151P COMPARISON OF THE VASODILATORY ACTION OF TESTOSTERONE IN ISOLATED HUMAN PULMONARY AND MESENTERIC ARTERIES AND VEINS

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Testosterone therapy has been shown to be of benefit to men with coronary artery disease (English *et al.*, 2000). This is proposed to be due to the vasodilatory effect of testosterone on human coronary arteries (Webb *et al.*, 1999). It has also been shown that testosterone therapy benefits men with heart failure (Pugh *et al.*, 2002). Pulmonary and systemic vasodilation is beneficial to patients with heart failure, but it is unknown if testosterone possesses this effect in human vessels.

Male patients were recruited from cardiothoracic (n=14, age=68±9) and gastrointestinal (n=8, age=70±3) operating lists in Sheffield Teaching Hospitals and gave full written consent. 2mm lengths of pulmonary conduit artery (PCA n=11, diameter=986±168□m), pulmonary conduit vein (PCV n=10, diameter=841±78□m), pulmonary resistance artery (PRA n=13, diameter=358±22□m) and pulmonary resistance vein (PRV n=16, diameter=387±20□m) and mesenteric resistance artery (MRA n=16, diameter=393± 31□m) were carefully dissected and loaded in a wire myograph to a tension equivalent to the *in vivo* pressure. Vessels were maintained in physiological saline solution at 37°C and bubbled with 95% O_2, 5% CO_2 to maintain pH 7.4. Vessel viability was confirmed by contraction to noradrenaline (10μM) or U46619 (1μM) and endothelial integrity was confirmed by subsequent dilatation to acetylcholine (1μM). Vessels were then exposed to increasing concentrations of KCl (0.1-100mM) followed by cumulative additions of ethanol vehicle. Vessels were then washed, the addition of KCl repeated and exposed to increasing concentrations of testosterone (T, 1nM-100μM).

Table 1 -Mean (S.E.M.) responses to KCl, Eth and T in PCA, PCV, PRA, PRV and MRA. * $p<0.05$, ** $p<0.01$ cf conduit vessels, ‡ $p<0.001$ cf PRA via Student's unpaired *t* test. † $p<0.001$ cf ethanol vehicle via Mann-Whitney U Test.

	Vessels	Patients	Emax KCl (mN)	Emax Eth (%)	Emax T (%)
PCA	12	8	10.56 (1.74)	10.6 (3.3)	-42.5† (3.2)
PCV	12	8	6.76 (1.06)	10.6 (5.2)	-40.7† (4.7)
PRA	11	9	3.66** (0.57)	18.0 (4.7)	-48.2† (5.7)
PRV	12	7	3.04* (0.79)	10.3 (5.8)	-52.0† (6.9)
MRA	16	8	15.98‡ (2.96)	9.7 (2.8)	-95.0†‡ (8.1)

Testosterone dilated pulmonary conduit and resistance arteries and veins at concentrations greater than 3μM. A significantly greater response was seen in mesenteric resistance arteries. This action may underlie the beneficial effects seen with testosterone replacement therapy in men with heart failure.

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152P MECHANISMS OF 17β-OESTRADIOL-INDUCED VASORELAXATION IN THE RAT AORTA

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Chen *et al.* (1999) have shown that 17β-oestradiol acutely activates endothelial nitric oxide synthase (NOS) in ovine pulmonary artery endothelial cells. However, Gonzales and Kanagy (1999) demonstrated that 17β-oestradiol caused vasorelaxation via endothelium- and NO-independent pathways in the ratmesenteric artery. The present study aims to investigate the roles of endothelial-derived vasorelaxants, K^+ efflux, and inhibition of Ca^{2+} influx in vasorelaxations to 17β-oestradiol in the rat aorta.

Male Wistar rats (250-300g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1}, i.p.) and exsanguinated. The thoracic aortae were dissected from the rats and cut into 5mm lengths. Each ring was bathed with oxygenated Krebs-Henseleit solution 37°C. The aortic rings were stretched to an optimal passive tension of 10mN. Following a 1-hour equilibration period, methoxamine (80-100μM) was added to increase tension by 5-10mN. 17β-oestradiol was added cumulatively (30pM-1mM). The vasorelaxant effects of 17β-oestradiol were assessed in the presence of 10μM indomethacin, a cyclooxygenase (COX) inhibitor, 300μM N^G-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor. To examine the role of the endothelium, some aortae were denuded by gently rubbing the luminal surface with a cocktail stick. The preparation was considered to be endothelium-denuded when the response to 10μM carbachol was less than 10%. To assess the effect of high extracellular K^+ on responses to 17β-oestradiol, 60mM KCl was added to induce tone. To investigate the calcium antagonistic effect of 17β-oestradiol, concentration-response curves to $CaCl_2$ (10μM-30mM) were obtained in the absence and in the presence of 17β-oestradiol (100μM). The aortae bathed in Ca^{2+}-free Krebs solution were depolarized by 100mM KCl. Mean responses at each concentration were compared by either Student's *t*-test for unpaired values or ANOVA.

17β-oestradiol (30pM-1mM) caused concentration-dependent vasorelaxations in the rat aorta. Vasorelaxations induced by 17β-oestradiol were not inhibited by removal of the endothelium or L-NAME (300μM). However, pre-treatment with indomethacin (10μM) significantly ($p<0.05$) enhanced vasorelaxations to 17β-oestradiol at concentrations from 3μM to 300μM. In the presence of indomethacin, 60mM KCl significantly inhibited relaxant responses to 17β-oestradiol at the concentrations from 10nM to 10μM (10nM-3μM 17β-oestradiol, $p<0.01$ and 10μM 17β-oestradiol, $p<0.05$), but there were no significant differences in vasorelaxations induced by 17β-oestradiol at the concentrations from 30μM to 1mM. $CaCl_2$ (10mM-30μM) caused contraction in a concentration-dependent manner in the rat aorta depolarized by 100mM KCl. Pre-incubation with 100μM 17β-oestradiol significantly inhibited contraction induced by $CaCl_2$ (maximal contraction: control=5.4±0.1mN, n=5; 17β-oestradiol= 3.5±0.1mN, n=5, $p<0.001$).

The present study showed, in the rat aorta, that 17β-oestradiol acutely induced concentration-dependent vasorelaxation through inhibition of Ca^{2+} influx, but not via endothelium- and NO-dependent pathways. In addition, the relaxant responses to 17β-oestradiol uncovered by COX inhibition occurred via activation of K^+ efflux.

This work is funded by The Thai Government.

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153P EP_4 PROSTANOID RECEPTORS MEDIATE RELAXATION OF HUMAN CEREBRAL ARTERY

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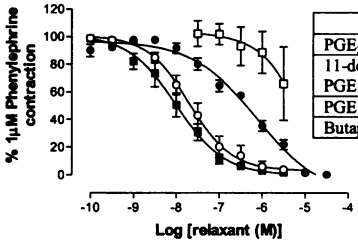
Enhanced levels of prostaglandin E_2 (PGE_2) are reported in migraineurs during an attack (Satchielli *et al*, 2000; Vardi *et al*, 1983). Also implicated in the pathophysiology of migraine are dilatation of the cerebral vasculature and stimulation of trigeminal sensory afferent nerves. The aim of the present study was to characterise the effects of PGE_2 on the regulation of vascular tone in human cerebral arteries.

Human cerebral arteries were obtained at autopsy with the informed consent of the donor's next of kin, and with the approval of the appropriate local ethics committee. Intact rings of middle cerebral artery were mounted under isometric conditions in 10 ml organ baths under an initial tension of 1g. All tissues were maintained in Krebs solution containing indomethacin (3 μM), at 37°C and gassed with 95% O_2 / 5% CO_2. Following a 90 min equilibration period, an approximate $EC_{60\text{-}80}$ concentration of phenylephrine (1μM) was added to obtain a stable contraction.

Administration of increasing concentrations of PGE_2 was found to concentration-dependently relax phenylephrine precontracted human cerebral arterial rings. Of the 4 known members of the prostanoid EP receptor family, only EP_2 and EP_4 mediate relaxatory responses of smooth muscle to PGE_2. Furthermore, the relative potencies of the EP_4>EP_2 agonist, 11-deoxy PGE_1, and the EP_2>EP_4 agonist, PGE_1-OH, and the lack of effect of the EP_2 selective agonist butaprost, suggest the involvement of EP_4 receptors. More direct evidence that PGE_2 was acting via EP_4 receptor stimulation was achieved by demonstrating a significant rightward shift in the PGE_2 concentration-response curve by the weak EP_4 receptor antagonist, AH23848 (Coleman *et al*, 1994), (10μM; apparent pKb -5.76 ± 0.22 (n=3; p=0.04, two-tailed T-test). Treatments with L-NAME (100μM) or denudation of the endothelium were without effect on PGE_2-mediated relaxations.



	pEC_{50} (M)	*n*
PGE_2 (■)	8.01 ± 0.06	6
11-deoxy PGE_1(○)	7.56 ± 0.26	6
PGE_1-OH (●)	5.90 ± 0.23	4
Butaprost (□)	< 6	3

Figure 1. Concentration-response curves to EP receptor agonists in phenylephrine pre-contracted human cerebral artery. *Table 1* denotes agonist pEC_{50} values and number of donors.

These data suggest that PGE_2 -induced relaxation of pre-contracted human cerebral artery is predominantly mediated via EP_4 receptors. These novel data are consistent with the hypothesis that EP_4 receptor antagonists will be of potential clinical value in the treatment of migraine.

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154P β_1-ADRENOCEPTORS MEDIATE ENDOTHELIUM-INDEPENDENT RELAXATION IN RAT FEMORAL RESISTANCE ARTERIES

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Vascular β-adrenoceptors were originally classed as β_2- (Lands *et al.*, 1967) and this is generally accepted to be the predominant subtype in most vascular smooth muscles. The aim of the present study was to characterise the β-adrenoceptor subtypes present in rat femoral resistance arteries and investigate the role of endothelium in β-adrenoceptor mediated relaxation.

Male Wistar rats (200 - 250 g) were stunned and killed by cervical dislocation. Third order femoral small arteries (normalised diameter of 224±6 μm, n=48) were dissected out and mounted on a small vessel wire myograph (Danish Myotech, Aarhus, Denmark) for isometric recording in Krebs physiological saline solution (PSS) gassed with 95/5 % O_2/CO_2 at 37 °C. In some preparations endothelium was denuded. Viability was checked by exposure to high potassium solution (60 mM) twice. Arteries were pre-constricted with phenylephrine (PE, 1 μM) before carrying out cumulative concentration-response curves (CRCs) to agonists. In endothelium denuded preparations the concentration of PE was reduced to 0.3 μM to produce a similar level of pre-constriction. The function of endothelium was checked using acetylcholine (3 μM). In antagonist studies, tissues were incubated with antagonist for 30 min before constricting. Values are mean±s.e.mean. Statistical analysis was carried out using Student's *t*-test.

Isoprenaline produced concentration dependent relaxation of PE-constricted arteries (pEC_{50}, 7.19±0.02, n=6). Pre-incubation with the selective β_2-adrenoceptor antagonist, ICI 118551 (30 nM), had no effect on isoprenaline-induced relaxation (n=6). A higher concentration (0.3 μM) produced a 4-fold shift of the isoprenaline CRC (pEC_{50}: ICI 118551, 6.58±0.01, n=6, P<0.05) with no change in the maximum response, giving an estimated pK_B of 6.97±0.12. Pre-incubation with the selective β_1-adrenoceptor antagonist, CGP 20712A (10 nM), produced a 9-fold shift of the isoprenaline CRC (pEC_{50}: CGP 20712A, 6.22±0.03, n=6, P<0.05) with no change in the maximum response, giving an estimated pK_B of 8.9±0.09. In the presence of a higher concentration of CGP 20712A (0.3 μM), isoprenaline produced no relaxation at concentrations ≤30 μM (n=6). CL 316243 (10 nM-30 μM), a selective β_3-adrenoceptor agonist, produced no relaxation.

Removal of endothelium had no effect on isoprenaline-mediated relaxations (pEC_{50}s: intact, 7.14±0.02; denuded, 7.18±0.02, n=3, P>0.05). In contrast, acetylcholine-induced relaxation was greatly reduced (% relaxation, n=3: intact, 91±3; denuded, 8±2, P<0.05).

In conclusion, β-adrenoceptors mediating relaxation in rat femoral resistance arteries are of the β_1-subtype, with no evidence for β_2- or β_3-adenoceptors. β_1-adrenoceptor-mediated relaxation in rat femoral resistance arteries is endothelium independent.

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155P DOES SIMVASTATIN INFLUENCE THE AGONIST-INDUCED DESENSITISATION OF β-ADRENOCEPTORS ?

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Statins, like simvastatin (SIM), block hepatic conversion of HMG-CoA to mevalonic acid (MA) in the cholesterol biosynthetic pathway. MA, however, serves as a precursor to isoprenoid metabolites that facilitates protein-protein interactions and membrane-associated protein trafficking. Therefore, depletion of MA leads to cytosolic sequestration and loss of intracellular signalling function of proteins posttranslational modified by isoprenoids. Membrane targeting of G protein coupled receptor kinase 2 (GRK2) occurs indirectly via binding to the Gβγ-subunits of trimeric G proteins, anchored by their own posttranslational attached isoprenoid-moiety at the γ-subunit (Penn *et al.*, 2000; Evanko et al., 2001) and is considered to be the first step involved in GRK-mediated agonist-induced homologous desensitisation of β-adrenoceptors (β-ARs) (Iaccarino & Koch, 1999).

The aim of the present study was, therefore, to determine whether desensitisation and down-regulation of β-ARs mediated by increased GRK activity after exposure to isoprenaline (ISO) might be altered in the presence of SIM. In order to study this, we assessed in adult rat cardiomyocytes (CM) pre-incubated ± 2 nM SIM (± 1 μM MA), β-AR density (assessed by [^{125}I]-Iodocyanopindolol (ICYP, 5-200 pM)-binding ± 1 μM CGP12177) and GRK activity (assessed by in vitro phosphorylation of rhodopsin, ROS) after stimulation with 10 μM ISO for 16 h. Additionally, we examined the ability of SIM to inhibit specific ICYP- binding by incubating CM-protein with 80 pM ICYP ± 10^{-12}- 10^{-6} M SIM.

As shown in Table 1 β-AR density was significantly reduced after ISO-treatment. This effect was abolished by pre-incubation with 2 nM SIM. Moreover, short-term administration of 2 nM SIM increased β-ARs expression and inhibited the ISO-induced increase in GRK activity. Additionally, assessment of the ability of SIM to inhibit specific ICYP binding revealed an IC_{50}-value of about 47 ± 2.3 nM.

	Controls	ISO	SIM	SIM/ ISO	SIM/ ISO/MA
β-AR	11.6±1	8.7±1*	19.5±1*	20.2±2[a]	6.6±1*
K_D	30±6	24±4	46±6*	48±6*,[a]	24±3
GRK_{cyt}	6.1±1	8.6±2	4.0±0.4*	9.1±2	8.3±2
GRK_{mem}	17.2±1	28.3±3*	12.8±1*	18.7±1[a]	24.1±2*

Table 1:β-AR density in fmol/mg protein; K_D [^{125}I]CYP-affinity in pM; GRK_{cyt} and GRK_{mem} cytosolic and membraneous GRK activity in [^{32}P]-ROS [cpm x 10^3/mg protein]. Values are means±SEM of 6 experiments each with *$p<0.05$ vs. controls and [a]$p<0.05$ vs. ISO.

Conclusion: These data indicate that SIM might alter agonist-induced desensitisation of β-AR.

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156P THE ROLE OF THE ENDOTHELIUM AND CONSTITUTIVE NO IN LPS-INDUCED VASORELAXATION

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Blood vessels exposed to bacterial lipopolysaccharide (LPS) express inducible nitric oxide synthase (iNOS) in smooth muscle cells and the fibroblasts of the adventitia (Stoclet *et al.* 1993) to produce large amounts of nitric oxide (NO), leading to the often-fatal, unresponsive hypotension observed in septic shock (Stoclet *et al.* 1993). Recent preliminary studies performed in our laboratory showed that LPS-induced relaxation was absent in blood vessels denuded of their endothelium. Therefore, the aim of the study was to examine the role of the endothelium and its production of constitutive NO on LPS-induced relaxation in rat aortic rings.

