00±0.02, Hill slope = 1.17±0.07; mean ± s.e.mean; n=3). Substitution of d- for l-amino acids at positions 7, 8, 11, 12, 13, 14 and 15 markedly reduced binding affinity (by >100-fold) compared with the native peptide (Figure 1). The [l-Ala^6], [l-Cit^{11}], [l-Ala^{13}] and [d-Ala^{13}] substitutions also decreased binding affinity (by >100-fold), whereas [d-Leu^9], [l-Phe^{13}], [l-Ala^{14}] and [l-Gln^{14}] substitutions were tolerated (Figure 1). Linearisation of the peptide (non-Cys^7-Cys^{16} bridged MCH) markedly reduced binding affinity (Figure 1).

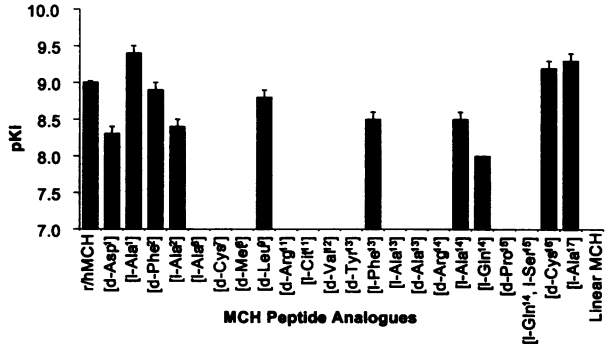

Figure 1. Affinity of MCH and substituted peptides for human MCH_1 receptors. pK_i values are mean±s.e.mean or <7; n=3-5.

These data support the finding that Arg^{11} in MCH is a critical residue for high affinity binding to MCH_1 receptors (MacDonald *et al.*, 2000). Furthermore, they demonstrate that the cluster of amino acid residues from 6-15, immediately adjacent to and within the Cys^7-Cys^{16} bridged loop of the MCH peptide, play a significant role in binding. These results are consistent with the functional data obtained by measuring forskolin-stimulated cAMP accumulation (Cumberbatch *et al.*, 2001).

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50P CHARACTERISATION OF [^{3}H]PHE^{13},TYR^{19}-MCH BINDING TO HUMAN MCH_1 (SLC-1) RECEPTORS

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Melanin concentrating hormone (MCH) has long been implicated in energy homeostasis and feeding behaviour. Intracerebroventricular administration of MCH increases food intake in the rat, MCH mRNA expression is increased by fasting in normal and obese *ob/ob* mice and MCH knock-out mice are lean due to hypophagia and increased metabolic rate (Tritos & Maratos-Flier, 1999). To date, two human MCH receptors have been identified, MCH_1 (SLC-1) and MCH_2 (Chambers *et al.*, 1999; Saito *et al.*, 1999; Hill *et al.*, 2001). In the present study, we have developed and validated a radioligand receptor binding assay using [^{3}H]Phe^{13},Tyr^{19}-MCH and CHO cells expressing the human cloned MCH_1 receptor.

Membranes (400µl) prepared from CHO-K1 cells expressing the human cloned MCH_1 receptor (Batch 1138; Euroscreen) were incubated with 50µl [^{3}H]Phe^{13},Tyr^{19}-MCH (saturation binding studies, 8 concentrations 0.025-3.2nM; competition studies, 0.23nM; Amersham 96Ci/mmol) and 50µl of incubation buffer (total binding) or MCH (1.5µM; non-specific binding) at 25°C for 60 minutes. Binding was terminated by rapid filtration through GF/C filters, pre-soaked in 0.5% polyethylenimine, using a Skatron cell harvester and radioactivity determined by scintillation counting. Data were analysed using a programme based on Ligand.

In preliminary experiments, binding of [^{3}H]Phe^{13},Tyr^{19}-MCH (0.029, 0.23 and 1.9nM) increased linearly with protein over the concentration range 4.46 to 13.37µg protein $tube^{-1}$. A protein concentration of 8.92µg protein $tube^{-1}$ was chosen for further experiments. Under these conditions specific binding was good (85±2%, 74±3% and 53±2%) and the total radioactivity bound as a percentage of the total radioactivity added was 22±2, 10±1 and 2.7±0.3 (values are mean±s.e.mean for 0.029, 0.23 and 1.9nM [^{3}H]Phe^{13},Tyr^{19}-MCH, respectively; n=3). Binding (0.019, 0.27 and 2.7nM) reached equilibrium within 30 minutes and remained constant up to 3 hours. Therefore, an incubation time of 60 minutes was used. Full saturation binding analysis revealed that binding approached saturation at the highest concentrations of radioligand. Binding was of high affinity and fitted well to a single site binding model (K_d = 0.23±0.01nM and B_{max} = 3.5±0.6pmoles mg $protein^{-1}$; n=3). Inhibition constants for human MCH, salmon MCH, $Phe^{13}Tyr^{19}$-MCH and MCH7-19 are summarised in table 1. Order of potency is salmon MCH > $Phe^{13}Tyr^{19}$-MCH = human MCH >> MCH7-19.

Table 1 - Affinity of human MCH and related peptides for MCH_1 receptors.

Peptide	K_i	pK_i	Hill slope
Human MCH	1.01±0.05	9.00±0.02	1.17±0.07
Salmon MCH	0.59±0.04	9.23±0.03	0.73±0.03
$Phe^{13}Tyr^{19}$-MCH	0.99±0.07	9.01±0.03	0.97±0.03
MCH(7-19)	14.4±2.7	7.86±0.07	0.70±0.03

Values are mean±s.e.mean; n=3-4. pK_i values are –logarithm K_i (nM).

These data show that [^{3}H]Phe^{13},Tyr^{19}-MCH is a suitable ligand for labelling MCH_1 receptors and represents a useful tool for identifying compounds with affinity for this receptor.

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51P ANTAGONISM OF ISOPRENALINE- AND SALBUTAMOL- STIMULATED CYCLIC AMP ACCUMULATION BY β2-ANTAGONISTS IN CHO-K1 CELLS EXPRESSING THE HUMAN β_2-ADRENOCEPTOR

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ICI 118551 is a selective β_2-adrenoceptor inverse agonist with an affinity for the β_2-adrenoceptor in the nanomolar range (Hopkinson *et al.*, 2000). CGP 12177 is also a high affinity compound with partial agonist properties at the human β_2-adrenoceptor (Baker *et al.*, 2001). We have previously reported that these two compounds are an order of magnitude more potent as antagonists of salbutamol-stimulated gene transcription than when isoprenaline is used as the agonist (Baker *et al.*, 2002a). In the present study we have compared the potencies of CGP 12177, ICI 118551, atenolol and propranolol as antagonists of isoprenaline- and salbutamol-stimulated cyclic AMP accumulation.

CHO-K1 cells expressing the human β_2-adrenoceptor at 300fmol/mg protein and a secreted placental alkaline phosphate (SPAP) reporter gene under the trancriptional control of six CREs (McDonnell et al., 1998) were used in the present study. Measurements of ^{3}H-cyclic AMP (cAMP) accumulation and SPAP secretion were made as described previously (McDonnell *et al.*, 1998).

Isoprenaline (EC_{50} 2.47 ± 0.37 nM; n=14) stimulated cAMP accumulation to yield a maximum response of 23.8 ± 3.8 fold over basal. Similar responses were seen with salbutamol (EC_{50} 42.04 ± 3.46nM n=18, E_{MAX} 92.57 ± 2.28% of maximum isoprenaline response).

ICI 118551, CGP 12177, atenolol and propranolol produced parallel shifts of the isoprenaline-induced concentration-response curves yielding apparent K_D values of 0.46 ± 0.13 nM (n=10), 0.28 ± 0.13 nM (n=6), 1.65 ± 0.23 μM (n=9) and 0.48 ± 0.07nM (n=8). When salbutamol was used as the agonist, apparent K_D values of : 0.55 ± 0.15 nM (n=5), 0.32 ± 0.12 nM (n=4), 1.58 ± 0.23 μM (n=8) and 0.27 ± 0.03nM (n=8) were obtained for ICI 118551, CGP 12177, atenolol, propranolol respectively.

It was notable however, that when ICI 118551 and CGP 12177 were used as antagonists of the salbutamol responses, there was a progressive reduction in the maximum agonist response as the concentration of antagonist was increased. In contrast, propranolol and atenolol produced parallel shifts of the salbutamol induced response curve. The most likely explanation for this is that slow dissociation of CGP 12177 from the human β_2-adrenoceptor (Baker *et al.*, 2002b) and ICI 118551 (Hopkinson *et al*, 2000) produces a hemiequilibrium that particularly influences the responses to the lower efficacy agonist salbutamol.

In summary, studies of cAMP accumulation in this cell line do not demonstrate the agonist specific differences in antagonist affinity previously observed from measurements of gene transcription in the same cells.

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52P THE EFFECT OF 5-HT DEPLETION ON CENTRAL CANNABINOID RECEPTOR FUNCTION.

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The pharmacological effects of cannabinoids in humans and animal models suggest that they could play a role in affective disorders and a recent study has shown that changes occur in CB_1 receptor function in the brains of depressed patients (Mato *et al* 2001). 5-HT is a neurotransmitter known to be intimately involved in affective disorders and several studies, both *in vitro* and *in vivo*, have shown that the 5-HT and cannabinoid systems interact (Nakazi *et al* 2000, Malone & Taylor 1998). The present study has determined the effects of 5-HT depletion on CB_1 receptor number and function using the tryptophan hydroxylase inhibitor pCPA (p-chlorophenyl-alanine) to deplete neuronal 5-HT.