Aortic rings from Sprague-Dawley rats (200-250 g) were isolated and mounted under 1 g tension in physiological salt solution aerated with 95% O_2 and 5% CO_2 at 37°C. Tissues were precontracted with phenylephrine (~EC_{80}) and endothelial integrity was assessed using acetylcholine (1uM): vessels giving a relaxation response ≥50% were considered endothelium-intact rings and those ≥10% endothelium-denuded rings. Following this vessels were precontracted and incubated with LPS (1 μg/ml) for 5h. Tension was monitored over this period, after which tissues were collected for iNOS protein determination using Western blotting. In separate experiments, constitutive NO was replaced by treating endothelium-denuded rings with the NO donor diethylenetriaamine NONOate (DetaNO; 0.1 μM). All data are expressed as mean ± SEM.

In endothelium-intact aortic rings, LPS produced a significant relaxation (52.3 ± 3.0%; n = 18; $P<0.001$, one-way ANOVA), while time-control vessels developed an increase in tension (4.0 ± 8.1%; n = 18). In endothelium-denuded vessels, the relaxation was not significantly different from time-control ($P>0.05$, one-way ANOVA). Interestingly, when endothelium-denuded rings were treated with the NO donor DetaNO to replace constitutive NO, LPS produced a significant relaxation (27.2 ± 7.3%; n = 9; $P<0.05$, Student's t-test) compared to vessels exposed to DetaNO alone (0.8 ± 5.7; n = 6). The results suggest that the presence of an intact endothelium and its constitutive NO are required for LPS-induced relaxation in rat aortic rings.

Western blot analysis of the endothelium-intact aortic rings at the end of 5h LPS treatment showed a clear band with an apparent molecular mass of ~130 kD (corresponding to iNOS protein), which was largely absent in endothelium-denuded vessels. Conversely, iNOS protein was again clearly detectable in endothelium-denuded vessels after co-treatment of tissues with LPS with DetaNO. There was no detectable iNOS protein in vessels which were not subjected to LPS treatment.

In conclusion, these results demonstrate that the endothelium is required for the induction of iNOS and associated functional relaxation observed in response to LPS. Furthermore, the study demonstrated that it is the constitutive NO released from the endothelium that is required for this effect.

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157P EFFECTS OF SPHINGOSINE-1-PHOSPHATE, SPHINGOSYLPHOSPHORYLCHOLINE AND CERAMIDE ON MESENTERIC ARTERY CONTRACTION AND RELAXATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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The contracting and relaxing effects of many agonists, including catecholamines, are enhanced and diminished, respectively, in mesenteric resistance vessels (MRV) of spontaneously hypertensive rats (SHR) (Kähönen et al. 1999; Mulvany et al. 1980). The sphingolipid metabolites sphingosylphosphorylcholine (SPC) and sphingosine-1-phosphate (S1P) also contract MRV (Bischoff et al. 2000), whereas the related C2-ceramide relaxes them (Czyborra et al. 2002). Therefore, we have determined whether vasoactive effects of SPC, S1P and C2-ceramide are also altered in MRV from SHR as compared to normotensive Wistar Kyoto rats (WKY); vascular effects of catecholamines were studied as controls.

Contraction and relaxation experiments in MRV were performed as previously described (Bischoff et al. 2000). Briefly, MRV (2nd branch from the gut) were isolated from 12-14 week old male SHR and WKY, and vessel segments (3 mm length) were mounted in a Mulvany-myograph in a Krebs-Henseleit buffer. Concentration response curves (CRC) were generated by addition of 10^{-9}–10^{-4}M noradrenaline (NA) and methoxamine (MET). Thereafter, vessels were challenged with SPC or S1P (10^{-8}–10^{-4}M). In a 2nd series of experiments, relaxation responses were determined by consecutive addition of 10^{-10}–10^{-4}M nitroprusside sodium (NPS) and isoprenaline (ISO) and 10^{-4} M C2-ceramide in MRV precontracted with MET (10^{-4} M). Data are means ± SEM of n tested vessels (2 vessels/animal).

As expected the E_{max} of NA and MET were significantly greater in SHR than in WKY (21.7 ± 0.3 vs. 14.8 ± 0.2 mN and 21.0 ± 0.2 vs. 14.1 ± 0.2 mN, respectively, n = 30-36, $p < 0.001$ each in t-test) whereas the pEC_{50} was not significantly altered (6.80 ± 0.02 vs. 6.68 ± 0.03 and 5.88 ± 0.01 vs. 5.75 ± 0.02, respectively). In contrast, the E_{max} (5.5 ± 0.3 vs. 6.6 ± 0.2 mN) and pEC_{50} (4.97 ± 0.06 vs. 5.30 ± 0.04) of SPC were significantly reduced in SPC (n = 18, $p < 0.01$). While CRC for S1P were not consistently explained by simple sigmoidal functions, its effects were decreased even more in SHR (e.g. 1.8 ± 0.2 vs. 7.4 ± 0.9 mN at 10^{-4} M, n = 14-18, $p < 0.001$).

In MET-precontracted MRV, relaxation responses to NPS (E_{max} 61.1 ± 3.8 vs. 77.5 ± 3.4%, pEC_{50} 6.82 ± 0.16 vs. 7.39 ± 0.16, n = 12, $p = 0.07$ and < 0.01, respectively) and ISO (E_{max} 52.9 ± 2.1 vs. 84.5 ± 3.5%, pEC_{50} 6.56 ± 0.08 vs. 7.16 ± 0.13, n = 12, $p < 0.05$ and < 0.001, respectively) were attenuated in SHR. Relaxation by 10^{-4} M C2-ceramide over time was modestly attenuated in SHR ($p < 0.001$ in two-way ANOVA, e.g. 62.3 ± 7.6 vs. 71.8 ± 6.3% after 10 min).

We conclude that in contrast to other vasoactive agents contracting effects of SPC and S1P are not enhanced but rather diminished in MRV from SHR. The relaxing effects of C2-ceramide are modestly diminished.

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Mulvany, M.J., *et al.* (1980) *Hypertension*, 2, 664-71

158P EFFECT OF COCAINE ON THE α_1-ADRENOCEPTOR SUBTYPES INVOLVED IN NEURALLY-EVOKED CONTRACTIONS OF RAT FEMORAL RESISTANCE ARTERIES

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Previous studies have shown that the adrenoceptor mediating contraction to electrical field stimulation (EFS) in rat femoral resistance arteries is predominantly the α_{1A}-subtype (Zacharia *et al.*, 2002). The aim of the present study was to investigate the effect of uptake blockade by cocaine on the α_1-adrenoceptor subtype(s) involved in response to EFS using antagonists prazosin (α_1-selective), 5-methyl urapidil (5 MU, α_{1A}-selective), BMY 7378 (α_{1D}-selective) and the preferential α_{1B}-alkylating agent, chloroethylclonidine (CEC).

Second and third order branches of rat femoral artery (normalised diameter $L_{0.9}$ = 226 ± 5 μm, n =31) were dissected out from male Wistar rats (200 - 250 g, 10-13 weeks old) and mounted on a small vessel wire myograph in physiological salt solution at 37°C continuously bubbled with carbogen. The vessels were activated by 123 mM KCl twice and 1 μM noradrenaline (NA). The vessels were stimulated with EFS at 20 V and 0.05 ms pulse width applied for 10 sec at frequencies of 5-30 Hz using platinum electrodes. Cocaine (3 μM) and antagonists were present for 15 minutes before EFS. For CEC, arterial segments were incubated with CEC (10μM) for 30 min followed by washing for 60 min (each wash every 15 min).

In the presence of cocaine, prazosin and 5 MU inhibited the nerve-evoked contractions with pIC_{50}s of 9.33 ± 0.07 (n=6) and 7.98 ± 0.22 (n=5) respectively at 20 Hz. There was no significant difference in the pIC_{50}s of prazosin or 5 MU at different frequencies. The pIC_{50}s were similar to those reported previously in the absence of cocaine (Zacharia *et al.*, 2002). In the presence of cocaine, BMY 7378 inhibited responses at 5 Hz with a pIC_{50} value of 7.34 ± 0.07, n =7. Higher frequencies were less sensitive to BMY 7378, e.g. the pIC_{50} at 20 Hz , 6.78 ± 0.12, n = 7, was significantly lower ($P < 0.001$). The pIC_{50} values obtained in the presence of cocaine at 10-30 Hz were not significantly different from those reported previously in the absence of cocaine (Zacharia *et al.*, 2002). However there was a significant difference in the threshold concentration of BMY 7378 required for inhibition in the presence of cocaine since 100 nM BMY 7378 produced a significant reduction in nerve-evoked contractions in the presence (e.g. 20 Hz, 32 ± 6 % reduction, n = 7, $P < 0.001$) but not in the absence of cocaine (20 Hz, 1 ± 3 % reduction, n = 5, $P > 0.05$). CEC (10 μM, n=4) had no significant effect on responses at all frequencies, similar to the results obtained in the absence of cocaine.

In conclusion, cocaine has little effect on the predominance of the α_{1A}-adrenoceptor subtype in mediating contractions to EFS in rat femoral resistance artery. There is however a significant difference in the sensitivity to low concentrations of BMY 7378 in the presence of cocaine, especially at higher frequencies, suggesting that a small α_{1D}-adrenoceptor component may be uncovered with uptake blockade.

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159P ENDOTHELIN-1 AND VASOPRESSIN ENHANCE α_2 ADRENOCEPTOR-MEDIATED VASOCONSTRICTION IN THE ISOLATED PORCINE EAR ARTERY THROUGH DIFFERENT MECHANISMS

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α_2 Adrenoceptor-mediated contractions in the porcine ear artery can be enhanced by pre-contraction with the thromboxane-mimetic U46619 (Bhattacharya & Roberts, *this meeting*). This enhancement is mediated through an enhancement of the ERK-MAP kinase pathway. The aim of this study was to determine whether α_2 adrenoceptor-mediated vasoconstriction can be enhanced by pre-constriction with other agonists, and to determine the involvement of the ERK signalling pathway.

Ears from pigs of both sexes were obtained from a local abattoir. Ear arteries were dissected into 5 mm segments and mounted in a tissue bath containing Krebs-Henseleit buffer maintained at 37°C, and gassed with 95% O_2/ 5% CO_2. Contractions were measured using an isometric force transducer linked to a PCLab data acquisition system. After reproducible responses to 60 mM KCl were obtained, responses were obtained to the selective α_2 adrenoceptor agonist UK14304 (1 μM) in the presence or absence of endothelin-1 or vasopressin (10-20 % of the KCl response). In some experiments 50 μM PD98059 (an inhibitor of ERK activation, Alessi *et al.*, 1995) was added 1 hr prior to the addition of agonists.

UK14304 (1 μM) alone produced a small response in the porcine ear artery (10.8 ± 1.6 % of 60 mM KCl response mean ± s. e. mean, n=14). Pre-contraction with endothelin-1 (1-5 nM) enhanced the response to 1 μM UK14304 to 31.2 ± 3.0 % (n=8, $p<0.01$, ANOVA followed by Dunnett's test). Pre-contraction with vasopressin (0.1-0.5 nM) also enhanced the response to 1 μM UK14304 (to 46.8 ± 5.3 %, n=6, $p<0.01$, ANOVA followed by Dunnett's test). PD98059 (50 μM) inhibited both the direct response to 1 μM UK14304 (64.3 ± 11 % inhibition, $p<0.01$, Student's 2-tailed, unpaired t-test, n=10), and the endothelin-1-enhanced response (52.8 ± 12.1 % inhibition, $p<0.01$, Student's 2-tailed, unpaired t-test). A higher concentration of endothelin-1 (50-80 nM) was required to induce a 20 % tone in tissues incubated with PD98059 compared to control tissues, indicating that endothelin-1 contractions are mediated through the ERK-MAP kinase pathway. In contrast to endothelin-1, the enhanced response to 1 μM UK14304 in the presence of vasopressin was unaffected by PD98059. The concentration of vasopressin required to induce equal tone was the same in both control, and tissues pre-treated with PD98059 indicating that vasopressin does not activate ERK-MAP kinase. This study demonstrates that endothelin-1 enhances α_2 adrenoceptor mediated vasoconstriction in the porcine ear artery through the ERK-MAP kinase signalling pathway. On the other hand, vasopressin enhances α_2 adrenoceptor mediated vasoconstriction through an ERK-MAP kinase-independent pathway.

Alessi D. R., Cuenda A., Cohen P., *et al.*, (1995). *J. Biol. Chem.*, **270**, 27489-27494.
Bhattacharya B. & Roberts R. E., *this meeting*
Supported by The Wellcome Trust.

160P UROTENSIN II STIMULATES PLASMA EXTRAVASATION IN MICE VIA UT RECEPTOR ACTIVATION

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The peptide urotensin II (U-II) is the cognate ligand of the G-protein coupled receptor UT (formerly named GPR14). A role in the regulation of cardiovascular functions has been proposed for this novel peptide / receptor system. Recently, Gendron *et al.* (2002) demonstrated that U-II stimulates plasma extravasation in rat tissues. In the present study, we evaluated the ability of U-II to induce plasma extravasation in mice. Moreover, a characterization of the receptor involved in this action was attempted using the novel UT receptor ligand [Orn^8]U-II which behaves in the rat aorta bioassay as a low efficacy partial agonist (Camarda *et al.*, 2002).

Evans blue dye was used to quantify plasma extravasation as described by Emanueli *et al.* (1998). Briefly, male Swiss mice (25-30 g) were anaesthetized with xylazine and ketamine (5 and 10 mg/100 g, respectively). Evans blue (30 mg/kg) was injected into the femoral vein. Antagonist ([Orn^8]U-II) was injected 5 min before, while agonist (U-II) was injected 2 min after Evans blue administration. Five minutes after agonist injection, a cannula was placed into the left ventricle with its tip in the aorta, the atria were opened, and mice were perfused with saline for 4 min at a flow of 10 ml/min. The various tissues (see Table 1) were removed, rinsed in saline, gently blotted and weighed. Evans blue was extracted by incubation in formamide for 24 h and quantified by colorimetric techniques. Data are expressed as ng Evans blue / mg of fresh tissue.

U-II (0.1, 1, and 10 nmol/kg) dose-dependently stimulated plasma extravasation in several mouse tissues. The effects of the peptide at 1 nmol/kg are reported in Table 1. [Orn^8]U-II at 30 nmol/kg was either inactive or produced a non significant increase in plasma extravasation (the only exception being the duodenum where the effect of [Orn^8]U-II reached the level of statistical significance). In the presence of [Orn^8]U-II the effects of U-II were reduced or, in same cases, fully prevented.

Table 1. Effects of U-II and [Orn^8]U-II on plasma extravasation from several mouse tissues.

	Saline	U-II (1 nmol/kg)	[Orn^8]U-II (30 nmol/kg)	[Orn^8]U-II plus U-II
Trachea	5.9 ± 0.3	12.6 ± 3.2*	7.9 ± 1.4	7.8 ± 1.0
Bronchus	12.3 ± 1.1	23.6 ± 4.6*	18.0 ± 4.0	15.8 ± 3.2
U. bladder	5.3 ± 0.5	15.7 ± 3.4*	5.4 ± 1.2	9.0 ± 2.1
Oesophagus	4.9 ± 0.3	8.9 ± 1.2*	6.6 ± 1.1	5.8 ± 0.6
Stomach	5.9 ± 0.9	17.5 ± 4.4*	6.5 ± 0.6	8.5 ± 1.3
Duodenum	7.0 ± 0.9	24.4 ± 2.3*	13.1 ± 1.6*	13.8 ± 2.4*
Ileum	5.4 ± 0.7	13.8 ± 1.8*	8.6 ± 1.2	7.6 ± 1.6
Colon	3.3 ± 0.4	10.4 ± 1.9*	4.8 ± 0.5	5.2 ± 0.9
Pancreas	4.2 ± 0.7	11.3 ± 2.5*	7.9 ± 1.9	7.2 ± 1.3
Spleen	12.8 ± 1.7	24.1 ± 2.2*	18.6 ± 3.7	17.1 ± 0.8
Liver	7.6 ± 1.3	14.9 ± 3.4*	6.7 ± 0.9	6.0 ± 0.3
Kidney	6.3 ± 1.3	18.6 ± 3.8*	6.6 ± 0.7	7.2 ± 0.9

Data are mean ± s.e.mean of 6-10 separate experiments. *$p<0.05$ vs saline, according to ANOVA plus Dunnett test.

In conclusion, the present data demonstrated that U-II stimulates plasma extravasation in several tissues of the mouse via UT receptor activation.