Male Lister-Hooded rats were treated either with saline ($2mlkg^{-1}$ i.p., n=10) or with pCPA ($150mgkg^{-1}$ i.p., n=10) once daily for 3 days and decapitated on day 4. 10 brains (5 of each group) were dissected into cortex, striatum, hippocampus and cerebellum and membranes prepared for [^{3}H]-CP55,940 binding assays carried out on all four brain areas. The other 10 brains were immediately frozen on dry ice; 20μm sections were cut using a cryostat and mounted on gelatin-coated glass slides for autoradiographic measurement of [^{35}S]-GTPγS binding as described by Sim *et al.*, 1995. Sections were incubated with [^{35}S]-GTPγS in the presence of the cannabinoid receptor agonist HU-210 (1μM) and/or the CB_1 antagonist SR141716A (1μM). The slides were exposed to photographic film which was developed after 3 days. [^{35}S]-GTPγS binding was measured in four brain areas using "NIH image" software.

pCPA treatment had no significant effect on [^{3}H]-CP55,940 binding in any of the four brain areas. The CB receptor agonist HU-210 increased [^{35}S]-GTPγS binding significantly in brains of saline treated rats to the following levels where basal is 100%: cortex: 299%; p=0.007, striatum: 297%; p=0.004, hippocampus: 232%; p=0.002, cerebellum: 194%; p=0.001 (n=5). This effect was antagonised by SR141716A (n=5, cortex: 119%; p=0.013, striatum: 133%; p=0.011, hippocampus: 98%; p= 0.002, cerebellum: 110%; p= 0.002). Pre-treatment with pCPA had no effect on its own (differences between basal values: cortex: p = 0.14, striatum: p= 0.32, hippocampus: p = 0.58, cerebellum: p= 0.94) but abolished HU-210 stimulated binding in the cortex and cerebellum (cortex: p=0.14, cerebellum: p=0.41) and decreased HU-210 stimulated binding in the striatum and hippocampus (striatum: 182%; p= 0.01, hippocampus: 175%; p= 0.015).

Depletion of 5-HT prevented cannabinoid agonist stimulated [^{35}S]-GTPγS binding in the cerebral cortex and cerebellum and decreased it in the striatum and hippocampus. This suggests that an intact 5-HT system is required for the full expression of CB_1 coupling to G proteins, although the mechanism underlying this effect is yet to be determined.

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53P CANNABIDIOL ATTENUATES RESPONSES OF THE MOUSE ISOLATED VAS DEFERENS TO WIN55212-2 AND NORADRENALINE

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(–)-Cannabidiol (CBD) attenuates several effects of Δ^9-tetrahydrocannabinol in rodents and man (see Karniol *et al.*, 1974). In the present experiments, we investigated if CBD affects the ability of the CB_1/CB_2 agonist, WIN55212-2 (WIN; Pertwee, 1997), to inhibit evoked contractions of the mouse vas deferens, a tissue in which prejunctional CB_1 receptors are thought to mediate inhibition of evoked release of noradrenaline (NA) and adenosine 5'-triphosphate (ATP) (Pertwee, 1997).

Experiments were performed with vasa deferentia from adult MF1 mice (30 to 40 g) as described by Ross *et al.* (2001). Supramaximal electrical stimuli were applied using 0.5 s trains of 3 pulses (train frequency 0.1 Hz; pulse duration 0.5 ms). Values for K_B, E_{max} and % inhibition of evoked contractions were calculated as described elsewhere (Pertwee *et al.*, 1995; Ross *et al.*, 2001). Drugs were dissolved in saline (disodium salt of the stable ATP analogue, β γ-methyleneATP), saline/ascorbic acid (NA bitartrate) or dimethyl sulphoxide (DMSO; WIN and CBD). CBD was added 30 min before the other drugs. Values are expressed as means and variability as s.e.mean or 95% confidence limits.

CBD attenuated the ability of WIN to inhibit electrically-evoked contractions, producing dextral shifts in the cumulative log concentration response curve of WIN that did not deviate significantly from parallelism and were not accompanied by significant changes in E_{max} ($P>0.05$; ANOVA). Dextral shifts after 0.32, 1.0, 3.2, 6 and 10 μM CBD with 95% confidence limits in brackets were 2.5 (1.2 & 5.1), 4.2 (2.1 & 8.9), 28.3 (13.2 & 59.5), 40.7 (19.9 & 87.7) and 88.6 (48.8 & 158.4) respectively (n=6). Corresponding values after 0.1 μM CBD were 1.2 (0.7 & 2.1; n=6). Data analysis yielded a Schild plot with a slope that did not deviate significantly from unity (1.2; 95% confidence limits of 0.8 & 1.6) and a mean K_B value for CBD of 120 nM (95% confidence limits of 99.7 & 141 nM), well below its reported K_i values for displacing ligands from CB_1 or CB_2 binding sites: >10 μM (Bisogno *et al.*, 2001). In the 30 min period before the first addition of WIN, the amplitude of evoked contractions increased in the presence of 0.32, 1.0, 3.2, 6 or 10 μM CBD ($P<0.05$; paired t test; n=6), mean increases being 53.3±12.2%, 51.6±7.6%, 204.8±68.6%, 77.5±12.0% and 129.7±43.9% respectively. Significant increases in amplitude were not induced by 0.1 μM CBD or DMSO (21.4±8.5% and 24.9±8.2% respectively; $P>0.05$; paired t test; n=6). The log concentration response curve of β γ-methyleneATP was not affected by 10 μM CBD (n=8) whilst contractile responses to NA were decreased by CBD at 1 and 10 μM but not at 0.1 μM ($P<0.05$; ANOVA and Dunnett's test). Mean responses to 10 μM NA were 419±47 mg (n=9) after DMSO and 320±59 mg (n=8), 159±70 mg (n=8) and 108±39 mg (n=7) after 0.1, 1 and 10 μM CBD respectively. In conclusion, CBD enhanced electrically-evoked contractions of the vas deferens but attenuated contractile responses to NA. It also antagonized WIN in a competitive, surmountable manner by acting on sites that are unlikely to be CB_1 receptors or to be located postjunctionally.

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54P THE CONVENTIONAL ISOFORMS OF PROTEIN KINASE C ARE TARGETTED IN THE ENDOGENOUS FACILITATION OF NORADRENALINE RELEASE IN THE RAT BRAIN CORTEX

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Protein kinase C (PKC) is a family of 11 enzymes that phosphorylate a variety of proteins which affect neurotransmitter release. PKC may be involved physiologically in the modulation of noradrenaline release, as inhibitors of the enzyme decrease transmitter release during a train of nerve impulses (Majewski & Iannazzo, 1998). The present study used isoform restricted inhibitors of PKC to determine which isoforms were involved in the process of endogenous noradrenaline release from the rat brain cortex.

Male Sprague Dawley rats (180-200 g) were decapitated, brains excised and slices of cortex (thickness approx. 400 μm) obtained. Slices were incubated with [^{3}H]-noradrenaline and transferred to a chamber where they were superfused with physiological salt solution (37 °C, 1 ml/min) containing selective PKC inhibitors. The electrically-stimulated (1Hz, 60s) spillover of radioactivity was taken as an index of endogenous transmitter release (Kotsonis *et al.*, 1996). In other studies the effect of PKC inhibitors on endogenous PKC activity was examined by measuring phosphorylation of B-50, a known PKC substrate present in synaptosomes derived from rat brain cortex. Synaptosomes were incubated with [^{33}P]-orthophosphoric acid, followed by SDS-PAGE (Kotsonis *et al.*, 2001).

Stimulation-induced noradrenaline release from slices of rat brain cortex was inhibited by the isoform non-selective PKC inhibitor polymyxin B (PXB, 21 μM; 53 ± 3% of control), the selective PKC α,β and γ inhibitor GO6976 (GO, 3 μM, 65 ± 4%) and bisindolylmaleimide I (BIS, 3 μM, 68 ± 4%), which is selective for PKC isoforms α,β, γ and δ. The diacylglycerol kinase inhibitor R59949 (1 μM) increased noradrenaline release (131 ± 4% of control) and each of the PKC inhibitors attenuated the R59949-induced facilitation of noradrenaline release (PXB, 61.5 ± 3.4%; GO, 71.3 ± 4.3% and BIS, 79.6 ± 4.8%, $p<0.05$ n=8-13 for all). In experiments measuring B-50 phosphorylation in rat cortical synaptosomes, R59949 increased B-50 phosphorylation (137 ± 8 % of control) and this effect was attenuated by GO (78 ± 3 %), BIS (86 ± 8 %) and the myristoylated substrate-derived inhibitor peptide $PKC_{\alpha/\beta\ 19\text{-}27}$, selective for PKC isoforms α and β (89 ± 6%; $P<0.05$, n = 6-9 for all).

These observations suggest that noradrenaline release from neurons in the rat brain cortex involves production of endogenous diacylglycerol and activation of PKC, most probably the conventional PKC-subfamily isoforms PKC α and/or β.

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55P ACTIVATION OF THE PRESYNAPTIC NICOTINIC ACETYLCHOLINE RECEPTOR MODULATES THE DEPOLARISATION-DEPENDANT RELEASE OF [^{3}H]D-ASPARTATE FROM RAT FRONTAL CORTEX SYNAPTOSOMES

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[^{3}H]Dopamine release from rat striatal slices in response to nicotinic acetylcholine receptor (AChR) agonists is proposed to include an indirect component via the release of glutamate (Kaiser & Wonnacott, 2000). Here we aimed to provide evidence for a direct nicotinic stimulation of glutamate release. To do this, we have examined the nicotinic AChR mediated release of [^{3}H]D-aspartate ([^{3}H]D-ASP) in frontal cortex P2 synaptosomes, from male Sprague-Dawley rats (250g). [^{3}H]D-ASP was chosen due to a minimum involvement in cellular metabolism and enzymatic degradation (Savage et al., 2001).