Gendron G., Simard B, D'Orleans-Juste P *et al.*, in *Endothelium et ses maladies cardiovasculaires*, Montreal, Canada, 10-11 March, 2002.
Camarda V, Guerrini R, Kostenis E, *et al.*, (2002) *Br J Pharmacol* **137**: 311-314.
Emanueli C, Grady EF, Madeddu P, *et al.*, (1998) *Hypertension* **31**: 1299-1304.

161P INFLUENCE OF BETA3-ADRENERGIC STIMULATION ON INTRACELLULAR CA^{2+} CONCENTRATION IN FRESHLY ISOLATED RAT AORTIC ENDOTHELIAL CELLS

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Beta3-adrenoceptors (beta3-AR) induce an endothelium and NO-synthase dependent vasorelaxation in rat aorta (Trochu *et al.*, 1999). Immunohistochemistry and RT-PCR experiments demonstrated specific endothelial expression of beta3-AR in rat aorta (Rautureau, Toumaniantz *et al*, 2002). As endothelial NO synthase activation could be dependent of an increase in intracellular calcium concentration ($[Ca^{2+}]_i$), we hypothesised that beta3-AR stimulation increases calcium concentration in endothelial cells. The present study was undertaken to investigate the role of beta3-AR stimulation on $[Ca^{2+}]_i$ variations in freshly isolated rat aortic endothelial cells (EC).

Wistar male rats (body weight 250-300g) were anaesthetised by pentobarbitone ip 30 mg/kg. Four rat aortae were isolated, the adventitia was removed, the vessels cut in fine rings and submitted to digestion with collagenase. After a brief mechanical dissociation, EC were centrifuged and put down on fibronectin coated coverglasses. Variations in $[Ca^{2+}]i$ were evaluated by fluorimetry. EC were loaded with the Ca^{2+} sensitive and ratiometric probe indo-1. Data are presented as mean ± s.e. mean. Statistical significance was determined by paired *t*-test, $P<0.05$ was considered as statistically significant.

Integrity of intracellular Ca^{2+} signalling pathway was confirmed by the presence of Ca^{2+} transient in response to acetylcholine (Figure 1). Acetylcholine 10 µM induced an increase in the fluorescent ratio from 0.65±0.03 to 1.08±0.06 ($P<0.01$; $n=13$).

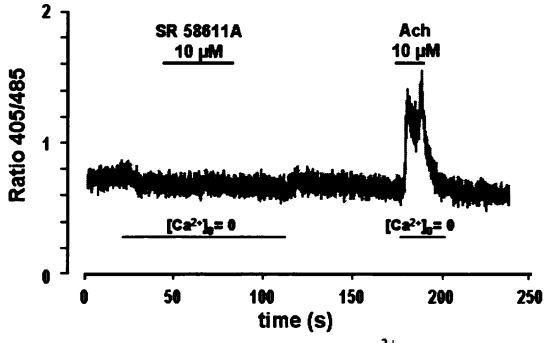

Figure 1: *Representative recording of $[Ca^{2+}]_i$ in response to SR 58611A and acetylcholine (Ach) in freshly isolated rat aortic endothelial cells.*

By contrast, in the same cells, the preferential beta3-AR agonist SR 58611A (gift from Sanofi Research, Montpellier, France) at 10 µM did not alter the $[Ca^{2+}]_i$ (Figure 1). The ratio before and during superfusion of SR 58611A, were not significantly different (0.66±0.03 *vs* 0.65±0.03 respectively). We did not observe $[Ca^{2+}]_i$ variation at a higher concentration of SR 58611A, 30 µM (data not shown).

The activation of beta3-AR expressed in rat aortic EC had no effect on global $[Ca^{2+}]i$. However, a localised elevation in $[Ca^{2+}]_i$ cannot be ruled out and further studies by confocal microscopy are required.

Trochu J.N. *et al.*, 1999 *Br. J. Pharmacol.* 128: 69-76.
Rautureau Y., Toumaniantz G. *et al.*, 2002 *Br. J. Pharmacol.* 137: 153-61.

162P EFFECTS OF BLOOD PRESSURE VARIATION ON ANGIOTENSIN RECEPTOR EXPRESSION IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

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We have previously demonstrated marked differences in densities of the two angiotensin receptor subtypes AT_1 and AT_2 in a variety of tissues taken from spontaneously hypertensive rats (SHR) and their normotensive controls, the Wistar Kyoto rat (WKY) (Bakhit et al., 2002). This study aimed to examine which of these changes were inherent strain differences and which were a direct consequence of the differences in the mean systolic blood pressure (SBP).

Male WKY (230-280g) received N^{ω}-nitro-L arginine methyl ester (L-NAME; 50mg Kg^{-1} day^{-1}) for 4w to elevate their blood pressure; male SHR (230-280g) received amlodipine (10mg Kg^{-1} day^{-1}) for 4w to reduce their blood pressure and angiotensin receptor binding was determined in aorta, liver, colon and heart. The group size was at least 6 in all cases. A control group of WKY were also treated with the same dose of amlodipine for 4w to determine whether it had any direct effects on angiotensin receptor expression in the absence of any blood pressure changes.

Treatment of WKY rats for 4w with L-NAME caused a significant elevation in SBP (tail cuff) from 116.5±3.3 to 181.9±3.9mm Hg ($p<0.001$). Similarly, SBP of SHR treated with amlodipine was significantly reduced from 213.0±3.4 to 125.0±3.7mm Hg ($p<0.001$). Treatment of WKY with amlodipine failed to alter SBP ($p>0.05$).

Binding studies on the aorta from untreated SHR demonstrated the presence of both AT_1R and AT_2R (B_{max} 0.120±0.007 and 0.098±0.055 Pmol mg^{-1} protein respectively). Similar studies on untreated WKY demonstrated the presence of AT_1R only (B_{max} 0.123±0.008 pmol mg^{-1} protein). Amlodipine treatment of SHR caused significant decrease in the B_{max} of the AT_1R (0.046+0.016 pmol mg^{-1} protein; $p<0.001$) and a complete absence of AT_2R. In normotensive WKY amlodipine caused the expression of AT_2R (B_{max} 0.148±0.056 pmol mg^{-1} protein) but no change in AT_1R B_{max}. In WKY, L-NAME caused a significant increase in AT_1R B_{max} (0.17±0.03 pmol mg^{-1} protein $p<0.05$) but did not induce expression of AT_2R..

AT_1R but not AT_2R, were found in liver from both WKY and SHR, with WKY exhibiting significantly greater expression (B_{max} 0.33+0.02 and 0.15+0.01pmol mg^{-1} protein respectively; $P<0.01$). In SHR amlodipine significantly increased AT_1R B_{max} (0.18+0.01 pmol mg^{-1} protein; $p<0.05$) but there was no significant effect in WKY. L-NAME treatment of WKY significantly decreased AT_1R B_{max} (0.25+0.02 pmol mg^{-1} protein; $p<0.01$).

There were no significant effects of alteration of SBP on angiotensin receptor expression in membranes taken from either heart or colon nor were there any effects of treatment on K_D values for either angiotensin receptor in any tissue.

These results indicate that alterations of SBP may explain the observed differences in angiotensin receptor density in liver, and to some extent aortae, from WKY and SHR, but that they cannot explain the observed differences in receptor expression in heart and colon.

Bakhit, D. *et al.* (2002) *J. Pharm. Pharmacol.* 54 S103

163P INFLUENCE OF 5-HYDROXYTRYPTAMINE (5-HT) UPTAKE ON 5-HT POTENCY IN RAT PULMONARY AND MESENTERIC ARTERIES: EFFECTS OF EXPOSURE OF RATS TO HYPOXIA

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Uptake of 5-hydroxytryptamine (5-HT) via the specific 5-HT transporter occurs in pulmonary vascular endothelial and smooth muscle cells. In both cell types uptake is altered by hypoxia (Eddahibi *et al.*, 1999; Jeffery *et al.*, 2000). In the present study the influence of uptake on the vasoconstrictor potency of 5-HT in intralobar pulmonary arteries and mesenteric arteries is examined using citalopram, a selective inhibitor of the 5-HT transporter. Arteries were obtained from male Wistar rats (335±14 g, n=17) exposed to room air (normoxia) or intermittent hypoxia (10% oxygen; 8 h per day; 5 days). Third generation pulmonary and mesenteric arteries with endothelium intact or removed, were set up in Mulvany myographs (modified Krebs' solution; 37°C; 95%O_2/5%CO_2). Contractile responses were obtained to potassium (80 mM) and, after washout, to 5-HT in the absence or presence of citalopram (100 nM). Potency (pEC_{50}) values and maximal responses (as % potassium response) were obtained (n=4 -5).

In pulmonary arteries from normoxic rats (endothelium intact), 5-HT had a pEC_{50} of 5.4±0.1 and a maximum response of 59±2%. Citalopram significantly ($P<0.05$) increased the potency of 5-HT (pEC_{50}=6.0±0.2). Endothelium removal also increased potency (pEC_{50}=6.1±0.2: $P<0.05$) but, in denuded tissues, citalopram caused no further increase in potency (pEC_{50}=6.1±0.1; $P>0.05$). In hypoxic rats, endothelium-intact pulmonary arteries were supersensitive to 5-HT (pEC_{50}=6.3± 0.1) when compared with normoxic control data ($P<0.05$). Also, in pulmonary arteries from hypoxic rats, in contrast to findings in normoxic rats, (i) removal of the endothelium did not enhance the potency of 5-HT (pEC_{50}=6.5±0.1) and (ii) citalopram enhanced ($P<0.05$) the sensitivity to 5-HT not only in endothelium-intact tissues (pEC_{50}=7.0±0.1) but also in endothelium-denuded (pEC_{50}=6.9±0.1) tissues.

In mesenteric arteries 5-HT had a pEC_{50} of 6.9±0.1 and a maximum contraction of 113±8%. The presence of citalopram had no effect on the potency of 5-HT in control tissues, endothelium-denuded tissues or tissues from hypoxic animals. Similarly in mesenteric vessels neither hypoxia itself nor endothelium removal affected the potency of 5-HT. Maximal responses to 5-HT were not affected by hypoxia, citalopram or endothelium removal in pulmonary or mesenteric vessels.

In conclusion, the 5-HT uptake inhibitor, citalopram, increases pulmonary artery but not mesenteric artery sensitivity to 5-HT. This effect of citalopram in pulmonary arteries is endothelium-dependent in normoxic rats but endothelium-independent in hypoxic rats. The data are consistent with reports that 5-HT uptake is reduced by hypoxia in pulmonary vascular endothelium (Jeffery *et al.*, 2000) but enhanced by hypoxia in pulmonary vascular smooth muscle (Eddahibi *et al.*, 1999).

Eddahibi *et al.* (1999) *Circ. Res.* 84, 329-336.
Jeffery *et al.* (2000) *Eur. J. Pharmacol.* 396, 137-140.

Supported by the National Health and Medical Research Council of Australia (JCW, AG) and Wellcome Trust (RC-W)

164P VASOCONSTRICTOR RESPONSES TO THE ELASTIN PEPTIDE VGVAPG IN AGING HUMAN ARTERIES *IN VITRO*

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The breakdown of elastin, as a consequence of aging or disease, results in the liberation of soluble elastin peptides that have potential pathophysiological activity. In the vasculature, elastin peptides promote vascular smooth muscle cell proliferation (Wachi *et al.*, 1995), with endothelium-dependent vasorelaxation observed in rat aorta (Faury *et al.*, 1995). Our aim was to determine the vascular effect of the elastin peptide VGVAPG in human radial artery.

Radial artery (RA) was obtained, with ethical approval, from 10 patients (6 male, 4 female, 64±2 years old) receiving coronary artery bypass grafts. Rings (4mm) of RA were set up for isometric force recordings in 5ml tissue baths containing oxygenated Krebs' solution, at 37°C. Arterial rings were constricted with 10μM phenylephrine (PE) and endothelial integrity assessed by addition of 10μM acetylcholine (ACh). ACh elicited 83±6% relaxation of the PE response. Cumulative concentration-response curves were then constructed to VGVAPG (10^{-10}-10^{-6}M) in arteries pre-constricted with PE or in the absence of constrictor tone. In the latter experiments VGVAPG was compared to endothelin-1 (ET-1) and angiotensin-II (Ang II). Experiments were terminated by the addition of 50mM KCl and agonist responses expressed as a % of this KCl response. Data were analysed using the iterative curve-fitting program Fig P (Biosoft, UK) to obtain values of potency (pD_2±s.e.mean) and efficacy (E_{max}±s.e.mean). Data were compared using Student's

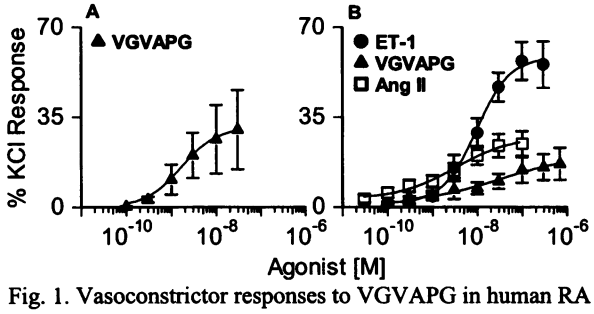

Fig. 1. Vasoconstrictor responses to VGVAPG in human RA two-tailed *t*-test, with significance at $P<0.05$. n-Values are the number of patients from whom arteries were obtained.

In contrast to rat aorta, VGVAPG elicited contraction of pre-constricted RA (Fig. 1A), with pD_2=8.43±0.33 and E_{max}=30.1±19.4% (n=3). The peptide also constricted 3 of 5 arteries tested in the absence of PE tone, with pD_2=7.73±0.12 and E_{max}=16.8±6.2% (n=3) (Fig. 1B). Compared to established vasoconstrictor peptides, VGVAPG was less potent than Ang II (pD_2 9.08±0.54, n=4) but equipotent to ET-1 (pD_2= 8.02±0.14, n=4). The maximum response to VGVAPG was not different from that to Ang-II (E_{max}=24.7±4.8%) but was significantly less than that to ET-1 (E_{max}=55.6±9.0%, $P<0.05$).

These data suggest that both the proliferative and vasoconstrictor properties of elastin breakdown peptides may contribute to age-related vascular remodelling and increased vascular tone observed in human arteries.

Faury, G. *et al.*, (1995). *J. Vasc. Res.*, 32, 112-119.
Wachi *et al.*, (1995). *FEBS Lett.*, 368, 215-219.

165P MOTILIN RECEPTORS IN THE HUMAN CARDIOVASCULAR SYSTEM

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Motilin was originally discovered in the gastrointestinal tract of animals where the 22 amino acid peptide affects smooth muscle tone. Motilin may also act centrally to modulate feeding behaviour. Recently, motilin has been paired with a previously designated orphan G-protein coupled receptor (GPR38, Feighner *et al.*, 1999). Motilin receptors have been characterised in the gastrointestinal tract of humans (Miller *et al.*, 2000) and in the human TE671 cell line of cerebellar origin (Thielemans *et al.*, 2001). However, receptors have not been studied in the human cardiovascular system. Our aim was to use [^{125}I]-motilin to quantify receptors in the human heart and visualise distribution within the vasculature.

Saturation binding experiments were carried out using cryostat sections (10μm) from surgical samples of left ventricle (n=4 individuals), with ethical approval. Following optimisation of the binding conditions, sections were pre-incubated for 15min in 50mM Hepes buffer, pH6.9 containing 5mM $MgCl_2$ and 0.3%BSA then incubated with increasing concentrations (15pM-8nM) of human [^{125}I]-motilin (Amersham Pharmacia Biotech) for 2 h. Unlabelled motilin (10μM) was used to determine non-specific binding within adjacent sections. Sections were washed for 3x5min in 50mM Tris-HCl, counted and analysed using the KELL suite of programmes (Biosoft, UK). For autoradiography, sections (n=4 individuals) were incubated with a fixed concentration of [^{125}I]-motilin (0.5 nM) using the same conditions and apposed to radiation sensitive film, with standards, for one week. Over the concentration range tested, [^{125}I]-motilin binding to ventricular tissue was saturable. The ligand bound with high affinity (2.2±0.9nM, mean±s.e.mean) with a density of 7.2±1.4fmol mg^{-1} protein. A one site fit was preferred to a two-site model using the F-test in the Ligand programme and Hill coefficients were close to unity (nH=1.1±0.1). [^{125}I]-motilin binding was localised using autoradiography to myocytes within the left ventricle. Binding was also detected to the smooth muscle layer of all vessels examined from a range of different vascular beds including large conduit arteries (main stem epicardial coronary, left internal mammary and intra-renal arteries) and veins (saphenous vein and vein grafts recovered following cardiac transplantation). The affinity of [^{125}I]-motilin binding in heart was comparable to the value for membrane fractions from human gastrointestinal tact (Miller *et al*, 2000) and the same as unlabelled motilin competing for the binding of the labelled analogue in cells expressing GPR38 (Feighner *et al.*, 1999). These results suggest that in humans, motilin receptors have a widespread distribution in the human vasculature, that may respond to motilin circulating in the plasma or released from the peripheral nervous system.