[^{3}H]D-ASP uptake (specific activity 14-21 Ci mmol^{-1}) by synaptosomes was optimal at 0.2 μM for 15 min at 37^{0}C. Loaded synaptosomes (approx. 0.21 mg ml^{-1} protein per superfusion chamber) were perfused with Krebs-Bicarbonate buffer (0.75 ml min^{-1}) and 2 min fractions were collected. Evoked release (by applying a 90 s stimulus pulse) was calculated as a percentage of the total radioactivity present at the point of stimulation. 4-Aminopyridine (10 mM), veratridine (10 μM) and KCl (12 mM and 30 mM) all evoked [^{3}H]D-ASP release (5.5 ±0.1%, 11.5 ±0.8%, 1.9±0.1% and 4.8±0.1% respectively compared to 0.15 ±0.1% for a buffer stimulus). Release evoked by KCl (12 mM) was Ca^{2+} dependent, as it was reduced to 47.3 ± 0.4% of control in Ca^{2+} free buffer (n= 3 or more experiments).

Nicotinic AChR agonists were compared for their abilities to evoke [^{3}H]D-ASP release. (-)-Nicotine (10 - 300 uM), (±)-anatoxin-a (0.01 - 0.3 μM), (±)-epibatidine (1-100 nM) and choline (1 - 10 mM) failed to increase [^{3}H]D-ASP release. However, when concomitantly applied with KCl (12 mM), a significant increase in [^{3}H]D-ASP release above the effect of KCl alone was observed. (±)Epibatidine (1nM) and (±)-anatoxin (0.3 μM) were the most effective agonists (Table 1). Nicotinic AChR specificity was confirmed by the block of the agonist evoked responses by mecamylamine (20 μM).

Stimulus	[^{3}H]D-ASP release (% of KCl control)
KCL (12 mM) control	100.0 ±8..3 n=8
KCl (12mM) + Epibatidine (1nM)	126.6±5.6 n=5
KCl (12 mM) Epibatidine (1 nM) + Mec 20 μM	84.9 ± 7.9 n=2
KCl (12mM) + Anatoxin (300 nM)	127.7±4.1 n=8 *
KCl (12 mM) Anatoxin (300 nM) + Mec 20 μM	97.6 ±4.9 n=8

Table 1. Evoked release of [^{3}H]D-ASP. * P<0.05, One way ANOVA with *post hoc* tukey test.

In summary we have validated the use of [^{3}H]D-ASP for investigating excitatory amino acid (EAA) release in vitro. Nicotinic agonists only increased [^{3}H]D-ASP release when co-applied with a depolarising agent. Thus, nicotinic AChRs may increase the probability of EAA release in response to depolarisation (Gray et al., 1996).

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56P BDNF FACILITATES POTASSIUM STIMULATED GABA RELEASE FROM THE ISOLATED RAT DORSAL HORN.

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Brain Derived Neurotrophic Factor (BDNF) is a polypeptide neuromodulator produced by primary afferent nociceptive neurones. These fibres terminate in the superficial laminae of the spinal cord and release BDNF after specific activity, (Lever *et al*, 2001). BDNF signalling is mediated by the tyrosine kinase receptor Trk-B, found on dorsal horn neurons in this area. When the dorsal horn is isolated *in vitro*, superfusion of BDNF reduces the excitatory release of Substance P after electrical stimulation of nociceptors, (Meyer-Tuve *et al.*, 2001). We hypothesized that BDNF could exert its inhibitory effects by promoting release of the inhibitory transmitter GABA from intrinsic neurons of the spinal cord. The same action could be used to explain the antinociceptive effect of exogenous BDNF in rat models of neuropathic pain, (Cejas *et al.*, 2000). We aimed to test this hypothesis by measuring the release of GABA from the isolated dorsal horn after acute superfusion of BDNF.

Spinal cord dorsal horn sections (10-15mm) were obtained from adult male Wistar rats (250-300g). These were mounted in chambers and superfused (1ml min^{-1}) with oxygenated Krebs' solution containing a GABA uptake inhibitor SKF 89976A (30μM). GABA content was measured in 3ml superfusate samples collected before, during and after two periods of stimulation with a high concentration of KCl (50mM superfused for 3 min), labelled K_1 and K_2. GABA content in superfusates was determined by high performance liquid chromatography (HPLC) coupled with o-phthalaldehyde pre-columns derivatisation and fluorimetric detection, (Lever *et al.*, 2001).

The GABA concentration in basal outflow fractions was 67.3 ± 13.3 nM, (n=8) and increased to 362.5 ± 35.5 nM, (n=8) following the first period of potassium stimulation (K_1). Evoked GABA release was lowered after the second potassium stimulus (K_2) and the K_2/K_1 ratio was 0.75 ± 0.1, (n=4). When BDNF (100ng/ml) was superfused for 3 min before and during K_2, the K_2/K_1 ratio was significantly increased to 1.17 ± 0.05 (n=4, p=0.014 Mann-Whitney-U test). Superfusion of the tyrosine kinase inhibitor K-252a (100nM) 3 min before and during BDNF superfusion, reversed the effect of BDNF on GABA release so that the K_2/K_1 ratio was 0.65 ± 0.028 (n=4). These data indicate that potassium stimulation increases the release of GABA from the rat dorsal horn and that acute BDNF treatment can facilitate this evoked release in a manner that requires the functioning of tyrosine kinase receptors. This study suggests the antinociceptive action of acute BDNF treatment may involve modulation of inhibitory GABAergic signalling in the spinal cord.

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57P GENE EXPRESSION FOR BRAIN-DERIVED NEUROTROPHIC FACTOR IN RAT HIPPOCAMPUS IS REDUCED BY BACLOFEN, TRANYLCYPROMINE AND PAROXETINE BUT NOT FLUNITRAZEPAM

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Recently we have shown a biphasic change in expression of mRNA for BDNF by antidepressant drugs in rat hippocampus. Thus BDNF mRNA expression is inhibited at 4 h and stimulated at 24 h after the last injection (Coppell & Zetterström, 2000). Although, the mechanism behind the initial inhibitory action is not known, the effect is seen after both single and repeated administration and is 5-HT dependent. GABAergic interneurons in hippocampus express excitatory 5-HT receptors which upon stimulation could increase release of GABA and inhibit BDNF gene expression. Here we tested whether two different GABAergic drugs, the benzodiazepine flunitrazepam and baclofen – acting via $GABA_A$ and $GABA_B$ receptors, respectively – modify the expression of BDNF at 4 h after single injections. For comparison, the action of two different antidepressant drugs, tranylcypromine (TCP) and paroxetine, was also tested on BDNF mRNA expresssion.

Groups of six male Sprague-Dawley rats (225-250 g) were administered either baclofen, flunitrazepam (both at 10 mg/kg, i.p.), paroxetine, TCP (both at 5mg/kg, i.p.) or saline (control group). After 4 h rats were killed, their brains removed and frozen in isopentane prior to being processed for *in-situ* hybridisation using ^{35}S labelled oligonucleotide probes specific to BDNF mRNA (Zetterström *et al*, 1998). Autoradiograms were quantified by computer-aided densitometry using NIH Image.

As shown in Table 1, the $GABA_B$ receptor agonist baclofen significantly inhibited BDNF mRNA expression in the dentate gyrus (DG) by 39% and in the CA3 by 29% of the hippocampus when compared to saline injected controls (100%) at 4 h after a single injection. In contrast flunitrazepam acting via $GABA_A$ receptors had no significant effect on BDNF mRNA expression. Similarly to baclofen, paroxetine and TCP reduced BDNF mRNA expression at 4 h after a single injection.

Table 1: Effect of drugs on BDNF mRNA expression

Drugs	CA1	CA3	DG
Flunitrazepam	101±4	98±2	97±3
Baclofen	94±8	71±2**	61±3**
Paroxetine	101±12	81±7	79±9*
TCP	94±6	80±3	57±4**

Data are mean±s.e.m. values (n=6) expressed as % of saline injected controls (100%). *p<0.01; **p<0.001 versus controls (Bonferroni post-hoc test following ANOVA). DG dentate gyrus.

In summary, these results show that activation of $GABA_B$ receptors and antidepressants inhibit mRNA expression for BDNF in rat hippocampus at 4 h after a single injection. In contrast, stimulation of $GABA_A$ receptors was ineffective.

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58P DIFFERENTIAL ROLES OF GROUP I AND GROUP II mGlu RECEPTORS IN THE REGULATION OF 5-HT RELEASE IN THE RAT FRONTAL CORTEX *IN VIVO*

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Using intracerebral microdialysis, we have recently suggested that 5-HT release in the rat frontal cortex is not subject to local regulation by presynaptic Group I metabotropic glutamate (mGlu) receptors (Lee & Croucher, 2002). To confirm that our initial findings were not due to desensitization of Group I receptors we now examine the effects of both shorter-term and lower dose administration of the Group I mGlu receptor agonist, (RS)-3,5-DHPG. Using the selective Group II mGlu receptor agonist, L-CCG-1, we also now report a positive modulatory role for Group II mGlu receptors in the same region.

Under halothane anaesthesia, concentric dialysis monoprobes (4mm, Gambro-Hospal GFE-9 membrane) were stereotaxically implanted into the frontal cortex of male Wistar rats (300-400g). The following day, probes were perfused at 1µl/min with artificial CSF (composition in mM: NaCl 145; KCl 2.7; Na_2HPO_4 2.0; $MgCl_2$ 1.0; $CaCl_2$ 1.2) containing 1µM citalopram. Following a 1 h equilibration period, samples were collected every 30 min. Ligands were added to the perfusion stream following at least four basal samples. Dialysate 5-HT was evaluated by HPLC separation with electrochemical detection. Data were calculated as the maximum percentage change compared to the mean of the three basal samples preceding drug treatment and expressed as mean ± s.e.mean of *n*=3-6 independent observations. Significance of differences was assessed using ANOVA with *post-hoc* Dunnett's test.