Feighner, S.D. *et al.* (1999). *Science,* 284, 2184-2188.
Miller, P. *et al.* (2000). *Am. J. Physiol. Gastrointest. Liver. Physiol.* 278, G18-23.
Thielemans, L. *et al.* (2001). *Brain Res.*, 895, 119-128.

166P VASCULAR ACTIONS OF ECSTASY: ROLES OF ADRENERGIC NEURONES, ENDOTHELIN, THROMBOXANE, 5HT, ANGIOTENSIN AND α_1-RECEPTORS

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3,4-methylenedioxymethamphetamine (Ecstasy) is a recreational drug of abuse, the use of which is increasing, and leading to increased numbers of fatalities. Ecstasy-related deaths are attributed to acute myocardial infarction as a result of its adverse cardiovascular effects (Dowling *et al.*, 1987). The aim of this study was to investigate the vascular actions of ecstasy and determine its possible mechanism of action.

Male Dunkin-Hartley guinea-pigs (250-350 g) were stunned and exsanguinated. The thoracic aorta was removed, cut into rings and suspended between wire hooks in an organ bath, as described previously (Ford & Broadley 1999). Following 1 h equilibration under 1 g tension, cumulative dose-response curves to ecstasy (3-300 μM), were obtained in the absence or presence of the uptake inhibitor cocaine (10 μM), or cocaine plus (a) the α_1 antagonist prazosin (1 μM), (b) the 5-HT_2 antagonist ketanserin (1 μM), (c) the endothelin ET_A/ET_B antagonist PD145065 (0.1 μM), (d) the thromboxane A_2 prostanoid (TP) antagonist ICI192605 (0.1 μM), or (e) the angiotensin AT_1 antagonist losartan (0.1 μM). These concentrations inhibited responses to the reference agonist. At the end of the experiment, tissues were contracted with 60 mM KCl and responses plotted as a % of this response. Contractions were recorded on a PowerLab/4SP computer system. Responses to 300 μM ecstasy are presented.

Ecstasy caused dose-dependent vascular contraction (300 μM ecstasy, 2.08±0.16g vs baseline 1.22+0.04 g: $P<0.05$),that was not inhibited or potentiated by 10 μM cocaine (1.85+0.19 g).

Likewise, cocaine in combination with prazosin, ketanserin, PD145065, ICI192605, or losartan did not influence the vasoconstrictor activity of ecstasy (Table 1).

	300 μM Ecstasy (% KCl response)
10 μM Cocaine	29.33±5.25
10 μM Cocaine + 1 μM Prazosin	26.55±9.55
10 μM Cocaine + 1 μM Ketanserin	26.95±6.06
10 μM Cocaine + 0.1 μM PD145065	32.53±5.35
10 μM Cocaine + 0.1 μM ICI192605	33.03±5.72
10 μM Cocaine + 0.1 μM Losartan	30.18±6.86

Table 1.The data represent aortic contraction to ecstasy. Values are mean+sem expressed as a % KCl response; n=6

Ecstasy is not an indirect sympathomimetic agent, as cocaine did not inhibit its vasoconstrictor actions. Likewise, ecstasy is not acting via an interaction with the α_1, 5-HT_2, ET_A/ET_B, TP or AT_1 receptors, or by mediating the release of catecholamines, 5-HT, endothelin-I, thromboxane A_2 or angiotensin-II, as none of these antagonists inhibited ecstasy-mediated vasoconstriction. The vasoconstrictor effects of ecstasy may therefore be the result of a direct interaction with a novel ecstasy binding site. The ability of ecstasy to cause vasoconstriction, suggests that it may contribute to the development of myocardial infarction via a direct vasoconstrictor action.

Supported by the British Heart Foundation

Dowling, G.P., *et al.* (1987). J.A.M.A. **257**, 1615-1617
Ford, W.R. & Broadley, K.J. (1999). *Gen. Pharmacol.* **33**, 143-150

167P RWJ-662733, A HISTAMINE H_3 RECEPTOR ANTAGONIST, DOES NOT AFFECT SYMPATHETIC NEUROTRANSMISSION IN THE PITHED RAT

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There is uncertainty regarding the ability of histamine H_3 receptors, located presynaptically on adrenergic nerve terminals innervating resistance vessels, to reduce neurally evoked pressor responses. For example, Malinowska & Schlicker (1991) reported that H_3 receptor activation reduces the neurogenic vasopressor response in pithed rats, whereas Hegde *et al.* (1994) found that H_3 receptors do not modify sympathetic transmitter output. The aim of the present study was to determine whether a novel H_3 receptor antagonist (4-[3-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-morpholine diHCl; RWJ-662733) affects sympathetic neurotransmission.

Male Wistar rats (306-454g) were anaesthetised with trichloroethylene and then pithed as described by Gillespie & Muir (1967). The trachea was cannulated and the animals were artificially ventilated with air and vagotomised. Blood pressure was measured from a cannulated carotid artery via a MacLab pressure transducer and recorder. Drugs were injected as a 0.1ml bolus, washed in with 0.2ml saline, via a cannulated femoral vein. The tip of the pithing rod was positioned at T4-6; electrical stimulation was for 15s at 0.25-4 Hz, 2ms pulse duration at 60 volts (Grass stimulator) with 3-5 min between successive stimuli. Pressor response curves were generated for a) electrical stimulation and b) norepinephrine (NE; 0.03-1μg i.v.) before and after a single dose of RWJ-662733 (10 $mgkg^{-1}$ i.v.): plasma drug concentration at 45 min = 0.98±0.15μM.

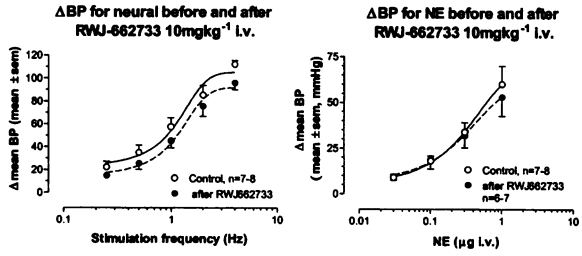

Figure 1. Pooled data showing pressor responses to neural stimulation and to injected NE before and in the period 5-45 min after a single injection of RWJ-662733. Basal mean BP averaged 42±2 before and 44±2 mm Hg 5 min after the H_3 antagonist. There was no significant difference in neural or NE responses obtained before and after RWJ-662733 ($P>0.05$, Kruskal-Wallis with Dunn's multiple comparison test).

Pressor responses were obtained for neural stimulation and, as a control, injection of NE in 8 experiments before and after injecting RWJ-662733 (see Fig.1), which itself caused a transient (2-3 min) rise in mean BP (23±3 mm Hg) and a fall in heart rate (-66 b.p.m; basal = 363 b.p.m.). There was no increase in vasopressor responses to nerve stimulation or NE after the antagonist, showing that RWJ-662733 does not affect sympathetic neurotransmission, and suggesting that H_3 receptors are not tonically inhibitory in this preparation.

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168P THE NOVEL CO RELEASING COMPOUND TRICARBONYLDICHLORORUTHENIUM (II) DIMER CONFERS PROTECTION FROM FREE RADICAL MEDIATED CYTOTOXICITY IN WKY 3M-22 CELL LINE AND PRIMARY RAT AORTIC SMOOTH MUSCLE CELLS

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Heme oxygenase (HO) metabolises heme to biliverdin, carbon monoxide (CO) and free iron (Maines et.al., 1982). CO can be an important messenger molecule, stimulating soluble guanylate cyclase, causing vasodilatation, reducing smooth muscle cell proliferation, and inhibiting platelet aggregation. Heme oxygenase induction is associated with protection from free radical mediated cytotoxicity, and it is possible that CO may be involved in this cytoprotection. A novel class of compounds (transition metal carbonyls) has been shown to release CO (Motterlini *et al.* 2002). Here we use one of these compounds, tricarbonyldichlororuthenium (II) dimer (TDRD), to investigate the effects of CO in preventing smooth muscle cell death induced by oxidative species.

Hydrogen peroxide (H2O2) is catalysed in the body by iron groups to form the hydroxyl radical, implicated in the pathophysiology of various states, including ischemia reperfusion injury, haemorrhagic shock, atherosclerosis, heart failure, acute hypertension and cancer. 3 morpholino-sydnonimine (SIN-1) is an NO donor that also produces equimolar amounts of superoxide anion (O2-). Together NO and O2- can generate peroxynitrite (ONOO-), a highly reactive and cytotoxic free radical species that is an extremely potent inducer of apoptosis.

WKY 3M-22 (Gordon et al., 1986; a gift from Dr. David Han, University of Washington, Seattle) and primary rat aortic smooth muscle cells (RASMCs) were grown to confluence in 96-well plates. Medium was then replaced with fresh serum-free medium, and the cells treated with TDRD (0-100μM) for 30 min before incubation with SIN-1 (3 mM) or H2O2 (1mM) for a further 16 h. The medium was again removed and the release of LDH determined.

TDRD caused a concentration dependent decrease in the cytotoxicity of SIN-1 and H_2O_2, in both WKY 3M-22 cells and primary RASMCs. The maximal protection was seen at 100μM and the cytotoxicity data at this concentration is shown below (Table 1).

	WKY 3M-22	Primary
SIN-1	66±6%	66±11%
SIN-1+100μM TDRD	35±4% *	22±7% *
H_2O_2	51±5%	54±7%
H_2O_2+100μM TDRD	16±3% *	21±10% *

Table 1. Cytotoxicity of SIN-1 in WKY 3M-22 and primary RASMCs in the presence or absence of TDRD(100μM). * = $p< 0.05$ (Anova plus Dunett's post test) compared to cells treated with SIN-1 only. (100% cytotoxicity =LDH release after treatment with 1% Triton-X 100)

Hence an increase in synthesis of CO by HO-1 could reduce the death of smooth muscle cells induced by endogenous NO and oxidative stress. HO-1 could therefore, aid plaque stability in atherosclerosis in which condition there is an increased production of free radical species and higher levels of smooth muscle cell death . There is also the possibility that these novel compounds could be used therapeutically in the treatment of vascular pathologies.

M.A.T. is the recipient of a British Heart Foundation Ph.D. studentship (FS/99058). D.B-B. holds a British Heart Foundation intermediate research fellowship (FS/99047).

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169P CYTOCHROME P450 2J2 OVER-EXPRESSION ACTIVATES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α

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Peroxisome proliferator-activated receptor (PPAR)-α is a fatty acid-activated nuclear receptor with roles regulating lipid metabolism, proliferation and inflammation (Bishop-Bailey, 2000). CYP2J2 is an abundant epoxygenase in human cardiovascular and pulmonary systems, whose metabolites are potent anti-inflammatory and vaso-dilatory mediators (Zeldin, 2000). We therefore investigated whether expression of CYP2J2 effected PPARα mediated transcriptional activation.

Human embryonic kidney cells (HEK)293 were maintained in DMEM supplemented with antibiotics/ anti-mycotics and 10% FCS (37°C; 5% CO2; 95% air). Cells were transfected with combinations (0.5μg of each) of pACO.luc PPAR luciferase reporter gene, CYP2J2, and mPPARα expression plasmids. Total transfected DNA was normalised using pcDNA. Promoter-less pGL2 basic and dominant negative PPARα (h6/29; a gift from Dr. Ruth Roberts; AstraZeneca) were used as respective controls for PPAR activation, and PPARα specificity. Plasmid details, transfections using NovaFector, and luciferase reporter gene activity normalised against protein, are described previously (Bishop-Bailey *et al.*, 1999 & 2002).

PPARα alone, but not CYP2J2, induced- PPAR transcriptional activation. PPARα combined with CYP2J2 synergistically activated PPAR responses. Little activation was seen with pGL2, while h6/29PPARα abolished PPARα, and PPARα + CYP2J2, mediated reporter-gene activation (figure1).

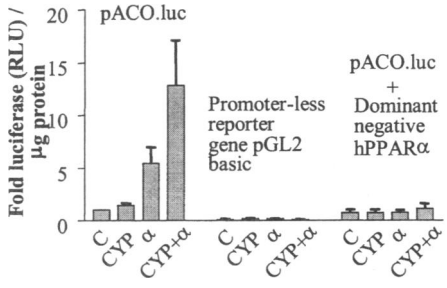

Figure 1.

Figure 1. CYP2J2 synergistically activates a PPARα mediated reporter gene (fold luciferase/μg protein). HEK293 were transfected with pACO.luc, pGL2, or pACO.luc with h6/29PPARα. Activation was measured in cells transfected with pcDNA control (C), PPARα (α), CYP2J2 (CYP), or CYP2J2 + PPARα. Data represents mean ± SEM of n=9-21 determinations from 7 experiments.

Over-expression of CYP2J2 activates PPARα. CYP2J2 may represent a novel endogenous pathway in the vascular and pulmonary systems for PPARα activation, and subsequent anti-inflammatory and anti-proliferative effects.

Funded by the William Harvey Medical Research Trust Foundation and the BHF (FS/99047)

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170P DIFFERENTIAL RESPONSIVENESS TO NORADRENALINE IN THE DISTAL COLON OF MAUDSLEY REACTIVE AND NON-REACTIVE INBRED RAT STRAINS

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Maudsley Reactive (MR) and Non-Reactive (MNRA) rats have been inbred for over 100 generations (Broadhurst, 1957; 1975), the selection criterion being high/low open field defaecation (OFD) rates. It has been suggested that the two strains may be models of the extremes of 'anxiety'-like behaviour. Amongst much behavioural data, some research has shown that tissue noradrenaline (NA) levels are higher in the colon of MNRA than MR rats (Blizard *et al.*, 1982). Blizard & colleagues have suggested that OFD differences between the strains may be due to differences in sympathetic transmission in the colon (Blizard & Adams, 2002) and also that the strains may constitute a model of irritable bowel syndrome (Blizard, pers. comm.). The aim of the present study was to determine whether the relaxant response to NA in the distal colon differed in MR and MNRA rats.

Distal colon segments from male Wistar, MR and MNRA rats (300-400 g) were suspended in oxygenated Krebs' buffer at 37°C in organ baths under isometric tension. One segment from each animal was incubated with nisoxetine (50 nM), a NA transporter (NAT) blocker. The maximal contractile response to KCl (KCl_{max}) was determined, before the tissues were tonically contracted with a submaximal (67 ± 3% [mean ± sem, n=26]) concentration of KCl and relaxed by cumulative application of NA (30 nM - 30 μM). Distal colon from four other rats of each inbred strain was processed, cut and stained for determination of the structure of the smooth muscle layers.

Two-way ANOVA indicated that strain ($P < 0.01$), but not nisoxetine, had a significant influence on KCl_{max}. Further analysis (by Student's t-test) indicated that in the presence (but not the absence) of nisoxetine, KCl_{max} was significantly lower in MNRA rats ($P < 0.05$) than in the other two strains.

NA produced concentration-dependent relaxation of KCl tone. In the absence of nisoxetine, MNRA colon had 7.8-fold lower sensitivity to NA than MR colon (mean EC_{50}s [range, n]: MNRA 1482 nM [163-4266, 5]; MR 191 nM [39.7-285, 4], $P < 0.0001$, 2-way ANOVA). Nisoxetine caused a 5.7-fold leftward shift in the NA concentration-response curve in MNRA rats ($P < 0.0001$, 2-way ANOVA), but was without effect in MR rats. In all respects, outbred Wistar rats were more similar to MR rats. Morphometry of the colon in the inbred strains showed that the longitudinal smooth muscle in MNRA rats was 59 ± 4% thinner than in MR rats ($P < 0.05$, Student's t-test, n=4).

The present results indicate that MNRA and MR rat distal colon have differential KCl and NA concentration-response relationships. The lower KCl_{max} in MNRA rats may be explained by there being significantly less muscle mass in that strain. The differential response to NA might be explained by increased *in vitro* sequestration of NA by the NAT in MNRA rats. The increased contractile response to KCl in MR rats fits with their behavioural trait of high OFD rates, but the differences in the NA response are at odds with a simple theory that increased NA release in MNRA rats leads to increased relaxation of the colon. *In vitro* preparations may not be the best model for the *in vivo* situation, where the input to the gut from the CNS is intact. Further work is therefore required to clarify whether differences in NA tissue content/release are important in mediating the contrasting OFD rates between these two strains.