Using this protocol, basal 5-HT release shows significant Ca^{2+}-dependency (Lee & Croucher, 2002) and was abolished in the current study by 1µM tetrodotoxin. 5-HT levels were not significantly (P>0.05) modified by short-term administration (15 min) of (RS)-3,5-DHPG (1mM; 127.0 ±17.8% of basal) or low concentrations of the agonist (100µM and 300µM for 1h; 80.6 ±17.1% and 124.9 ±16.2%, respectively). Similarly, L-CCG-1 (100µM) showed no significant effect on basal 5-HT release (119.2 ±13.8%; P>0.05). However, at 500µM, L-CCG-1 evoked a marked facilitation of release (144.7 ±17.8%; P<0.05). This was fully reversed by the Group I/II antagonist (S)-MCPG (3mM; 70.7 ±12.8%), whilst (S)-MCPG alone was inactive (116.0±20.9%; P>0.05). The concentration-dependency of L-CCG-1 appeared to be bell-shaped in character, as a higher concentration of the agonist (1mM) failed to significantly influence 5-HT efflux (108.1 ±23.1%; P>0.05).

While the current data consolidates our initial contention that Group I mGlu receptors do not regulate 5-HT release in the rat frontal cortex, we now provide evidence that the Group II subtype mediates a facilitation in serotoninergic transmission. An identical differentiation in mGlu receptor-mediated control of 5-HT release has been observed in the rat periaqueductal gray matter (Maione *et al.,* 1998) suggesting a heterogeneous distribution of mGlu receptors which may be neurone-specific in its complexity.

Supported by The Sir Jules Thorn Charitable Trust.

Lee, J.J. & Croucher, M.J. (2002). *Br. J. Pharmacol.*, **135**, P99.

Maione, S. *et al.*, (1998). *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **358**, 411-417.

59P CHANGES IN GROUP I, II AND III METABOTROPIC GLUTAMATE (mGlu) RECEPTOR GENE EXPRESSION IN THE BASAL GANGLIA AND THALAMUS OF RATS WITH A NIGROSTRIATAL TRACT LESION.

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Degeneration of the substantia nigra pars compacta (SNc) in Parkinson's disease (PD) leads to abnormally increased glutamatergic and GABAergic transmission in a number of regions of the basal ganglia (BG). Activation of Group I metabotropic glutamate ($mGlu_{1+5}$) receptors can facilitate neurotransmitter release, whilst Group II ($mGlu_{2+3}$) and III ($mGlu_{4,6,7+8}$) mGlu receptors function as auto- or heteroreceptors in many BG regions (e.g. Cartmell et al 2000). This study aimed to establish whether changes in the expression of groups I, II and III mGlu receptors may serve to exacerbate or to help counteract the pathological changes in neurotransmission in a rodent model of PD.

Under general anaesthesia, male Sprague Dawley rats (250-270g) were unilaterally injected with 6-hydroxydopamine (6-OHDA; 12.5µg in 2.5 µl) in the median forebrain bundle. Three weeks later rats were killed, the brains frozen (-45°C), coronally sectioned (15µm) then stored at –70°C until processing. Sections were then fixed, acetylated, dehydrated and defatted. ^{35}S-labelled oligonucleotide probes, complementary to $mGlu_{1,2,3,4,5,6,7\&8}$ receptor mRNA species were diluted in hybridisation buffer to a specific activity of 3 x 10^6 cpm ml^{-1}. Sections were hybridised overnight at 37°C then washed in a series of standard saline citrate solutions (SSC; 0.15M NaCl, 0.015M NaCitrate, pH 7.2) to a maximum stringency of 60 °C and 0.1x SSC. Sections, together with ^{14}C standards, were exposed to Biomax MR film (Kodak) for between 2-12 weeks. Relative levels of each mGlu receptor expression were semi-quantified using image analysis.

Significant reductions in mGlu mRNA levels were observed in the striatum (STR; $mGlu_3$), globus pallidus (GP; $mGlu_3$) and SNc ($mGlu_1$) following nigrostriatal tract lesioning. No other changes were found in any region of the BG or thalamus.

Table 1: % Changes in mGlu mRNA in 6-OHDA-lesioned rat

mGlu receptor subtype	Region	6-OHDA lesioned rat brain		
		Intact Hemisphere (nCi/g)	Lesioned Hemisphere (nCi/g)	%change
mGlu1	SNc	9.5 ±1.1	4.9±1.6	-51.8±11.5*
mGlu3	STR	26.1±3.6	22.8 ±9	-11.7 ± 2.8
	GP	19.2±1.4	15.6 ±1.2	-18.9 ± **1.4

Values are means ± SEM (n=5-6). ** indicates a significant difference between lesioned and intact hemispheres in the 6-OHDA lesioned versus sham lesioned rats; $p<0.01$; unpaired t-test. Abbreviations as in text.

The loss of $mGlu_1$ expression in SNc suggests that $mGlu_1$ receptors are expressed by vulnerable dopaminergic neurones. The reductions in mRNA encoding $mGlu_3$ receptors seen in the BG may reflect the elevation in glutamate release found in these regions in PD. Such reductions may in turn reduce trophic support within the BG (Matarredona *et al.* (2001). Whether this reduction exacerbates the pathophysiology of a nigrostriatal tract lesion requires further investigation.

MJM is supported by an MRC Studentship.

Cartmell J. & Schoepp D.D. (2000) J. Neurochem. 75, 889-907.
Matarredona E.R. (2001). J. Neurochem. 76, 351-360.

60P ANTICONVULSANT ACTIVITY OF TETRAHYDROISOQUINOLINE DERIVATIVES AS POTENTIAL NEW AMPA RECEPTOR ANTAGONISTS

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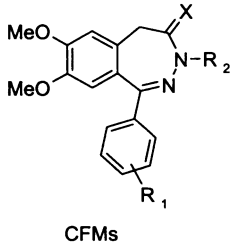
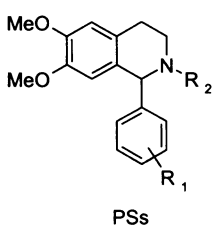
Our previous publications reported the chemical and pharmacological properties of some 7,8-dimethoxy-2,3-benzodiazepines (CFMs), GYKI 52446 analogues, which have shown antiepileptic activity in several seizure models *via* AMPA receptor non-competitive antagonism (De Sarro *et al.* 1995; Chimirri et al., 1997; 1998).

The aim of the present study was to evaluate the anticonvulsant activity of some novel dimethoxytetrahydroisoquinoline derivatives (PSs) structurally related to CFMs, following administration in audiogenic-sensitive DBA/2 mice and the comparison between the two series of compounds.



CFMs



PSs

DBA/2 mice, 22-26 days old, were pretreated i.p. 30 min before auditory stimulation (mixed frequency of 12-16 kHz, 109 dB intensity for up to 60 sec.) with PS derivatives according according to De Sarro et al. (1995). ED_{50} values (± 95% confidence limits) were evaluated according to the method of Litchfield and Wilcoxon (1949).

The results obtained (expressed as µmol/kg) reveal that all PSs tested were effective anticonvulsant agents with similar potency to CFM-2 and GYKI 52466 (Table 1), apart from PS3NAC, which was approximately an order of magnitude more potent.

Table 1. ED_{50} values (± 95% confidence limits) of PS derivatives studied (at least n = 40 per compound).

Compound	Clonus	Tonus
PS2	44.9 (23.9-84)	19.3 (9-41.3)
PS3	20.1 (9.6-41.9)	19.3 (11.8-31.5)
PS3NAC	4.2 (2.2-7.8)	2.4 (1.3-4.4)
PS4	30.6 (19.4-48.2)	12.5 (6.9-22-6)
PS4NAC	43.1 (21.9-84.6)	16.5 (7.8-34.9)
PS6	47.6 (28-77.4)	28.6 (14.4-56.8)
PS6NAC	32.1 (17.7-58.3)	21.1 (11-40.4)
PS10	19.3 (6.1-61.2)	7.2 (2.4-21.2)
PS10NAC	37.2 (18.9-73.4)	12.8 (7.8-21)
GYKI 52466	35.8 (24.4-52.4)	25.3 (16-40)
CFM-2	15 (9-24)	12.6 (8-19)

Electrophysiological studies to evaluate the mechanism of action of the new PS derivatives are in progress.

De Sarro GB. *et al.*, (1995). *Eur.J. Pharmac.* **292**, 411-422.
Chimirri A. *et al.*, (1997). *J. Med. Chem* **40**, 1258-1269.
Chimirri A. *et al.*, (1998). *J. Med. Chem* **41**, 3409-3416.
Litchfield J.T. and Wilcoxon F. (1949) J. *Pharmac. Exp. Ther.* **96**, 99-113.

61P THE ANTI-EMETIC ACTIVITY OF S(-)-ETICLOPRIDE AGAINST MORPHINE- AND IPECACUANHA-INDUCED EMESIS IN THE CONSCIOUS FERRET

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The dopamine D_2/D_3 receptor antagonist (S)-eticlopride has been demonstrated to inhibit emesis evoked in ferrets by the dopamine receptor agonist R(+)-7-OH-DPAT (Yoshikawa *et al.*, 1996). In this study we have determined the anti-emetic activity of S(-)-eticlopride against two emetogens with clinical relevance, morphine and ipecacuanha (ipecac).

Ferrets (albino and polecat) of either sex (0.6-1.7kg) were dosed subcutaneously (s.c.) with antagonist or vehicle 15-60min prior to challenge with either morphine (0.25mg/kg s.c.) or ipecac syrup (0.75ml/kg p.o.). Ferrets were observed continuously for 6h and emesis quantified as number of animals showing an emetic response, total retches, total vomits and latency to first emesis. Statistical analysis (Students unpaired t-test) compared data from the vehicle- treated group with that from antagonist-treated groups.