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171P BLUNTED PHARMACOLOGICAL MODULATION OF 5-HT RELEASE FROM RAT MESENCEPHALIC NUCLEI AFTER PRIOR TISSUE 'RADIOLABELLING' INDICATES NON-PHYSIOLOGICAL AMINE ACCUMULATION

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In early studies of monoamine release from brain tissue (Baldessarini & Kopin, 1967), detection methods for *endogenous* amines were too insensitive to measure amine release. Therefore radiolabelling of the tissues was used, on the assumption that egress of the tritiated monoamine reflected that of the endogenous transmitter. The present study sought to determine the extent to which exogenous serotonin (5-HT), applied to mesencephalic slices in the manner used during a typical radiolabelling protocol (e.g. Moret & Briley, 1997), might be released from physiologically inappropriate loci.

Fast cyclic voltammetry at carbon fibre microelectrodes was used to determine the effects of prior tissue incubation with 5-HT on subsequent electrically-stimulated 5-HT efflux and reuptake in rat mesencephalic slices. Slices were incubated in artificial cerebrospinal fluid (aCSF) at 37°C for 30 minutes. In one group, the aCSF also contained 5-HT (100 nM) to label the tissue. The control group had no added 5-HT.

5-HT efflux and uptake were studied in the dorsal raphe nucleus (DRN), deep mesencephalic nucleus (DpMe), the oral part of the pontine reticular nucleus (PnO), the paralemniscal nucleus (PL), the ventromedial pontine nucleus (VMPn) and both dorsomedial and ventrolateral aspects of the periaqueductal grey (PAGdm & PAGvl). The effects of citalopram and 5-carboxamidotryptamine (5-CT) on 5-HT release was compared in DRN and PnO.

In the absence of prior labelling, stimulated 5-HT efflux (20 pulses, 0.1 ms, 10 mA100 Hz stimuli) was detectable only in DRN, PAGdm and, occasionally in PnO (1/5 slices). In all other areas, 5-HT efflux was below detection limits (5 nM).

Following incubation for 30 minutes in 5-HT (100 nM), stimulated 5-HT efflux was detectable also in DpMe, PL, VMPn and PAGvl. In such slices, the selective 5-HT reuptake inhibitor citalopram (75 nM) increased stimulated 5-HT efflux (20 pulses, 100 Hz trains) in DRN to 201 ± 21 % ($P < 0.05$ vs pre-drug values, t-test, n=5) but had no effect in PnO (112 ± 14%, n=4). Citalopram also slowed the reuptake of 5-HT, increasing the uptake half-time to 487 ± 117 % of pre-drug values ($P < 0.05$, n=4) in DRN, without significant effect in PnO. The 5-HT_1 agonist 5-CT (100 nM) decreased stimulated 5-HT efflux (10 pulses, 200 Hz trains) in DRN by 54 ± 6 % after 30 minutes ($P < 0.05$ vs pre-drug values, t-test, n=4) but was without effect (10 ± 14 %, n=3) in PnO.

Previous studies from this lab have shown that citalopram increases whilst 5-CT decreases stimulated *endogenous* 5-HT efflux in the DRN (Davidson, Hopwood & Stamford, unpublished). The present results show that, although prior labelling affects the magnitude of stimulated 5-HT efflux in DRN, it does not modify its pharmacology. However, pre-incubation in 5-HT unmasks 5-HT release in other regions of the mesencephalon without appreciable serotonergic innervation. In these areas (e.g. PnO), 5-HT efflux is apparently not influenced by 5-HT transporters or 5-HT_1 receptors, suggesting that its release is ectopic, an artefact of the pre-incubation process.

In summary, incubation of rat mesencephalic tissue in 5-HT, in the manner of a typical radiolabelling protocol, results in 5-HT release from non-physiological sites. The results of such transmitter release studies should thus be interpreted with some caution.

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172P PHARMACOLOGICAL PROFILE OF THE SYMPATHO-INHIBITORY EFFECTS OF MOXONIDINE AND RILMENIDINE: POSSIBLE INVOLVEMENT OF PREJUNCTIONAL IMIDAZOLINE I_1 RECEPTORS

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Intravenous (i.v.) infusion of moxonidine, rilmenidine and BHT933 (6-Ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d] azepin-2-amine dihydrochloride) elicited inhibition of the electrically-induced vasopressor responses in pithed rats, but not those induced by noradrenaline (Centurión *et al.*, 2002). This study has analysed the pharmacological profile of the sympatho-inhibition induced by moxonidine, rilmenidine and BHT933. For this purpose, male Wistar-Kyoto rats were pithed and blood pressure and heart rate were measured, as previously reported (Villalón *et al.*, 1998). Table 1 shows that moxonidine and BHT933 inhibited the electrically-induced vasopressor responses in animals treated with saline. Interestingly, cimetidine and efaroxan (I_1 receptor antagonists), but not rauwolscine (α_2-adrenoceptors antagonist), blocked the inhibition elicited by moxonidine. This pharmacological profile was also observed with rilmenidine (not shown). The above antagonists, except cimetidine, blocked the inhibition induced by BHT933. These results suggest that moxonidine and rilmenidine may activate a component insensitive to rauwolscine which could involve prejunctional I_1 receptors, whilst BHT933 activates prejunctional α_2-adrenoceptors.

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Table 1. Effect of various compounds (i.v.) on the inhibition of the electrically-induced (0.03-3 Hz) vasopressor responses (Δ mm Hg) induced by infusion of: (a) moxonidine (3 μg kg^{-1} min^{-1}) or (b) BHT933 (100 μg kg^{-1} min^{-1}) in pithed rats.

Compounds	n	Dose	0.03 Hz		0.1 Hz		0.3 Hz		1 Hz		3 Hz	
		(mg kg^{-1})	Before	After	Before	After	Before	After	Before	After	Before	After
(a) Moxonidine												
Saline[a]	6	-	12±2	7±2*	20±3	8±2*	33±6	14±4*	79±7	50±5*	106±8	94±6*
Rauwolscine	6	0.3	14±2	8±1*	25±4	10±2*	38±5	21±4*	92±6	65±7*	124±7	104±5*
Cimetidine	6	0.1	12±2	8±2	17±3	10±2*	30±3	17±5*	63±7	48±9	110±7	102±10
Efaroxan	6	0.1	18±3	16±3	22±3	23±2	38±5	37±5	87±6	82±2	123±7	125±9
(b) BHT933												
Saline[a]	6	-	17±4	12±3*	27±6	16±3*	41±6	25±5*	75±7	56±9*	127±4	99±9*
Rauwolscine	6	0.3	14±3	13±3	20±4	23±6	34±4	35±6	69±10	78±7	101±9	110±5
Cimetidine	6	0.1	13±1	10±2	21±3	15±4*	38±4	27±4*	69±3	60±4*	107±3	101±10
Efaroxan	6	0.1	14±2	10±1	25±4	14±3*	35±4	28±5	76±8	83±9	113±8	125±9

[a], 0.1 ml kg^{-1} , *, $P<0.05$ vs corresponding value before antagonists.

173P IDENTIFICATION OF A PUTATIVE IMIDAZOLINE-2 BINDING PROTEIN

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Imidazoline-2 (I_2) receptors or I_2 binding proteins are known to be involved in feeding and various brain disorders such as depression. Despite the intensive studies *in vivo* and *in vitro*, the molecular properties of these proteins remain elusive. Several biochemical techniques have isolated I_2 binding proteins of several sizes and it is now established that they exist in heterogeneous population with some evidence suggesting monoamine oxidase as one of them (for review see Eglen *et al.*, 1998). The aim of this study was to isolate and identify I_2 binding proteins from rabbit brain using a highly selective I_2 ligand, 2-(2-benzofuranyl)2-imidazoline (2BFI), to synthesise an affinity column.

Rabbit crude whole brain (New Zealand White, either sex, 2.5-5kg) P_2 membranes were prepared as described previously (Lione *et al.*, 1996). This preparation was solubilised in 0.5% CHAPS (Escribá *et al.*, 1999) in the presence of protease inhibitor cocktail for 2 hours on ice, constantly stirred. The mixture was then spun at 100,000g for 30 min. The supernatant containing solubilised I_2 binding proteins was loaded onto an affinity column prepared with 2BFI (PharmaLink, PerbioScience UK, Ltd). I_2 binding proteins were eluted with another I_2 ligand, idazoxan (20mM), and these were subjected to SDS-PAGE analysis. The proteins separated by SDS-PAGE were then transferred onto PVDF membranes and stained with Amido Black. I_2 binding proteins of relevant sizes were subjected to N-terminal sequencing by Edman degradation reaction.

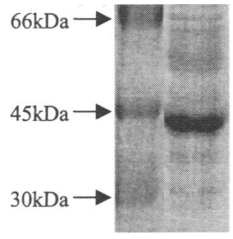

Figure 1. Solubilised I_2 binding proteins blotted onto PVDF membrane. These were isolated by 2BFI affinity column and separated by SDS-PAGE.
Left lane = Standard markers.
Right Lane = I_2 binding proteins.

Analysis revealed a strong band at around 45kDa (Figure 1). This and other proteins were then subjected to N-terminal sequencing. The first 10 amino acids were determined and the resulting peptide sequence was used to search for corresponding protein in the Protein Information Resources-International Protein Sequence Database. There was only one match for the 45kDa protein, rabbit brain creatine kinase (EC 2.7.3.2) (43kDa). The I_2 binding protein of around 45kDa has been isolated previously using other approaches (Escrib· *et al.*, 1999) but the identity remained unknown until now. This study has isolated a number of I_2 binding proteins using a 2BFI affinity column and identified one of them to be creatine kinase. Further studies are required to confirm the identification of the other protein bands and roles they play in various brain disorders.

This study and A. Kimura are supported by Yamanouchi UK Ltd. We thank Dr G. Kemp at University of St Andrews for his assistance with the protein sequencing.

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174P SOME RESIDUAL ALPHA-2-ADRENOCEPTOR MEDIATED INHIBITION OF SEROTONIN TURNOVER IN STRIATUM OF ALPHA-2A KNOCKOUT MICE

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Over the past few years we have been using genetically-manipulated mice strains to unravel the role of the sub-types of alpha-2-adrenoceptors. A strain of mice which do not express the alpha-2A-receptor (Altman et al. 1999), do not show the characteristic behavioural or neurochemical effects of alpha-2-receptor activation when challenged with non-subtype selective alpha-2-agonists like dexmedetomidine (DEX). In this study we have examined the alpha-2-mediated inhibition of serotonergic nerve activity in more detail to see if we could discern any residual alpha-2-effects.

Male alpha-2A knockout (KO) mice were used (n=29). An equivalent number of age and sex-matched C57BI/6J mice were used as wild-type controls. Since the experiments were conducted at different times, we only compare drug treatment to the saline control within each strain and not between strains. The animals were divided into 3 groups; controls (n = 10, saline s.c.); DEX 30 µg/kg s.c. (n=9) and DEX 100 µg/kg s.c. (n = 10). One hour later, the mice were sacrificed and frontal cortex, hippocampus, thalamus/hypothalamus and striatum dissected. The samples were stored at -80°C until assayed for brain biogenic amines and metabolites by HPLC-EC. In this presentation only the ratio of the concentrations of 5-hydroxyindoleacetic acid (5-HIAA) to serotonin (5-HT) is presented. This is more sensitive to DEX than either of the parameters alone. Statistical analysis was ANOVA followed by Dunnet's test with $P<0.05$ statistically significant.

In the wild-type mice, both doses of DEX caused major decreases in 5-HT turnover (reflected as a decrease in 5-HIAA/5-HT ratio) (Table 1). In the KO mice, the decrease in this ratio was virtually absent, only in striatum did it reach statistical significance. In other brain regions, the decrease was between 9% (cortex) and 13% (hippocampus, hypo/thalamus)

Table 1: Brain regional values for 5-HIAA/5-HT ratio 1h after DEX in alpha-2A-KO mice and their wild type controls

KO-mice	CORTEX	HIPPOCAMPUS	HYPO/ THALAMUS	STRIATUM
Saline	0.56±0.04	1.01±0.07	0.88 ±0.06	0.88±0.07
DEX 30 µg/kg	0.57± 0.02	1.00±0.05	0.84 ±0.05	0.77±0.03
DEX 100µg/kg	0.51±0.02	0.88±0.07	0.76 ±0.06	0.69±0.07*
F / p	1.0/0.37 NS	1.4/0.26 NS	1.5 /0.23 NS	3.84 / 0.03
Wild-type				
Saline	0.72±0.05	1.19±0.07	0.98 ±0.04	0.91±0.04
DEX 30 µg/kg	0.40±0.04**	0.62±0.03**	0.65 ±0.03**	0.57±0.03**
DEX 100µg/kg	0.31±0.03**	0.44±0.03**	0.48 ±0.02**	0.44±0.03**
F / p	26.7/ 0.001	70.2 / 0.001	86.9 / 0.001	48.9 / 0.001

Results are in units (± s.e. mean) * $P < 0.05$; ** $P < 0.01$ compared to saline treated mice from the same strain with Dunnet's test; NS = not significant in ANOVA

It is concluded that there may be some residual modulation of 5-HT turnover in striatum in alpha-2A-KO mice. Since this region contains a relatively high density of alpha-2C-receptors, it is possible that these receptors can modulate 5-HT turnover, though even in striatum, the major alpha-2-receptor involved in this process is the alpha-2A-subtype.

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

175P IDENTIFICATION OF DOPAMINE D_2 AUTORECEPTORS ON STRIATAL NEURONES OF BROILER CHICKENS

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A review of the welfare of poultry during stunning and slaughter has provoked renewed interest in the neurophysiology and pharmacology of chickens (Manteca, 1998). The presence of functional $\forall_2$-adrenoceptors on chick neurones has previously been reported by Boehm and Huck (1996). The present study has sought to determine the nature of the dopamine autoreceptor present within the chicken brain by examining the effect of a selective D_2 agonist quinpirole and the D_2 antagonist AJ76 ((1S,2R)-cis-5-methoxy-1-methyl-2-(N-propylamino)tetralin hydrochloride) on the *in vitro* release of [^{3}H]dopamine.

Striatal brain tissue of broiler chickens (Cobbs, either sex, 8 weeks old, 2.0-2.5 kg) was dissected *post mortem*, cross chopped (0.3 mm^3) and preloaded with [^{3}H]-dopamine (0.1 μM), in oxygenated Krebs for 30 min, 37°C (ascorbate, 0.4 mM). Tissue was placed in a superfusion apparatus (Harvard) and perfused (0.4 ml min^{-1}) with oxygenated Krebs and electrically stimulated (3 Hz, 20 mA, 2 msec width for 2 min) at three time points S_1, S_2 and S_3. The tissue was incubated with quinpirole, AJ76 or both quinpirole and AJ76, between t=36min and t=64min, the effect of which is apparent at S_2. The perfusate was collected in 4 min fractions and [^{3}H] content determined using liquid scintillation counting.

Electrical stimulation was able to evoke a transient release of [^{3}H]dopamine from the tissue (Figure 1) and in initial experiments the evoked release of [^{3}H]-dopamine was found to be Ca^{2+} dependent and tetrodotoxin sensitive. The D_2 agonist quinpirole significantly reduced the evoked release of [^{3}H]-dopamine (Fig 1, ANOVA F=4.965, P>0.05 n=5. Dunnett's post hoc test P=0.029) with no effect on basal release. This effect was reversible on removal of the drug (S_3, Figure 1). Following incubation with a D_2 dopamine antagonist AJ76, the affect of quinpirole was attenuated and S_2 fractional release did not differ significantly from controls (Figure 1).

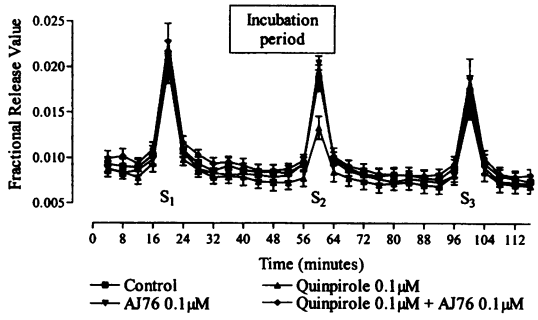

Figure 1. The effect of D_2 selective drugs on the release of [^{3}H]-dopamine. Data represent the mean ± s.e.mean (vertical bars) for 5 separate determinations performed in duplicate.