Table 1. Anti-emetic activity of S(-)-eticlopride (mg/kg s.c.) vs. morphine-induced emesis (* $P \leq 0.05$)

Treatment	Dose	Emesis/ tested	Retches (± s.e.m)	Latency (min ± s.e.m)
Vehicle	-	39/39	48.8 ± 2.6	1.9 ± 0.2
S(-)-eticlopride	0.1	4/4	39.8±11.2	2.8 ± 0.6
S(-)-eticlopride	0.3	5/6	20.0 ± 9.9*	4.4 ± 0.8*

All control ferrets treated with morphine developed emesis within 4min (Table 1). (S)-eticlopride (0.3mg/kg) inhibited the retching response to morphine by over 50%, also significantly extending the latency. 90% of animals treated with ipecac developed emesis, with a mean latency of 26.4 ± 2.6min (n=18) (Table 2).

Table 2. Anti-emetic activity of S(-)-eticlopride (mg/kg s.c.) vs. ipecac-induced emesis (* $P \leq 0.05$)

Treatment	Dose	Emesis/ Tested	Retches (±s.e.m)	Latency (min ± s.e.m)
Vehicle	-	18/20	43.5 ± 6.0	26.4 ±2.6
S(-)-eticlopride	0.1	3/3	21.3 ± 9.2	70.0 ± 27.4
S(-)-eticlopride	0.3	5/7	3.0 ± 1.4*	40.2 ± 15.4

S(-)-eticlopride (0.3mg/kg) produced a significant reduction in the number of retches, compared to vehicle-treated animals. The maximum dose of S(-)-eticlopride was limited to 0.3mg/kg due to sedative effects.

These data strongly suggest that the non-selective D_2/D_3 antagonist S(-)-eticlopride can decrease emesis evoked by clinically-significant emetogens, although it is less potent compared to the inhibition of emesis evoked by a dopamine agonist (Yoshikawa *et al.*, 1996). In this study we were unable to define the maximum degree of inhibition afforded by this mechanism, so the potential utility is difficult to determine. Whether an antagonist with selectivity for either dopamine receptor subtype would preserve anti-emetic activity but avoid the appearance of adverse events remains to be investigated.

Yoshikawa, T., Yoshida, N. & Hosoki, K. (1996) Eur. J. Pharmacol. 301 143-149

62P THE ALPHA-2A-ADRENOCEPTOR IS ACTIVATED AFTER AMPHETAMINE ADMINISTRATION AND PROTECTS HIPPOCAMPAL NORADRENERGIC NERVES IN MICE FROM TRANSMITTER DEPLETION

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There are three receptor subtypes of the alpha-2-adrenoceptor, termed alpha-2A, 2B and 2C. Genetically manipulated mice lacking one receptor subtype have been generated and proven useful in matching drug effects to subtype function. Recently a true knock-out strain of the alpha-2A subtype became available (Altman *et al.* 1999) and we have been examining the properties of these mice. In the course of these experiments, a novel aspect of amphetamine neuropharmacology emerged.

A total of 36 male alpha-2A knockout (KO) mice were used. An equivalent number of age and sex-matched C57Bl/6J mice were used as wild-type controls. The animals (weight range 25 – 35 g) were divided into 4 groups; controls (n = 10, saline s.c. and i.p.); atipamezole (ATI) (n=8, 1 mg/kg, s.c.); amphetamine (AMP) (n = 10, 10 mg/kg, i.p.) and ATI+ AMP (n=8, s.c. and i.p.). The injections were arranged with ATI or saline administered at time 0, followed 10 min later by AMP (or saline). Fifty min later, the mice were sacrificed and hippocampus dissected. Noradrenaline (NA), other biogenic amines and metabolites were analysed by HPLC-EC. Statistical analysis was ANOVA followed by Student or Dunnett's t- test with $P<0.05$ considered as statistically significant.

In the hippocampus of wild type mice, ATI, a selective alpha-2-adrenoceptor blocking drug which does not differentiate between the subtypes, caused a significant increase in the levels of the NA metabolite, 3-methoxy-4- hydroxyphenylglycol (MHPG), but did not alter the level NA (Table 1). AMP, in contrast, evoked a major decrease in the levels of MHPG. When ATI and AMP were combined, the levels of MHPG reached those achieved by ATI alone but this was accompanied by a 36% decrease in the hippocampal NA concentration. In the KO mice, MHPG levels were elevated in the saline treated mice compared to the wild type controls but ATI caused no further elevation. AMP, when given alone caused a 24% decrease in brain NA levels, and supplementation with ATI caused no further significant changes.

Table 1: Hippocampal levels of NA and MHPG one hour after drug administration in alpha-2A-KO mice and their wild type controls. Results are expressed as nmol/g ± s.e mean

Treatment	Wild-type mice		Alpha-2A-KO mice	
	NA	MHPG	NA	MHPG
Saline	1.76±0.12	0.17±0.02	1.60±0.04	0.23±0.03§
ATI	1.58±0.13	0.29±0.02*	1.57±0.13	0.24±0.03
AMP	1.77±0.03	0.08±0.01*	1.27±0.03*	0.20±0.07
ATI+AMP	1.12±0.08*	0.27±0.02*	1.14±0.09*	0.23±0.02

§ refers to comparison versus wild-type ($P<0.05$; Student test)
* refers to comparison versus saline ($P<0.01$ Dunnett's test)

It is concluded that AMP initially releases NA from noradrenergic neurones and this NA activates alpha-2A-adrenoceptors to inhibit further NA release. If the alpha-2A receptor is blocked or has been genetically removed, then AMP causes a marked depletion of hippocampal NA stores.

Altman, J.D., Trendelenburg, A.U., MacMillan, L. et al. *Mol. Pharmacol.* 56, 154-161, 1999.

63P ACUTE METHYLENEDIOXYMETHAMPHETAMINE ADMINISTRATION: EFFECTS ON LOCAL CEREBRAL BLOOD FLOW AND GLUCOSE UTILISATION IN THE RAT

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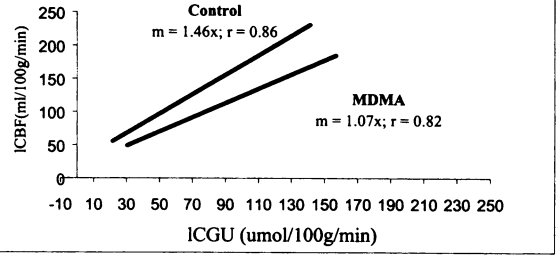
Acute administration of 3,4,-Methylenedioxymethamphetamine (MDMA; "ecstasy") results in an efflux of cerebral monoamines, including the vasoconstrictor, 5-HT. However, the acute functional and cerebrovascular consequences of this action are poorly understood.

Conscious, lightly restrained adult Dark Agouti rats were injected with either 15mg/kg i.p. MDMA (n=10) or saline (n=9). Local cerebral blood flow (lCBF) and glucose use (lCGU) were measured 25-min post-injection in 68 brain areas using [^{14}C]- iodoantipyrine and [^{14}C]-2-deoxyglucose quantitative autoradiography respectively (Kelly *et al.*, 1995). Mean arterial blood pressure (MABP) and rectal temperature were monitored throughout. Data (mean ± standard error) were analysed using appropriate *t*-tests ($p<0.05$).

MDMA produced significant increases in rectal temperature (37.7±0.2 to 39.5±0.2°C) and MABP (142±3 to 182±4 mmHg). MDMA produced significant increases in lCGU in 21 brain areas, most markedly in components of the motor system (cerebellar vermis: +69%; globus pallidus: +82%; and medial striatum: +71%). In no area of the brain were any significant increases in lCBF measured. Significant decreases in lCBF were observed in 20 brain areas, with the most marked decreases occurring in primary sensory nuclei (superior colliculus: -32%; medial geniculate: -25%) and limbic areas (anterior thalamus: -34%; dorsal subiculum: -30%).

Fig 1. The relationship between mean lCMRglu and mean lCBF. Best fitting straight lines are illustrated.



A global analysis of all 68 brain areas (Fig.1), revealed a close correlation (r=0.86) between lCGU and lCBF with a ratio of 1.46 in the control groups. Despite the divergence of lCGU (increases) and lCBF (decreases) in MDMA treated groups there was a similar close correlation (r=0.82), but the ratio was decreased to 1.07.

This study provides direct evidence that acute exposure to MDMA has the potential to disrupt cerebrovascular control. The uncoupling of cerebral blood flow from underlying metabolic demand, possibly as a result of the vasoconstrictor action of 5-HT, could provide the basis for oligaemia-induced pathological changes in the brain.

Kelly, P.A.T., Ritchie, I.M., McBean, D.E. *et al.* (1995) *J. Cereb .Blood Flow Metabol.*,**15**,706-713.

64P *IN VITRO* EFFECTS OF (±)-4-METHYLTHIOAMPHETAMINE, (±)-4-METHYLTHIOMETHAMPHETAMINE AND (±)-MDMA ON VASCULAR RESPONSES TO NORADRENALINE AND PHENYLEPHRINE

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Relatively little is known regarding the direct influence of the psychoactive ring-substituted amphetamine (±)-3,4-methylenedioxymethamphetamine (MDMA) on vascular function, though there are accounts of adverse cardiac effects in humans (see Cannon *et al.*, 2001). The structurally related amphetamine 4-methylthioamphetamine (4-MTA) reportedly lowers blood pressure in rats and we have recently shown that MDMA and 4-MTA inhibit vascular responses to 5-HT (Murphy *et al.*, 2001). This study compared *in vitro* vascular effects of 4-MTA, the corresponding N-methyl analogue 4-methylthiomethamphetamine (4-MTMA) and MDMA, on responses to noradrenaline and the selective α_1-agonist phenylephrine.

Synthesis of compounds was carried out as described previously (Murphy *et al.*, 2001). Rings dissected from descending rat thoracic aorta (male Wistar rats, 220-240 g) were mounted in a tissue bath and maintained at 37°C in Krebs' bicarbonate medium continuously bubbled with 95% O_2/5% CO_2. Rings were pre-loaded to a basal tension of 1.3 g. Cumulative concentration-response curves to noradrenaline (NA) or phenylephrine (PE) were established in tissues pre-incubated for 15 min ± MTA, 4-MTMA or MDMA (all at 100 μM). The significances of differences between responses were analysed using an unpaired Students' t test and a p value <0.05 considered significant. All data were expressed as mean values ± s.e.m., derived from n experiments using n rats.

There was no significant difference in maximal contractions to NA or PE in the absence or presence of 100 μM 4-MTA, 4-MTMA, or MDMA. pD_2 values for NA were decreased significantly ($p<0.05$) by 4-MTA (6.84 ± 0.12 vs 7.33 ± 0.11, n=5) and 4-MTMA (6.74 ± 0.03 vs 7.26 ± 0.02, n=6), but not by MDMA (6.84 ± 0.08 vs 7.01 ± 0.07, n=4). In contrast, pD_2 values for PE were decreased significantly ($p<0.05$) by all 3 agents. Reductions for 4-MTA, 4-MTMA and MDMA were respectively, 6.60 ± 0.04 vs 7.15 ± 0.06, n=4; 6.35 ± 0.08 vs 7.24 ± 0.08, n=4; 6.49 ± 0.10 vs 7.32 ± 0.04, n=4.

These results suggest that 4-MTA and 4-MTMA are non-selective α-adrenoceptor antagonists in rat aorta, while MDMA exhibits some selectivity for α_1-adrenoceptors. Thus it appears that abuse of 4-MTA (and potentially 4-MTMA), not only mimics adverse effects of MDMA on noradrenergic systems in the brain (Huang *et al.*, 1992), but may have added potential to compromise vascular reactivity.

Supported by the Conway Institute

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Murphy, J.E.J., Cannon, D.M., Guiry, P.J. *et al.*, (2001), Br. J. Pharmacol., **134**, 35P

65P PAROXETINE MODULATES THE EXPRESSION OF THE IMMEDIATE EARLY GENE ARC INDUCED BY MDMA IN RAT BRAIN

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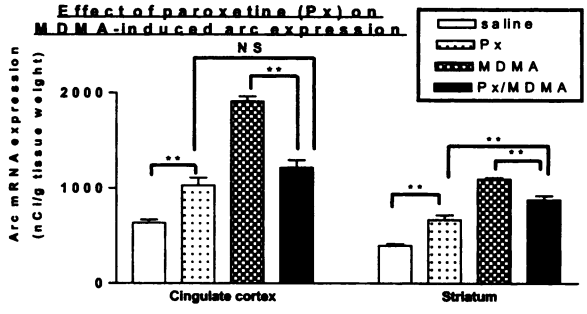
Arc (activity-regulated cytoskeleton-associated gene) is an effector immediate early gene whose mRNA is selectively localised in neuronal dendrites. Its expression is induced by neuronal excitation and by stimulation of 5-HT_{2A} and D_1 receptors. MDMA (3,4-methylenedioxymethamphetamine) induces the release of monoamines, principally 5-HT and dopamine (Shulgin, 1986). Blockade of the 5-HT transporter inhibits the induction of 5-HT release by MDMA (Berger et al., 1992). In this study we have investigated the effects of pretreatment with the 5-HT transporter antagonist paroxetine on the arc mRNA response to MDMA.

Male Dark Agouti rats (190-210g) were administered (i.p.) either saline or paroxetine (5mg/kg) 30 min before either saline (1ml/kg) or MDMA (10 mg/kg). Rats were killed 2 hours later and the brains isolated and flash-frozen in cooled isopentane. Arc mRNA expression was analysed by in situ hybridisation histochemistry using [^{35}S]-dATP labelled oligonucleotide probe as described previously (Pei et al., 2000). Abundance of arc mRNA in selected areas was determined by densitometric quantification of autoradiograms using NIH-Image software. Statistical analysis of the results was made by ANOVA and Newman-Keuls post-hoc test with signficance set at $p<0.05$.

MDMA induced a significant increase in arc mRNA expression (range 177-275%) in cingulate and orbital frontal cortex, striatum, dorsal endopiriform nucleus (DEn) and lateral dorsolateral amygdaloid nucleus (LaDL). Paroxetine itself induced a smaller (range 39-115%) but still significant increase in arc expression in these same brain regions. Administration of paroxetine prior to MDMA significantly reduced the arc expression induced by MDMA (range 51-79%) in all areas except LaDL (31% decrease, NS). Expression of arc mRNA induced by paroxetine/MDMA was significantly greater (range 23-51%) than that observed in animals receiving paroxetine/saline in all areas except cingulate cortex (18% NS).



**p<0.001 Newman-Keuls test

Since paroxetine is known to inhibit the release of 5-HT caused by MDMA, these results suggest that the induction of arc mRNA expression induced by MDMA is mediated either directly or indirectly by 5-HT in all brain areas examined except LaDL. In cingulate frontal cortex the effect of MDMA appears to be mediated exclusively by 5-HT. In striatum, DEn, LaDL and orbital frontal cortex the increase in arc expression induced by MDMA which is resistant to paroxetine suggests mediation by additional agent(s), possibly including dopamine. Further studies using selective receptor antagonists should resolve the pharmacological basis of this response.

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66P UROTENSIN-II, THE NEUROPEPTIDE LIGAND FOR GPR14, INDUCES C-FOS IN THE RAT BRAIN

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Urotensin-II (U-II) is an agonist at the G-protein-coupled receptor GPR14 (Ames *et al.*,1999). Consistent with the localisation of both U-II and GPR14 mRNA in the CNS (Ames *et al.*, 1999: Gartlon *et al.*, 2001), central effects of intracerebroventricular (ICV)-administered U-II have been described. U-II increases motor activity and grooming behaviour, increases plasma prolactin and TSH levels, but does not affect DA or 5-HT metabolism in various brain regions (Gartlon *et al.*,2001). Therefore, the aim of this study was to investigate anatomical substrates for centrally mediated effects of U-II by measuring the induction of an immediate early gene, c-fos, following ICV-administered U-II.

Male CD rats (200-250 g) were implanted with a unilateral cannula directed at the lateral ventricle (anaesthesia was Domitor 0.4 mg/kg sc, and Sublimaze 0.45 mg/kg ip). Rats were habituated to the ICV dosing procedure once daily for 5 days. U-II (1 and 10 µg, n=6) or vehicle (saline, 5 µl) were injected ICV. 2 h later rats were deeply anaesthetised with sodium pentobarbitone (200 mg/kg ip) and intracardially perfused with heparinised saline solution, followed by 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer. Fos immunohistochemistry was carried out according to the methods of Leslie *et al.* (1993). Sections were examined by bright-field microscopy and cells which exhibited fos-like immunoreactivity (FLI) in pre-selected brain regions (table 1). were counted. U-II dose-dependently increased FLI in the cingulate cortex and periaqueductal grey (table 1).

Brain Region	U-II (µg ICV)		
	Vehicle	1	10
Cingulate cortex	6 +/- 1.9	***24.2 +/- 5.6**	***52.2 +/- 5.3**
Paraventricular thalamus	19.2 +/- 6.1	23.8 +/- 5.6	30.5 +/- 3.7
Basolateral amygdala	2 +/- 0.7	3.5 +/- 0.8	3.5 +/- 0.7
Caudate	1.3 +/- 0.4	4.7 +/- 1.1	3.5 +/- 1.4
Nucleus accumbens	2.5 +/- 0.6	6.8 +/- 2.3	4.2 +/- 1.6
Hypothalamus	11.7 +/- 3.8	17.5 +/- 1.8	18.3 +/- 3.9
Periaqueductal grey	4.75 +/- 0.8	***11.5 +/- 0.9**	***13.5 +/- 2.6**
Habenular	2.75 +/- 0.5	2.25 +/- 0.9	3.5 +/- 0.9
Septum	2.75 +/- 0.5	8.25 +/- 2.4	8.25 +/- 2.5

Table 1: Number of cells showing FLI following U-II (1- 10 µg ICV). Means are shown +/- SEM. Significant differences from vehicle are shown by *(P<0.05) after 1-way ANOVA followed by Duncan's New Multiple Range *post-hoc* analysis.

Marked fos expression in the cingulate cortex, an area involved in regulating motor output, is consistent with GPR14 mRNA expression in cortical areas (Gartlon *et al.*, 2001) and provides a potential substrate for U-II stimulated behaviours. Taken together, U-II-induced FLI in the periaqueductal grey and cingulate, along with CNS localisation of GPR14 and effects on behaviour and neuroendocrine hormones, suggests important roles for U-II and GPR14 in the CNS. Future studies may provide more detail on neuroanatomical substrates for these effects by examining FLI, and other immediate early genes, in more discrete brain regions.

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67P N^3-TETRAZOLYLALKYLWILLARDIINE ANALOGUES ARE AMPA AND KAINATE RECEPTOR ANTAGONISTS

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Increasing the inter-acidic group chain length of willardiine by adding N^3-substituents to the uracil ring has been shown to convert its agonist action at AMPA receptors into antagonism (More *et al.* 2001a,b). The current study aims to determine the activity of two tetrazole-substituted willardiine derivatives, (*S*)-3-tetrazolylmethyl-willardiine ((*S*)-3-TMW) and (*S*)-3-(2-tetrazolylethyl)willardiine ((*S*)-3-TEW), as antagonists of AMPA and kainate receptors. The results are compared to previously reported data for similar compounds with a carboxyl group as the terminal acidic group (More *et al.* 2001b,c).