Therefore, these results indicate the presence of functional D_2 autoreceptors in chicken striatal tissue.

Gillian Jackson is a PhD student funded by Department of Environment, Food and Rural Affairs (DEFRA), UK.

Boehm, S. and Huck,S. (1996) *Eur. J.Neurosci.*, 8(9), 1924-1931.
Manteca X. (1998) *Meat Science.*, 49:S205-S218 Suppl.

176P MICE OVER-EXPRESSING THE 5-HT TRANSPORTER SHOW EVIDENCE OF INCREASED CENTRAL 5-HT_{2A} FUNCTION

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Allelic variation in the 5-HT transporter (5-HTT) gene leading to increased 5-HTT expression has been linked to vulnerability to mood disorder (Lesch and Mössner 1998) and the response of patients to antidepressant drugs (Smeraldi *et al.* 1998). At present the functional consequence of 5-HTT over-expression is unknown. This study assessed 5-HT function in a novel strain of mice genetically engineered to over-express the human 5-HTT. The induction of the immediate early gene c-fos by direct 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and indirect 3,4-methylenedioxymethamphetamine (MDMA, fenfluramine) acting agents was used as a marker of 5-HT function.

Wild-type and transgenic male CBAxC57/B16J mice (30-50 g) were injected s.c. with MDMA (5 mg/kg), fenfluramine (5 mg/kg), DOI (2 mg/kg) or saline. After 1 h brains were removed, snap frozen and stored (-70°C) prior to sectioning (12μm). c-Fos mRNA was measured by *in situ* hybridisation using oligonucleotide probes labelled with [^{35}S]-dATP (Pei et al. 2000). Abundance of c-fos mRNA was determined in 3 brain regions using an MCID image analysis system.

Fenfluramine, MDMA and DOI increased c-fos mRNA, an effect blocked by the 5-HT_{2A} antagonist, MDL100907 (0.3 mg/kg s.c.). These drugs also increased c-fos mRNA in transgenic and wild-type mice, but the magnitude in the transgenics was greater (Table 1). Repeated measures two way ANOVA showed a significant effect of region (p<0.001), treatment (p<0.001) and genotype (p<0.001). The effect of genotype was statistically significant in most regions.

Table 1. Fos expression in wild-type and transgenic mice after 5-HT drug challenges. Data are mean ± s.e.mean expressed as % of saline; n=5-6; *p< 0.05 vs wildtype, Student's t-test).

Brain region	Treatment	Fos mRNA Transgenic	Wild-type
Prelimbic	Fenfluramine	194.7±24.0*	122.6±16.2
cortex	MDMA	186.2±10.6	149.8±19.6
	DOI	188.2±14.9*	129.1±11.9
Cingulate	Fenfluramine	152.3±13.2*	123.6±10.4
cortex	MDMA	225.3±15.4*	134.2±10.4
	DOI	166.0±14.7*	115.4±6.8
Orbital	Fenfluramine	192.7±21.0	159.3±26.6
cortex	MDMA	285.4±21.2*	179.2±14.3
	DOI	194.5±19.0*	124.2±12.8

DOI - 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; MDMA - 3,4-methylenedioxymethamphetamine

These data indicate that compared to wild-type controls, mice over-expressing the human 5-HTT show enhanced 5-HT_{2A} receptor-mediated increases in cortical c-fos mRNA. We conclude that 5-HT transporter over-expression leads to enhanced central 5-HT_{2A} receptor function.

Lesch K. & Mössner R. (1998) *Biol. Psychiatry* **44**: 179-192.
Pei Q., *et al.* (2000). *Neuropharmacology* **39**: 463-470.
Smeraldi E., *et al.* (1998). *Molecular Psychiatry* **3**: 508-511.
Katie Jennings is funded by an MRC Priority Studentship

177P 5-HT$_7$ RECEPTORS INCREASE FIRING ACTIVITY IN THE CA3 REGION OF MOUSE HIPPOCAMPUS

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Even though the hippocampus contains a high density of 5-HT$_7$ receptor binding sites and mRNA (Gustafson *et al.*, 1996), the physiological consequences of 5-HT$_7$ receptor activation in this region have not been extensively studied. The 5-HT$_7$ receptor antagonist, SB 269970 has been shown to modulate firing activity in disinhibited slices of rat hippocampus (Gill *et al.*, 2002). To further investigate the role of the 5-HT$_7$ receptor in the hippocampal CA3 region, we have characterised the functional consequences of disrupting the 5-HT$_7$ receptor gene on spontaneous firing activity in disinhibited mouse brain slices.

Hippocampal slices (parasagittal, 350 μm thick) were prepared from female wild-type (WT) and 5-HT$_7$ knock out (KO) mice and maintained using methods as described previously (Seabrook *et al.*, 1997). All aspects of animal care, and use, were in accordance with the UK Animals (Scientific Procedures) Act 1986. Extracellular recordings were made from pyramidal cells of the CA3 sub-field. Drugs were introduced to the bath for 5-8 minutes by superfusion. Data are expressed as mean ± S.E.M. 100 and Student's *t*-test was used to test for significance; $P<0.05$ was considered to be significant.

Application of the GABA$_A$ receptor antagonist (-)-bicuculline methiodide (30 μM), consistently induced spontaneous firing activity in hippocampal CA3 neurones from WT (n=42 slices from 7 animals) and KO (n=42 slices from 7 animals) mice. Spontaneous firing activity (2.9 ± 1.7 Hz) was abolished by the selective 5-HT receptor agonist, 5-carboxyamidotryptamine (5-CT, 100 nM) in 62% of slices from WT mice (n=26). This effect was significantly reversed by WAY 100635 (1 μM) to 2.1 ± 0.3 Hz, consistent with activation of the 5-HT$_{1A}$ receptor subtype. In the presence of WAY 100635, 5-CT (100 nM) significantly increased firing activity in 6 of these slices from 2.2 ± 0.6 Hz to 3.5 ± 0.5 Hz. In the remaining 16 slices from WT mice (38%), 5-CT (100 nM) increased firing activity from 5.0 ± 1.0 Hz to 10.1 ± 2.1 Hz. This effect was reversed by SB 269970 (10 μM), consistent with activation of the 5-HT$_7$ receptor subtype. In the presence of SB 269970, 5-CT (100 nM) abolished firing activity in 10 of these slices from WT mice.

Spontaneous firing activity (6.2 ± 1 Hz) was abolished by 5-CT (100 nM) in 98% of slices from KO mice (n=41). This effect was reversed by WAY 100635 (1 μM) to 4.3 ± 0.7 Hz, consistent with activation of the 5-HT$_{1A}$ receptor subtype. In the remaining slice, 5-CT was without effect. In contrast to slices from WT mice, 5-CT did not increase firing activity in either the absence or presence of WAY 100635 in KO slices.

These studies confirm that activation of 5-HT receptors can either increase (5-HT$_7$) or decrease (5-HT$_{1A}$) spontaneous neuronal activity in the CA3 region of hippocampus.

Gill, C.H., *et al.* (2002) *Neuropharmacol.* **42**, 82-92.
Gustafson, E.L., *et al.* (1996) *Br.J.Pharmacol.* **117**, 657-666.
Seabrook, G.R., Easter, A., Dawson, G. (1997) *Neuropharmacol.* **36**, 823-830.

178P FROVATRIPTAN PREVENTS GTN-INDUCED INCREASES IN FIRING RATE OF NEURONES IN NUCLEUS TRIGEMINALIS CAUDALIS

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The nitric oxide donor glyceryl trinitrate (GTN) triggers migraine attacks in migraineurs (Thomsen et al., 1994) and we have previously shown that GTN potentiates evoked responses of neurones in rat nucleus trigeminalis caudalis (NTC) to electrical stimulation of the dura or facial skin (Jones et al., 2001). Here, we describe the GTN-evoked acceleration in the ongoing discharge of NTC neurones and test the ability of frovatriptan succinate, a 5-HT$_{1B/1D}$ receptor agonist antimigraine drug, to block this effect.

Experiments were performed in 43 male Wistar rats (200-250g) anaesthetised with urethane (1.5g/kg i.p.). A craniotomy and laminectomy were performed to expose the middle meningeal artery and superior sagittal sinus, and caudal brainstem and upper cervical cord respectively; exposed tissue was covered with mineral oil or agar solution. Extracellular recordings were made from spontaneously firing NTC neurones receiving input from craniovascular afferents. Receptive territories were established from responses to electrical (single or 500Hz twin pulse, 0.4msec, 0.1-2.0mA) and mechanical (fine polythene probe) stimulation of the exposed cerebral vasculature. Vehicle for all drugs was 0.9% saline. Vehicle or GTN, 100μg/kg/min, were infused into the common carotid artery for 15min. Drug or vehicle injections were given into the femoral vein 10 minutes prior to infusions.

To quantify the effect on firing of vehicle or frovatriptan injection (**Treatment**), mean rates over 10min pre- and post-treatment were compared. To measure the effect of GTN (**GTN**) after vehicle or frovatriptan, mean rates over 10min post-treatment and post-infusion were compared. Mean percentage changes (±sem) are given in the table (n=5 all groups).

		Frovatriptan (μg/kg)		
	Vehicle	**0.3**	**3**	**30**
Treatment	-1±6	+16±28	-2±6	-15±18
GTN	+43±8[a]	+50±23	-6±9[b]	+2±13[b]

In statistical tests, P values of less than 0.05 were considered significant. Subsequent to vehicle treatment, infusion of GTN, but not saline (n=4, data not shown), caused a significant change in firing ([a]Student's t-test). Significant block of the GTN-evoked firing rate increase was achieved at doses of 3 and 30μg/kg frovatriptan ([b]1-way ANOVA, Student Newman Keuls post-hoc test). Changes in rate due to vehicle treatment or frovatriptan alone were not significant (1-way ANOVA).

Thus, frovatriptan blocks the potentiating effect of the migraine-inducing agent GTN on trigeminovascular neurotransmission. Such activity may therefore be predictive of a therapeutic potential in this class of compounds.

Jones, M.G., Lever, I., Bingham, S. et al. (2001). *Cephalalgia*, **21**, 643-655.
Thomsen, L.L., Kruuse, C., Iversen, H.K. et al. (1994) *Eur. J. Neurol.*, **1**, 73-80.

179P THE 5-HT_{1A} PARTIAL AGONIST ANTIPSYCHOTIC DRUGS ZIPRASIDONE AND CLOZAPINE DOWN-REGULATE POST-SYNAPTIC 5-HT_{1A} RECEPTORS IN RAT BRAIN

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The receptor mechanisms underlying the clinical effects of the atypical antipsychotic drugs are far from understood. A few of these drugs have effects at 5-HT_{1A} receptors in addition to a variety of other receptor actions; such 5-HT_{1A} effects may well contribute to the efficacy of some of these drugs in ameliorating symptoms in schizophrenia. We have shown that clozapine and quetiapine are weak partial agonists at human 5-HT_{1A} receptors (Elliott and Reynolds, 1999); ziprasidone is also a partial agonist with higher affinity (Elliott and Reynolds, 2000).

We have investigated the consequences of sub-chronic antipsychotic drug administration on 5-HT_{1A} receptor density in rat hippocampus. Two experiments were undertaken with adult hooded Lister rats. In one ziprasidone (5mg/kg), clozapine (5mg/kg) or vehicle were administered i.p. by daily injection to groups of 6 males and 6 females for 26 days. In the second risperidone (1mg/kg), haloperidol (1mg/kg) or vehicle were administered i.p. daily to groups of 6 females for 21 days. One day after the last dose, brain tissue was removed following decapitation, dissected and frozen at –70°C.

5-HT1A receptor densities were determined by saturation analysis of specific binding of [^{3}H]WAY100635 to membrane preparations from hippocampus and (for the second series) frontal cortex, otherwise following the method of Elliott and Reynolds (1999). Data from each experiment were analysed by ANOVA followed by Dunnett's t-test.

The results are shown in table 1. No significant effect of risperidone or haloperidol on 5-HT_{1A} receptor density was found in hippocampus or frontal cortex. However significant deficits were found following ziprasidone and clozapine administration. No significant differences in Kd values or between sexes (in the first series) were observed.

Table 1. 5-HT_{1A} receptor densities following antipsychotic drug administration

	Hippocampus	Frontal cortex
Control I	19.7 ± 2.1	
Ziprasidone	15.6 ± 3.4*	
Clozapine	15.9 ± 2.2*	
Control II	22.0 ± 5.4	7.2 ± 1.8
Risperidone	22.1 ± 3.3	7.4 ± 4.0
Haloperidol	19.9 ± 1.8	6.8 ± 2.8

Data expressed as mean ± SD in pmol/g tissue.
*$p<0.01$ vs. Control I.

The data are consistent with a selective effect of the 5-HT_{1A} partial agonist antipsychotic drugs on this receptor. 5-HT_{1A} receptor down-regulation is a feature common to antidepressant drug treatment; in addition to indicating a possible mechanism underlying antidepressant effects of these drugs, the regulation of post-synaptic 5-HT_{1A} receptors controlling glutamatergic neuronal firing may have consequences on cognitive function.

Elliot J, Reynolds GP (1999) Eur J Pharmacol 386, 313-315
Elliot J, Reynolds GP (2000) Int J Neuropsychopharmacol 3 (Suppl 1), S131.

180P SB-656104-A, A NOVEL 5-HT_7 RECEPTOR ANTAGONIST, INHIBITS REM SLEEP IN RATS

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We have previously reported that the 5-HT_7 receptor-selective antagonist, SB-269970-A, inhibits rapid eye movement (REM) sleep in rats (Hagan *et al.*, 2000), suggesting a role for 5-HT_7 receptors in the modulation of sleep architecture. SB-269970-A is, however, not an ideal tool for *in vivo* studies due to rapid metabolism ($t_{1/2}$ < 0.5 h, Hagan et al., 2000). Structure-activity studies around SB-269970-A were therefore undertaken leading to the synthesis of SB-656104-A (6-((R)-2-{2-[4-(4-Chloro-phenoxy)-piperidin-1-yl]-ethyl}-pyrrolidine -1-sulfonyl)-1H-indole hydrochloride) predicted to show an improved pharmacokinetic profile. In the present study we have characterised SB-656104-A as a novel 5-HT_7 receptor antagonist *in vitro* and *in vivo* and investigated its effect on REM sleep in comparison with SB-269970-A.

Binding studies were carried out as described by Lovell et al. (2000) and pharmacokinetic and pharmacodynamic assays as described by Hagan *et al.* (2000). For sleep studies, SB-269970-A or SB-656104-A or (10 and 30 mg kg^{-1} i.p.) or vehicle (saline or 10% Captisol/saline respectively) were administered to rats (male, Sprague Dawley, 275-300g, n=8 per group) at the start of the sleep phase (circadian time (CT) = 0, 1400 h). Effects on REM and non-REM sleep parameters were assessed over the first 5 h of the physiological sleep period using continuous electroencephalogram and electrooculogram recordings.

SB-656104-A showed high affinity for the human 5-$HT_{7(a)}$ receptor (pK_i 8.7 ± 0.1) and at least 100-fold selectivity versus other human 5-HT receptor subtypes, apart from the 5-HT_{1D} (10-fold) and 5-HT_{2A} (30-fold) receptors. The compound competitively antagonised 5-CT-stimulated cAMP production in HEK293 cells stably expressing the human 5-$HT_{7(a)}$ receptor (pA_2 8.4 ± 0.2) and antagonised 5-CT-induced hypothermia in the guinea pig (ED_{50} 2.0 ± 0.01 mg/kg i.p.) a putative pharmacodynamic model of 5-HT_7 receptor interaction (Hagan *et al.*, 2000). Following i.p. administration to rats (10mg kg^{-1}), the compound was brain penetrant and displayed a plasma $t_{1/2}$ of 1.4 h. SB-656104-A, administered to rats at CT = 0 did not affect non-REM sleep, but produced a significant increase in latency to REM sleep at 30mg kg^{-1} i.p. (193 ± 29% vs vehicle, $p<0.01$, ANOVA) and a significant reduction in time spent in REM at both 10 and 30mg kg^{-1} i.p. (60 ± 8.8% and 35 ± 9.4% vs vehicle respectively, both $p<0.01$). In a parallel study, SB-269970-A significantly increased latency to REM sleep at 30mg kg^{-1} i.p. (202 ± 42% vs vehicle, $p<0.05$) and significantly reduced time spent in REM (53 ± 10% vs vehicle, $p<0.01$) but had no significant effect at 10mg kg^{-1} i.p. Interestingly, this selective REM inhibitory profile mimics that seen for the majority of antidepressant agents.