To measure GluR5-containing kainate receptor activity recordings were made from dorsal roots of spinal cords taken from 2-5 day old Wistar rats of either sex (4-13 g). This allowed measurement of depolarisations evoked by the exogenously applied agonist, kainate (1 min applications) (Agrawal & Evans, 1986). Non-cumulative concentration-response curves (CRCs) were constructed to kainate in the absence and presence of the antagonists (100 μM; 30 min pre-incubation). To assess AMPA receptor antagonist activity the ability of the compounds to block the fast component of the dorsal root evoked ventral root potential (fDR-VRP) in the neonatal rat hemisected spinal cord preparation was measured (Evans et al., 1982; More *et al.*, 2001a). CRCs were constructed for the antagonists (5 min applications), in the presence of 2 mM $MgSO_4$ / 50 μM (*R*)-AP5 (30 min pre-incubation) to block NMDA receptors. Results are expressed as mean ± s.e.m., n=3.

Table 1 shows the apparent K_D values for antagonism of kainate responses on dorsal roots and IC_{50} values for the depression of the fDR-VRP, an indication of AMPA receptor antagonism. The compounds are compared to previously reported data for (*S*)-3-carboxymethylwillardiine ((*S*)-3-CMW) and (*S*)-3-(2-carboxyethyl)-willardiine ((*S*)-3-CEW) (More et al, 2001b).

Table 1: Antagonist activity at kainate and AMPA receptors

Compound	Apparent K_D vs. kainate (μM)	IC_{50} for depression of the fDR-VRP (μM)
(*S*)-3-CMW	n.d.	287 ± 34
(*S*)-3-CEW	73.1 ± 4.5	23.8 ± 3.9
(*S*)-3-TMW	60.2 ± 4.5	45.4 ± 8.4
(*S*)-3-TEW	53.7 ± 5.1	6.87 ± 1.39

These preliminary data indicate that converting the terminal acidic group from a carboxyl to a tetrazolyl group could increase the potency of AMPA receptor antagonism, but only slightly increase antagonism at GluR5-containing kainate receptors. The data also support earlier findings for carboxyl-substituted compounds which suggest a chain length equivalent to 2 methylene groups as the inter-acidic spacer unit is preferable to a shorter chain length for AMPA receptor antagonism.

Changing the terminal acidic group from a carboxyl to a tetrazole group enhances AMPA receptor antagonism, however little improvement in GluR5-containing kainate receptor antagonism is seen. Thus, tetrazole substitution is a useful way of increasing the potency and selectivity of AMPA receptor antagonists based on the willardiine structure. Further structure activity studies on willardiine derivatives may lead to optimisation of compounds for selective antagonism of either AMPA or kainate receptors.

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68P ROPINIROLE, BROMOCRIPTINE AND L-DOPA HAVE A DIFFERENTIAL EFFECT ON STRIATAL DOPAMINE D-2 AND D-3 BUT NOT D-1 RECEPTOR mRNA EXPRESSION IN MPTP-TREATED MARMOSETS

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In Parkinson's disease (PD) repeated administration of L-DOPA induces dyskinesia (Bedard *et al.*, 1986) which is associated with an imbalance between striatal output pathways. In contrast, the D_2/D_3 agonists bromocriptine and ropinirole produce a low or negligible incidence of dyskinesia respectively (Pearce *et al.*, 1996). This may be related to differential effects on dopamine receptors. We now report the effect of these drugs on dopamine (DA) D_1, D_2 and D_3 receptor mRNAs by *in situ* hybridisation histochemistry in 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP)-treated common marmosets.

Adult common marmosets (n=16, 260-360g, *Callithrix jacchus*, either sex) were divided into 4 groups after MPTP treatment and received 10% sucrose solution, bromocriptine (0.5 mg/kg, p.o.), L-DOPA plus carbidopa (12.5 mg/kg plus 12.5 mg/kg, p.o.) or ropinirole (0.3-0.5 mg/kg, p.o.) once daily for 4 weeks. A further 4 animals were used as naive controls. At the end of the study, brains were removed under terminal anaesthesia and flash-frozen. Coronal sections (20 μm) were incubated with ^{35}S-labelled oligonucleotide probes for human cDNA for DA D_1 (Zhou *et al.*, 1990), D_2 (Grandy *et al.*, 1989) and D_3 (Sokoloff *et al.*, 1990) receptor as described previously (Tel *et al.*, 2002). Data were analysed by one way ANOVA followed by *post hoc* Dunnett's test.

Neither MPTP treatment nor drug administration altered D_1 receptor mRNA expression in the caudate nucleus (CN) or putamen (PUT). In both CN and PUT, MPTP treatment increased D_2 receptor mRNA expression compared to naïve controls (Table 1). L-DOPA administration tended to further increase D_2 receptor mRNA expression compared to MPTP. Ropinirole but not bromocriptine, reversed the increased levels of D_2 receptor mRNA. D_3 receptor expression decreased in MPTP-treated animals in the CN and PUT. This was reversed by L-DOPA and ropinirole but not by bromocriptine, but only L-DOPA was effective in CN (Table 1).

Table 1. D_2 and D_3 R mRNA expression (nCi/mg) relative to ^{14}C standards in the CN and PUT of the MPTP-treated marmosets. §p<0.05 vs naïve control, +p<0.01 vs MPTP,*p<0.05 vs MPTP (Data is mean±SEM)

	D-2		D-3	
	Caudate	Putamen	Caudate	Putamen
Naïve Control	44.8±5.1	54.8±2.5	11.8±0.2	11.4±0.3
MPTP	60.5±1.3§	64.1±1.2§	7.7±0.6§	8.0±0.6§
L-DOPA	68.4±2.7	69.7±2.0	13.1±1.5+	13.0±1.4+
Bromocriptine	63.7±0.6	65.8±1.0	9.6±0.8	8.9±0.6
Ropinirole	52.2±3.2*	53.0±3.3*	10.4±0.9	11.8±0.8*

The present study showed a differential effect of L-DOPA, bromocriptine and ropinirole on DA receptor mRNA in MPTP-treated marmosets. D_1 receptor mRNA was unaltered suggesting no effect on the direct output pathway. However, ropinirole normalised both D_2 and D_3 receptor mRNA, whereas, L-DOPA normalised only D_3 and bromocriptine had no effect on either D_2 or D_3 receptor mRNA compared to MPTP-treated animals. These data suggest that the D_2 receptor mediated indirect pathway may play an important role of the genesis of the dyskinesia.

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69P EVIDENCE THAT EXCITATORY SPINAL ACTIONS OF A TRIPTAN ARE MEDIATED THROUGH 5-HT$_7$ RECEPTORS

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In pentobarbitone-anaesthetized, spinalized rabbits low doses (< 100 μg kg^{-1}) of the triptan L-741,604 potentiates reflexes evoked in the ankle flexor tibialis anterior (TA) by stimulation at the toes, an effect that is not reversed by the selective 5-HT$_{1B/1D}$ receptor antagonist GR 127935 (Jenkins *et al.*, 2000). It was proposed that this effect might be mediated via 5-HT$_7$ receptors, an idea that has now been tested directly.

Experiments were performed in 9 rabbits anaesthetized with pentobarbitone sodium (average dose 41 mg kg^{-1}, followed by a continuous i.v. infusion at 5 mg kg^{-1} h^{-1}). A flexor withdrawal reflex (FWR) evoked by electrical stimulation of the toes, and the jaw depressor reflex (JDR) evoked by electrical stimulation of the tongue were recorded from the left TA and digastric muscles, respectively. Heart rate was measured from ECG recordings and arterial blood pressure was recorded from the carotid artery. All drugs were given i.v.. The selective 5-HT$_7$ receptor antagonist SB-269970 was given at 0.5 mg kg^{-1}, followed at an interval of 24 min by L-741,604 10 μg kg^{-1}. After a further 24 min a supplementary dose of SB 269970 0.3 mg kg^{-1}, followed after the same delay by L-741,604 at 30 μg kg^{-1}. The reflex and cardiovascular effects of L-741,604 were compared with data from Jenkins *et al.* (2000).

SB 269970 alone had no significant effects on the FWR, JDR (Wilcoxon tests, $p > 0.05$) or blood pressure (paired t test, $p > 0.7$). After blockade of 5-HT$_7$ receptors, L-741,604 10 μg kg^{-1} had no significant effect on the FWR or JDR (Wilcoxon, $p > 0.2$), but did increase blood pressure by a mean (± s.e.m.) of 8 ± 2 mmHg over pre-injection levels (paired t test, $p < 0.01$). At 30 μg kg^{-1}, L-741,604 increased the FWR to a median of 109% or pre-injection values (inter-quartile range, IQR 102 – 115%, Wilcoxon $p < 0.05$), decreased the JDR to a median of 80% (IQR 66 – 86%) of immediate pre-dose values (Wilcoxon, $p = 0.008$), and increased the blood pressure by a mean of 18 ± 2 mmHg (paired t test, $p < 0.0001$). The increases in blood pressure were *not* accompanied by compensatory falls in heart rate. In animals not treated with SB-269970 (Jenkins *et al.*, 2000), both doses of L-741,604 increased the FWR to greater levels than seen in the present study (Mann-Whitney, $p < 0.01$), whereas effects on the JDR and blood pressure were not significantly different (Mann-Whitney and t test respectively, $p > 0.4$). In that study, increased blood pressure was accompanied by significant decreases in heart rate.

These data indicate that SB 269970 depressed the ability of L-741,604 to increase the FWR, and may also have compromised the baroreceptor reflex. It did not inhibit the effectiveness of the triptan to decrease trigeminal transmission or cause vasoconstriction. The findings are consistent with the view that the spinal reflex enhancing action of L-741,604 is mediated via 5-HT$_7$ receptors.

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70P INVESTIGATION OF THE MECHANISM OF TOLERANCE TO THE ANOREXIGENIC EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8OH-DPAT) IN FOOD-DEPRIVED RATS

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5HT$_{1A}$-selective agonists such as 8OH-DPAT exert an acute anorexigenic effect in food-deprived rats that is decreased by the 5HT$_{1A}$ receptor antagonist, WAY 100635 (Ebenezer, 1992; Arkle & Ebenezer, 2000). In this study we have shown that food-deprived rats display tolerance to chronic treatment with 8OH-DPAT. Possible mechanisms include receptor down-regulation and increased metabolism of 8OH-DPAT. We have investigated this by measurements of 8OH-DPAT metabolism and the effects of WAY 100635 on development of tolerance.

Food intake was measured in 22h food-deprived male Wistar rats (200-250g) as previously described (Arkle & Ebenezer, 2000). Rats were assigned to 3 treatment groups (n=6) and injected s.c. with saline (S/S group) or 8OH-DPAT (50μg.kg^{-1}; S/DPAT and WAY/DPAT groups) daily for 24 days. On days 1, 8, 12, 15 and 19 the rats were given either saline (S/S and S/DPAT groups) or 300 μg.kg^{-1} WAY 100635 (WAY/DPAT group) s.c. 15 min prior to injection with saline or 8OH-DPAT and measurement of food intake. On day 21 all animals received only the saline or 8OH-DPAT injection immediately prior to measurement of food intake. Food intake is expressed as % of mean for the S/S group on the same day. Rats were killed on day 24 and liver microsomal fractions prepared. Metabolism was assayed as aldehyde production (determined colourimetrically by reaction with NASH reagent) in microsomes incubated with 100 μM 8OH-DPAT or anisole or no substrate. Metabolism was also measured in rats treated as above but killed on day 1. Values are expressed as mean aldehyde production ± s.e.m.; n=6. Differences between means were tested by ANOVA with *post hoc* Newman-Keuls test.

8OH-DPAT reduced feeding on day 1 in S/DPAT but not in WAY/DPAT rats (S/DPAT 67±14%, $P<0.05$; WAY/DPAT 89±14% of S/S). Tolerance developed by day 19 (S/DPAT 97±3%, WAY/DPAT 105±3% of S/S). On day 21 the S/DPAT group remained tolerant but 8OH-DPAT, given without WAY 100635, decreased feeding in WAY/DPAT rats (S/DPAT 94±4%, WAY/DPAT 74±11%, $P<0.05$, of S/S) Metabolism of 8OH-DPAT was higher on day 1 (S/S, 5.5±0.7; S/DPAT, 4.5±0.5; WAY/DPAT, 4.6±0.2 nmol min^{-1}.mg protein^{-1}) than day 24 (S/S, 3.4±0.3; S/DPAT, 3.4±0.4; WAY/DPAT, 3.3±0.1 nmol min^{-1}.mg protein^{-1}) ($P<0.05$) but unaffected by drug treatment. Parallel changes were seen in anisole metabolism (day 1, S/S, 9.9±1.1; S/DPAT, 9.6±0.7; WAY/DPAT, 9.3±0.4; day 24, S/S, 5.3±0.3; S/DPAT, 5.5±0.3; WAY/DPAT, 5.1±0.2 nmol min^{-1}.mg protein^{-1}) ($P<0.05$).

We conclude that tolerance to 8OH-DPAT occurs during repeated treatment with this drug. This is not due to an increase in its hepatic metabolism and changes in metabolism during repeated food deprivation are neither specific to nor influenced by 8-OH-DPAT. The protection to tolerance afforded by WAY 100635 may indicate that the mechanism of tolerance is related to stimulation of 5HT$_{1A}$ receptors.

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71P PRETREATMENT WITH THE CCK_1 RECEPTOR ANTAGONIST DEVAZEPIDE ATTENUATES THE SUPPRESSANT EFFECT OF THE $5HT_{1A}$ RECEPTOR AGONIST GEPIRONE ON FOOD INTAKE IN HUNGRY RATS

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It has been previously shown that 5-HT_{1A} receptor agonists decrease food intake in food deprived rats by an action at central 5-HT_{1A} receptors (Arkle and Ebenezer, 2001). There is also growing evidence that central cholecystokinin plays an important inhibitory role in the control of feeding behaviour (Baldwin et al., 1998). In order to see if 5-HT_{1A} receptor agonists mediate their hypophagic actions via a central CCK mechanism, the present study was undertaken to investigate whether pretreating rats with the CCK_1 receptor antagonists devazepide or napthalenesulphanyl-l-aspartyl-2-(phenyl) amide (2-NAP) would abolish or attenuate the inhibitory effects of the 5-HT_{1A} receptor agonist gepirone on food intake in hungry rats.

Male Wistar rats (n=16; body mass 230 – 300g) were divided into 2 equal group. The rats were fasted for 22h each day but had free access to water at all times. The rats in Group 1 were injected with either vehicle followed by saline, devazepide (0.25 mg kg^{-1}) followed by saline, vehicle followed by gepirone (4 mg kg^{-1}), or devazepide (0.25 mg kg^{-1}) followed by gepirone (4 mg kg^{-1}). The rats in Group 2 received 2-NAP (4 mg kg^{-1}) instead of devazepide. Both injections were given i.p. An interval of 30 min separated the two injections. Immediately after the second injection the animals were put into experimental cages, as described previously (Ebenezer, 1990), and food intake measured. A repeated measures design was used with each rat receiving all 4 treatments assigned to its group. Three to four day separated successive drug treatments. The data were analysed by ANOVA for repeated measures.

The effects of pretreatment with devezepide on the effects of gepirone on food intake is shown in Fig.1. As reported previously (Ebenezer & Tite, 1995) gepirone (4 mg kg^{-1}) significantly decreased food intake in the first 30 min after administration ($P<0.01$).

Devazepide (0.25 mg kg^{-1}) significantly attenuated the hypophagic action of gepirone. By contrast, 2-NAP (4 mg kg^{-1}) did not attenuate the inhibitory effect of gepirone on food intake (data not shown). This dose of 2-NAP has been previously reported to abolish the hypophagic effect of exogenous peripheral CCK (see Baldwin *et al.*, 1998).

The results of this study show that the while CCK_1 receptor antagonist devazepide attenuates the hypophagic effects of gepirone, the CCK_1 receptor antagonist 2-NAP has no effect. It has been well established that devazepide can enter the CNS from the systemic circulation, while 2-NAP does not cross the blood brain barrier (see Ebenezer and Baldwin, 1995; Baldwin et al., 1998). Thus, the present findings suggest that 5-HT_{1A} agonist may mediate, at least, part of their inhibitory effects on food intake in hungry rats by interacting with central, but not a peripheral, CCK.

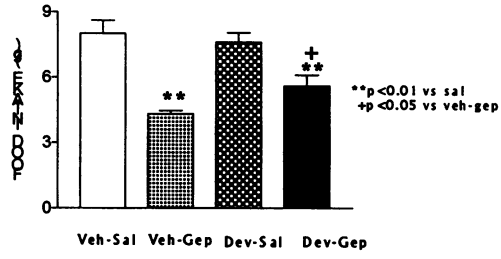

Fig. 1. Effect of devazepide pretreatment on the hypophagic effect of gepirone in food deprived rats.

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72P EFFECTS OF CHRONIC ADMINISTRATION OF THE $GABA_B$ AGONIST BACLOFEN ON FOOD INTAKE IN RATS

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It has been previously demonstrated that acute central or systemic administration of the $GABA_B$ agonist baclofen increases short-term food intake in rats (Ebenezer, 1990, Ebenezer and Pringle, 1992). The present study was undertaken to investigate the effects of chronic administration of baclofen on food intake measured over 24h in rats.

Adult male Wistar rats (n=12; b. wt.= 430-450g) were divided into two equal groups. The rats were injected i.p. daily at 10.30h with either saline solution or baclofen (2 mg kg^{-1}). On experimental days 1, 12 and 21, the rats were placed into separate experimental boxes immediately after their treatments and presented with food and water, as described previously (Ebenezer, 1990). Food intake was measured at intervals over 24 hours. The dose of baclofen used in this study has previously been shown to increase food intake in rats (Ebenezer and Pringle, 1992).

The results are shown in Fig. 1. Baclofen (2 mg kg^{-1}) did not significantly increase cumulative food intake at any of the measurement intervals on day 1. However, following chronic treatment with baclofen, the rats displayed significant and long-lasting increases in cumulative feeding (i.e. 16h after treatment with baclofen for 12 days, and 12h after treatment with baclofen for 21days). Nevertheless, the animals appeared to be able to regulate their daily food intake quite accurately, and there were no significant differences in the amount eaten at 24h between the two groups irrespective of the treatment day.

Two important conclusions can be drawn from the present study. Firstly, it appears that chronic administration with baclofen sensitises the animals, so that there is a prolonged increase in cumulative food

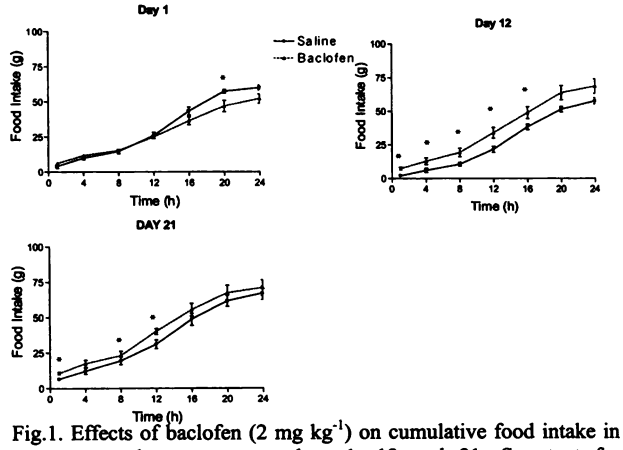

Fig.1. Effects of baclofen (2 mg kg^{-1}) on cumulative food intake in rats measured on treatment days 1, 12 and 21. See text for experimental details. Vertical lines represent ± s.e. mean. *$P<0.05$ (2-tailed t-test)

intake when they are subsequently injected with baclofen and given free access to food. The mechanisms involved are not known. Secondly, the 24h daily intake of the rats are fairly accurately regulated irrespective of prior treatment with baclofen. The latter observation may explain why chronic administration of baclofen to rats does not result in increases in body mass (Patel & Ebenezer, 2002).

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