In conclusion, SB-656104-A is a novel selective 5-HT_7 receptor antagonist and this study provides further supportive evidence for a role for 5-HT_7 receptors in the control of sleep.

Lovell P.J. *et al.* (2000) *J. Med. Chem.* 43, 342-345.
Hagan J.J. *et al.* (2000). *Br. J. Pharmacol.*, 130, 539-548.

181P EFFECT OF ENDOGENOUS 5-HT ON [^{11}C]MDL 100907 BINDING INVESTIGATED IN RAT BRAIN USING PET

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Radioligand displacement by endogenous 5-HT offers a route to measure 5-HT release in the brain *in vivo* using positron emission tomography (PET). The PET ligand [^{11}C]MDL 100907, shows specific binding to central 5-HT$_{2A}$ receptors (Ito *et al.*, 1998). Here, small animal PET was used to test the effect of fenfluramine-evoked 5-HT release on [^{11}C]MDL 100907 binding in rat brain. Expression of immediate early genes (IEG) c-*fos* and *Arc* was used to demonstrate 5-HT$_{2A}$ receptor activation.

For PET studies, male Sprague-Dawley rats (280-300 g) were anaesthetised using isoflurane and injected i.v. with ~10 MBq of [^{11}C]MDL 100907, given either alone or ~30 min after fenfluramine (10 mg/kg i.p.); n=5 for each group. This dose of fenfluramine causes a 5-15 fold increase in brain extracellular 5-HT (Hume *et al.*, 2001). Each rat was scanned for 60 min in a quad-HIDAC tomograph (Jeavons *et al.*, 1999) and a 40 min time frame beginning 20 min after scan start was reconstructed into 0.5 mm cubic voxels. Using a volume of interest (VOI) template, 7 regions were sampled, including frontal cortex, hippocampus and cerebellum. Total:non-specific binding was estimated as VOI:cerebellum count ratio. For the IEG studies, groups of 4-6 anaesthetised rats were injected i.p. with vehicle, fenfluramine (10 mg/kg i.p.) or MDL 100907 (0.2 mg/kg i.v.) 5 min before fenfluramine (10 mg/kg i.p.). Rats were kept under anaesthesia for a further 60 min and then euthanased. Brains were rapidly snap-frozen, sectioned (12 μm) and then prepared for in situ hybridisation using [^{35}S]-labelled oligonucleotides complementary to mRNA encoding gene for c-*fos* and *Arc* (Pei *et al.*, 2000). Sections were exposed to β-max film for 3-7 days and mRNA abundance was measured in selected forebrain regions using a MCID image analysis system.

The level of specific binding (VOI:cerebellum ratio, mean ± SD) of [^{11}C]MDL 100907 was highest in frontal cortex (2.9 ± 0.2; n=5) and moderate in hippocampus (1.7 ± 0.1; n=5). Pre-treatment with fenfluramine did not alter specific binding in any of the regions sampled (Student's t-test). Compared with vehicle controls (mean ± s.e.m), fenfluramine significantly increased the abundance of c-*fos* mRNA in frontal (by 150 ± 35%; n=8), parietal (by 107 ± 25%; n=8) and piriform (by 56 ± 14%; n=4) cortex (One-way ANOVA with Bonferroni correction, P<0.05). This effect was blocked by MDL 100907. Similar data were obtained for *Arc* mRNA.

In summary, our data show that fenfluramine evoked a 5-HT$_{2A}$ receptor mediated increase in IEG expression but, under similar conditions did not alter [^{11}C]MDL 100907 binding. The lack of effect of fenfluramine on [^{11}C]MDL 100907 binding may be due to low affinity of 5-HT at 5-HT$_{2A}$ binding sites or the primarily intracellular localisation of the 5-HT$_{2A}$ receptors (Cornea-Herbert *et al.*, 1999).

Cornea-Herbert et al., (1999) J. Comp. Neurol., 409, 187-209.
Hume S et al., (2001) Synapse, 41, 150-159.
Ito H et al., (1998) J. Nucl. Med., 39, 208-214.
Jeavons AP et al., (1999) IEEE Trans. Nucl. Sci., 46, 468-473.
Pei Q et al., (1997). Neuropharmacology 39, 463-470.

182P A TYROSINE-FREE AMINO ACID MIXTURE ATTENUATES AMPHETAMINE-INDUCED DISPLACEMENT OF [^{11}C]RACLOPRIDE IN STRIATUM: A RAT PET STUDY

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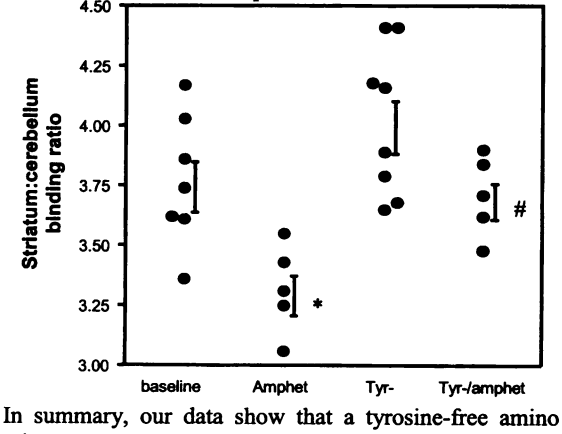
Tyrosine depletion using a tyrosine-free amino acid mixture offers promise as a treatment for acute mania through decreasing dopaminergic neurotransmission (McTavish et al. 2001). In rats, tyrosine depletion attenuates the dopamine-releasing and psychostimulant properties of amphetamine (McTavish et al. 1999). Here, positron emission tomography (PET) was used to investigate the effect of tyrosine depletion on amphetamine-induced [^{11}C]raclopride displacement.

Male, Sprague Dawley rats (290-310 g), were anaesthetised with isoflurane and nitrous oxide:oxygen and scanned using a quadHIDAC scanner for 60 min following i.v. injection of 10 MBq [^{11}C]raclopride (specific activity 44±25 MBq/nmol). Prior to scanning, rats received one of the following treatments: saline, amphetamine (2 mg/kg i.p.), a tyrosine-free amino acid mixture (1 g/kg i.p. ; see McTavish et al. 1999), or the same mixture followed by amphetamine. Data were reconstructed into 3D images, and ANALYZE software was used to calculate volume of interest (VOI) data (Hume et al. 2001). VOI:cerebellum ratios were calculated for the period 20-60 min after injection of [^{11}C]raclopride. Data were analysed statistically using one way ANOVA followed by Bonferroni's multiple comparison test. Fig. 1 shows the striatum:cerebellum ratios for each rat. Amphetamine caused a 16 ± 5 % (mean ± SD) reduction in [^{11}C]raclopride binding ratio compared to saline controls. Whereas the tyrosine-free amino acid mixture had no significant effect alone, the treatment reduced the effect of amphetamine.

Fig. 1. Effect of amphetamine and a tyrosine-free amino acid mixture (Tyr-) on striatal [^{11}C]raclopride binding in the rat. Vertical bars are s.e.mean values; n=5-8. *P< 0.05 vs. baseline, #P<0.001 vs. amphetamine.



In summary, our data show that a tyrosine-free amino acid mixture attenuates amphetamine-induced displacement of [^{11}C]raclopride in striatum *in vivo*. These data are in keeping with neurochemical studies showing that the same mixture decreases amphetamine-evoked dopamine release (McTavish et al. 1999) and serve as a platform for future PET studies testing tyrosine depleting paradigms in humans.

Hume S. et al. (2001) *Synapse* **41**: 150-9.
McTavish S.F. et al. (1999) *Psychopharmacology* **141**: 182-188.
McTavish S. F. et al. (2001) *Br J Psychiatry* **179**: 356-360.

183P INHIBITION OF THE HUMAN TWO-PORE DOMAIN K^+ CHANNEL TREK-1 (hTREK-1) BY FLUOXETINE

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Fluoxetine, a widely prescribed selective serotonin reuptake inhibitor, has been shown to inhibit a variety of voltage-gated K^+ channel currents in native and recombinant systems, including $K_V1.1$ and $K_V1.3$ (Yeung *et al.*, 1999, Choi *et al.*, 1999). In this study, we ask whether fluoxetine can also inhibit a member of the novel two-pore domain background K^+ channel superfamily, hTREK-1 (KCNK2).

TREK-1 currents were measured using whole-cell and outside-out patch-clamp recordings from stably transfected HEK-293 cells at room temperature (see Meadows *et al.*, 2000). Cells were subjected to step and ramp voltage protocols, normally in the range –120 to +60 mV. On perfusion with 1 - 100 μM fluoxetine, whole-cell TREK-1 currents, evoked by a ramp voltage protocol, were inhibited in a concentration-dependent manner, with an IC_{50} of 14.0 μM and a predicted maximal inhibition close to 100%. TREK-1 current inhibition was observed at all voltages from –120 to +60 mV. Although TREK-1 is a background or leak K^+ current, it does show some voltage-dependence, the degree of which depends upon the phosphorylation state of the channel (see Bockenhauer *et al.*, 2001). The whole-cell TREK-1 current at +60 mV can be divided into an instantaneous current and an evoked current (the former measured 0.5 ms after the step to +60 mV). In whole-cell recordings the instantaneous current was only 58 ± 8% (mean ± S.E.M., n = 8) of the total current. However, in outside-out macropatches all of the current activates instantaneously.

Interestingly, fluoxetine was found to be much less effective at inhibiting TREK-1 currents in outside-out macropatch recordings compared to whole-cell recordings. In a series of alternating experiments, fluoxetine (10 μM) inhibited the sustained whole-cell current at +60 mV by 58 ± 8 % (n = 8) but inhibited the sustained current in outside-out macropatches by only 11 ± 7 % (n = 8). While TREK-1 currents in outside-out patches have a larger instantaneous component than that in whole-cell currents, this probably cannot explain the differential effect of fluoxetine, since fluoxetine was equally effective at inhibiting both the instantaneous (47 ± 8 %, n = 8) and the sustained (58 ± 8 %, n = 8) current components in whole-cell recordings.

Given fluoxetine's therapeutic plasma level (0.5 - 1.5 μM) and the much higher levels that can accumulate in the brain and that can be attained during chronic treatment (Karson *et al.*, 1993), block of TREK-1 leak channels may be of clinical significance and may be linked to the convulsions which are sometimes observed following fluoxetine overdose.

Supported by the MRC. hTREK1 was kindly supplied by GSK.

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184P DO LOW CONCENTRATIONS OF HIGH EFFICACY μ-OPIOID AGONISTS REVEAL DESENSITISATION AND INTERNALISATION OF μ-OPIOID RECEPTORS BY MORPHINE?

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μ-Opioid receptors (MOR) exhibit rapid desensitisation and internalisation on exposure to agonists such as Met-enkephalin (ME) and DAMGO. Morphine, however, induces neither rapid desensitisation nor internalisation (Alvarez *et al.*, 2002). A recent study in HEK293 cells stably transfected with FLAG-tagged MOR (He *et al.*, 2002) described MOR internalisation when morphine (5 μM) was combined with a low concentration of DAMGO (100 nM) that did not itself induce internalisation. In the present study, we have examined MOR desensitisation in rat locus coeruleus (LC) neurones and MOR internalisation in HEK293 cells.

Whole cell patch clamp recordings were made from visually identified LC neurones in 200 μm thick brain slices prepared from male Wistar rats (120-150g) as described previously (Fiorillo & Williams, 1996). HEK293 cells stably expressing MOR1 or MOR1B splice variants (Koch *et al.*, 1998) were created. Internalisation of surface receptor was quantified by an immunosorbent assay (ELISA; Daunt *et al.*, 1997), making use of a T7 epitope tag inserted into the extracellular domain of the rat MOR1 and MOR1B. Statistics were performed using Student's t-test. $P<0.05$ was deemed significant. Data are given as mean ± S.E.M.

In LC neurones held at –60mV, a saturating concentration of ME (30 μM) evoked an outward potassium current (202 ± 16 pA; n=7) that rapidly desensitised. The amplitude of the evoked current after 7 min of ME exposure was 49 ± 3% (n=7) that of the initial peak current. In contrast, a saturating concentration of morphine (30 μM) that evoked a current of 138 ± 10 pA; n=5) produced little or no desensitisation, the amplitude of the response after 7 min of morphine exposure was 92 ± 3 % (n=5) that of the peak response. Co-perfusion of DAMGO (100 or 500 nM) and morphine (5 or 30 μM) for up to 30 min did not increase desensitisation compared with morphine alone (Table 1).

Exposure of HEK293 cells to ME or DAMGO for 10 min caused rapid internalisation of both MOR1 and MOR1B. With ME (30 μM) the level of MOR1 remaining on the plasma membrane was 48 ± 4% and MOR1B was 64 ± 14% of control (n=4 for both) and with DAMGO (10 μM) the level of MOR1 remaining on the plasma membrane was 72±3% and MOR1B was 77 ± 1% (n=7 for both). On the other hand, morphine (30μM) produced very little receptor internalisation (amount remaining after 30 min for MOR1 was 92 ± 8%; and for MOR1B was 89 ± 2% of control, n=5). Co-application of DAMGO (100 or 500 nM) and morphine (30μM) for 30 min did not increase morphine-induced internalisation (Table 1).

Table 1

	Desensitisation	Internalisation	
	LC	MOR1	MOR1B
500 nM DAMGO+ 30 μM morphine	105 ± 5%	101 ± 8%	106 ± 13%
100 nM DAMGO+ 5 μM morphine	102 ± 6%	92 ± 7%	99 ± 10%

Table 1 shows desensitisation or internalisation of MOR following DAMGO/morphine treatment as a percentage of morphine alone. n=3-6

These data provide no evidence to support the notion that low concentrations of high efficacy μ receptor agonists can 'reveal' μ receptor desensitisation in mature neurones and internalisation of recombinant receptors by morphine.

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185P *IN VIVO* MODULATION OF [^{35}S]TBPS BINDING IN MOUSE BRAIN

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$GABA_A$ (γ-aminobutyric acid) receptors are inhibitory neurotransmitter gated ion channels which are pentameric structures formed from combinations of α1–6, β1-3, γ1-3, δ, θ, π and ε subunits. The radioligand [^{35}S]TBPS (t-butylbicyclophosphorothionate) binds to the convulsant site located in the channel pore of the $GABA_A$ receptor. [^{35}S]TBPS binding can be modified *in vitro* by a variety of compounds acting at the $GABA_A$ receptor, such as the benzodiazepine diazepam, the convulsant pentylenetetrazole (PTZ) and the anaesthetic etomidate (Im *et al.*, 1994; Zezula *et al.*, 1995).

The aim of this study was to determine if [^{35}S]TBPS binding could be modulated by $GABA_A$ receptor ligands *in vivo* using an *in vivo* binding receptor occupancy assay (Atack *et al.*, 1999). The *in vivo* receptor occupancy assay measured total bound [^{35}S]TBPS to the $GABA_A$ receptor. [^{35}S]TBPS (NEN, specific activity approx. 200 Ci/mmol) was administered i.v. to male Swiss-webster mice (26-30 g) (5 μl/g of a 150 μCi/ml saline solution). To determine if [^{35}S]TBPS binding could be modulated *in vivo*, mice were pretreated for 30 min with diazepam (30 mg/kg in 0.5% methocellulose (M.C.), i.p.), a dose that will occupy 100% of $GABA_A$ receptors; for 5 min with etomidate (Hypnomidate®, Janssen-Cilag) (30 mg/kg in 35% polyethyleneglycol (PEG) 300, i.p.) and for 30 min with PTZ (10 mg/kg and 50 mg/kg in 0.5% M.C. s.c.) the maximum sub-convulsant dose. An additional *in vivo* receptor occupancy assay was performed using doses of etomidate from 0.3–30 mg/kg in 35% PEG 300 i.p. with a pretreatment time of 5 min. [^{35}S]TBPS was administered i.v. 1 min before culling (a time which gave optimal brain [^{35}S]TBPS radioactivity in a separate time course experiment). Whole brains were removed, weighed, homogenised in 10 vols of ice cold buffer (50 mM Tris citrate/0.2 M NaBr buffer, pH 7.4) and aliquots (300 μl) were filtered over Whatman GF/B filters, washed with 10 ml ice cold buffer, placed in vials with scintillation fluid and radioactivity measured using a scintillation counter. Data was shown as the mean % of vehicle ± S.E. Statistical differences were analysed using an ANOVA with a Dunnetts post test.

Maximal [^{35}S]TBPS binding to $GABA_A$ receptors in mouse brain was observed 0.5-1 min after i.v. injection (n=5). Diazepam and etomidate significantly inhibited [^{35}S]TBPS binding by 39 ± 6% (n=6) and 70 ± 11% (n=6) respectively compared to vehicle group (P<0.01). PTZ at 10 and 50 mg/kg inhibited [^{35}S]TBPS binding by 21 ± 2% (n=6) and 41 ± 4% compared to vehicle groups (P<0.01; n=6). A dose dependent inhibition of [^{35}S]TBPS binding by etomidate was also demonstrated with a significant decrease in [^{35}S]TBPS binding between vehicle and 3 and 30 mg/kg etomidate of 48 ± 6% and 82 ± 9% respectively (P<0.01; n=5).

In this study, we have demonstrated that [^{35}S]TBPS can be used to measure the *in vivo* binding of a variety of classes of compounds acting at various sites on the $GABA_A$ receptor.

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186P $GABA_A$ RECEPTOR BLOCKADE POTENTIATES THE INHIBITORY NEUROMODULATION BY ADENOSINE IN THE HIPPOCAMPUS

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Adenosine and GABA are major inhibitory neuromodulators/ neurotransmitters in the CNS. Activation of inhibitory adenosine A_1 receptors potentiates activation of $GABA_A$ receptors in the hippocampus (Akhondzadeh & Stone, 1994), suggesting that adenosine A_1 receptors control GABAergic responses. In the present study, we evaluated whether endogenous GABA could influence the inhibitory action of adenosine in the hippocampus.

Field-excitatory post-synaptic potentials (fEPSPs) were recorded from the CA1 area of hippocampal slices from male Wistar rats (5-7 weeks old). The slices were kept under continuous perfusion (3ml/min) with gassed (95% O_2/5% CO_2) Krebs solution (mM: NaCl 124; KCl 3; NaH_2PO_4 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; $CaCl_2$ 2; glucose 10). Responses were evoked by stimulation (rectangular 0.1 ms pulses, once every 15 s) of the Schaffer collateral/commissural fibres, through a concentric bipolar electrode. fEPSPs were recorded through a microelectrode (4M NaCl, 3-5 MΩ) placed in *stratum radiatum*. Two consecutive cumulative concentration-response curves for the adenosine stable analogue 2-chloroadenosine (CADO 0.15-1 μM), causing a reduction in fEPSP, were performed in the absence (1st curve) and in the presence (2nd curve) of a selective $GABA_A$ antagonist (bicuculline 10 μM) or a selective $GABA_B$ antagonist (CGP 55845 1 μM). Control experiments without GABA receptor antagonists were also performed. In all experiments, construction of the two curves was separated by 60 minutes. Values are given as mean pIC_{50} ± S.E.M. from n experiments. Significant differences were taken at P<0.05 (unpaired Student's t-test).

CADO (0.15 - 1 μM) caused a concentration dependent reduction in the fEPSP slope, with a pIC_{50} value of 6.400 ± 0.065 (n=6), corresponding to an IC_{50} of 398 nM. When the same slices were superfused with bicuculline (10 μM), the concentration-response curve for CADO was displaced to the left by a factor of 2, the pIC_{50} value being changed to 6.677 ± 0.076 ⇒ IC_{50}=210nM (P<0.05, n=6). In contrast, superfusion of slices with CGP 55845 (1 μM) did not significantly alter the concentration-response curve for CADO (P>0.05, n=5). When two consecutive concentration-response curves for CADO were performed, in control conditions, the second and the first applications of CADO caused similar inhibition of the fEPSPs (P>0.05, n=4).

The present results show that the blockade of $GABA_A$ receptors (but not $GABA_B$ receptors) potentiates the adenosine A_1 receptor mediated inhibition of synaptic transmission. These findings, together with previous evidence that adenosine potentiates activation of $GABA_A$ receptors (Akhondzadeh & Stone, 1994), suggest a potential regulatory loop between these two inhibitory neuromodulators/neurotransmitters, in which adenosine potentiates $GABA_A$ activity, and is in turn inhibited by endogenous GABA, through $GABA_A$ receptors.

Work supported by FCT.
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187P BRAIN DERIVED NEUROTROPHIC FACTOR FACILITATION OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS IS DEPENDENT ON A_{2A} ADENOSINE RECEPTOR ACTIVATION

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Brain derived neurotrophic factor (BDNF) is expressed at high levels in the hippocampus and plays an important role in neuronal survival, differentiation and in plasticity through activation of TrkB receptors. In the hippocampus BDNF has been reported to enhance excitatory synaptic transmission (Kang & Schuman, 1995) but this has not been consistently observed (Frerking, *et al.*, 1998). At the developing neuromuscular junction the excitatory action of BDNF on synaptic transmission is facilitated by pre-depolarisation (Boulanger & Poo, 1999). Activation of TrkB in hippocampal neurones might be under control of A_{2A} adenosine receptors (Lee & Chao, 2001), which finely tune neurotransmission (Sebastião & Ribeiro, 2000). We have now investigated whether the action of BDNF on hippocampal synaptic transmission is facilitated by pre-depolarisation and the activation of A_{2A} adenosine receptors in this mechanism.

Field-excitatory post-synaptic potentials (fEPSP) were recorded from the CA1 area of hippocampal slices of male Wistar rats (3-4 weeks old). The slices were kept under continuous perfusion (3ml/min) with gassed (95% O_2 / 5% CO_2) Krebs solution (mM:NaCl 124; KCl 3; NaH_2PO_4 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; $CaCl_2$; glucose 10). Responses were evoked by stimulation (rectangular 0.1 ms pulses, once every 15 s) of the the Schaffer collateral/commissural fibres through a concentric bipolar electrode. fEPSPs were recorded through a microelectrode (4M NaCl, 3-5 MΩ) placed in *stratum radiatum*.

BDNF (20ng/ml) increased fEPSP slope (41 ± 9.8% increase after 46 min, n=9, $P<0.05$, Students' t test) in slices that had been briefly depolarised by a 2 min pulse of high K^+ (10 mM), 46 min before application of BDNF. The Trk receptor inhibitor, K252a (200nM, added 30 min before the high K^+ pulse) prevented this BDNF effect (n=3, $P<0.05$). Without the pre-depolarising pulse BDNF (20ng/ml) was virtually devoid of excitatory action (% change in slope: 11 ± 10%, n=5, $P>0.05$) on fEPSPs. In the presence of the A_{2A} adenosine receptor agonist, 2-[p-(2-carbonyl-ethyl)-phenylethylamino]-5′N-ethylcarboxamidoadenosine (CGS 21680, 10 nM), BDNF increased the slope of fEPSP by 36 ± 8.6% (n=4, $P<0.05$) even in slices that had not been predepolarised by the high K^+ pulse. Blockade of A_{2A} adenosine receptor by the selective antagonist, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl) phenol (ZM 241385, 50 nM, added 30 min before BDNF), prevented ($P<0.05$) the excitatory effects of BDNF in conditions of pre-depolarisation (n=3), as well as in the presence of CGS 21680 (n=3).

The results suggest that the excitatory action of BDNF on synaptic transmission in the hippocampus is dependent on A_{2A} adenosine receptor activation and that this might be achieved by a brief depolarisation, which induces release of endogenous adenosine. Supported by FCT, Portugal. BDNF was a kind gift from Regeneron

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188P TOPIRAMATE MODULATES L-TYPE CALCIUM CURRENTS IN RAT OLFACTORY CORTICAL NEURONES *IN VITRO*

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Topiramate (TPM) is a structurally novel 'newer generation' anticonvulsant drug, that is effective in a variety of animal seizure models (Shank *et al.*, 1994) and is currently used as an adjunctive therapy for patients with refractory partial and secondarily generalized seizures (see Reife *et al.*, 2000). Although its effects on voltage-activated Na^+, Ca^{2+}, as well as $GABA_A$ and AMPA/KAI ligand-gated ion channels have already been described (Shank *et. al.*, 2000), the relative importance of these actions in accounting for the anticonvulsant activity of TPM remains unclear. We have previously reported that TPM enhances and prolongs the slow afterhyperpolarization (sAHP) that follows a burst of action potentials in olfactory cortical neurones *in vitro* (Russo & Constanti, 2002). In the present study, we wished to examine the mechanism underlying this novel effect of TPM under voltage clamp conditions. Transverse brain slices of olfactory cortex were prepared from 160-200g Wistar rats (either sex) as previously described (Constanti *et al.*, 1993) and intracellular recordings made from neurones in the deep piriform cell layer II-III using 4M K acetate (60-80 MΩ) or 3M Cs acetate (50-70 MΩ)-filled microelectrodes connected to an Axoclamp 2A amplifier. Data are presented as mean ± S.E.M.

Cells were routinely voltage clamped at -70 mV and stepped to -20 mV for 1.5s to observe outward current relaxations (in the presence of 1 μM TTX to block fast Na^+ conductances). In 20 K acetate-recorded cells, this protocol evoked a slow outward relaxation (1.77 ± 0.7 nA peak amplitude) and slow outward tail current (I_{AHP}) (115 ± 23 pA peak, 3.37 ± 0.64 s duration), largely due to activation/decay of a slow Ca^{2+}-activated K^+ conductance. In TPM (20 μM, 20 min; n=17) this outward relaxation was increased in amplitude (2.23 ± 0.9 nA), with an enhancement (153 ± 34 pA) and dramatic prolongation (7.83 ± 1.32 s) of the I_{AHP} tail (means significantly different from control; $P<0.05$; Student's t-test). On further adding Cd^{2+} (200 μM; n=15), both enhanced components were abolished confirming their Ca^{2+}-dependence. In 22 Cs acetate-loaded cells (~30 min, to minimise outward currents) TPM still enhanced the residual outward current relaxation (1.84 ± 0.48 nA *vs* 1.56 ± 0.42 nA amplitude: control), but now, an enhanced and prolonged slow *inward* after tail current was revealed (124 ± 47 pA amplitude, 7.52 ± 1.15 s duration *vs* 114 ± 52 pA amplitude, 3.18 ± 0.32 s duration:control) most likely due to turn-off of an enhanced high voltage-activated Ca^{2+} current. Accordingly, both the TPM-induced outward relaxation component and inward tail current were eliminated by further adding Cd^{2+} (200 μM; n=17) or nifedipine (20 μM; n=6), indicating their dependence on Ca^{2+} entry through L-type channels. In conclusion, we propose that the observed enhancement/prolongation of the sAHP by TPM might be largely explained by an *enhancement* of an underlying L-type Ca^{2+} current. It is interesting to speculate that such a novel effect on the sAHP may contribute to the potent anticonvulsant activity of TPM in the brain.

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Supported by Johnson & Johnson Pharmaceutical Research & Development LCC (USA), who also kindly supplied topiramate.

189P MUSCARINIC MODULATION OF $GABA_A$ RECEPTOR-MEDIATED IPSPS IN ADULT AND IMMATURE RAT OLFACTORY CORTEX *IN VITRO*

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Assimilation of odour responses into memory by the piriform cortex (PC) is subject to differential presynaptic muscarinic acetylcholine receptor (mAChR) modulation at the level of the lateral olfactory tract (LOT) and intrinsic fibre excitatory nerve terminals. Thus, mAChR activation suppresses intrinsically-evoked EPSPs, but not those elicited by LOT stimulation (Hasselmo *et al.*, 1990). In adult rat PC, this intrinsic suppression is M_1-receptor mediated, whereas in immature (P13 to P24) animals it is M_2-receptor mediated, and less efficient (Whalley & Constanti, 2002). In the present study we investigated whether inhibitory synaptic transmission in the PC was also susceptible to mAChR modulation. PC brain slices were prepared from male and female adult (P>40) or immature Wistar rats as previously described (Postlethwaite *et al.*, 1998). Stable intracellular recordings were made from presumed deep (layer III) pyramidal neurones with 2 M KAc-filled microelectrodes (50-80 MΩ). To isolate IPSPs, 10 μM CNQX + 100 μM DL-APV + 1 μM CGP-52432 were used to suppress glutamatergic and $GABA_B$ receptor-mediated responses respectively. The mAChR agonist, oxotremorine-M (OXO-M; 10 μM) was bath-applied and synaptic responses elicited using bipolar stimulating electrodes placed in either layer I to stimulate the LOT fibres and in layer II-III to stimulate intrinsic fibres. IPSPs were recorded from a membrane potential of –40 mV (maintained by positive current injection), following stimulation of local interneurones using either electrode position. In each case, the IPSP stimulus strength (3-30 V) was pre-determined in control solution as the intensity sufficient to elicit an EPSP of ~10 mV at –90 mV. A total of 18 adult and 20 immature neurones were recorded in current clamp mode; average resting membrane potentials (± s.e.m.) were -84 ± 1 mV (adult) and -82 ± 1 mV (immature). The peak amplitudes of layer I (LI) and intrinsic layer IPSPs elicited in immature slices (LI: 9.1 ± 0.3 mV; intrinsic: 5.3 ± 0.4 mV; n=20) were significantly smaller than those recorded in adults (LI: 18.1 ± 0.7 mV; intrinsic: 12.6 ± 0.8 mV; n=18); (P<0.01; Students t-test). Rather surprisingly, all IPSPs elicited by *intrinsic* layer stimulation were *unaffected* by OXO-M, whereas, those elicited from LI were consistently suppressed. In adult slices, the percentage degree of suppression was significantly smaller (35.2 ± 5.5 %) than in immature slices (72.1 ± 5.2%) (means ± s.e.m. of peak IPSP amplitude; P<0.01 by Wilcoxon signed rank test). Moreover, the M_1-receptor selective antagonists, pirenzepine (100 nM; 8/9 cells) or telenzepine (20 nM; 9/9 cells) reversed the OXO-M-induced LI IPSP inhibition in adult slices, but not in immature slices (10/10 cells: pirenzepine; 10/10 cells; telenzepine). Conversely, the M_2-receptor selective antagonists, AFDX-116 (1 μM; 9/9 cells) or methoctramine (300 μM; 9/9 cells) did not affect the mAChR-induced inhibition of LI IPSPs in adults, but reversed the inhibition in immature slices (10/10 cells: methoctramine; 10/10 cells: AFDX-116). We conclude that (1) $GABA_A$-receptor mediated inhibition in both LI and intrinsic layers of the PC is reduced in immature slices; (2) only LI-elicited IPSPs are suppressed by OXO-M, more so in immature slices than in adults and (3) this presynaptic muscarinic suppression is M_2-receptor mediated in immature slices but M_1-receptor mediated in adults. We have previously reported a comparable developmental change in mAChR subtypes mediating *intrinsic* EPSP suppression in the PC (Whalley & Constanti, 2002). Theoretically, this difference in mAChR-mediated modulation of excitatory and inhibitory transmission maintains the immature PC in a heightened excitatory state following presynaptic mAChR activation. This may play a role in driving synaptic growth, learning, memory and also be important for the generation of muscarinic agonist-induced epileptiform bursting seen in some cells of the immature PC (Postlethwaite *et al.*, 1998).

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190P MUSCARINIC INDUCTION OF THE POST-STIMULUS AFTERDEPOLARIZING TAIL CURRENT (I_{ADP}) IN RAT OLFACTORY CORTICAL NEURONES *IN VITRO* REQUIRES G-PROTEIN ACTIVATION

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Muscarinic acetylcholine receptor (mAChR) activation in olfactory cortical neurones converts the slow post-stimulus afterhyperpolarization (sAHP) into a slow afterdepolarization (sADP) and underlying inward tail current (I_{ADP}), recorded under whole-cell patch clamp (Sciancalapore & Constanti, 1995). This unusual tail current is calcium dependent, and most-likely K^+ mediated (Constanti *et al.*, 1993; Postlethwaite *et al.*, 2000); however, the second messenger systems involved in its generation remain unknown. In light of evidence that some mAChRs may act independently of G-proteins (Guerineu *et al.*, 1995), we investigated whether a G-protein signalling mechanism was involved in I_{ADP} induction.

Transverse slices of Wistar rat (P14-P22, either sex) olfactory cortex were prepared as previously described (Sciancalapore & Constanti, 1995). Whole-cell recordings were taken from visually identified pyramidal cells in Layers II-III of these slices using electrodes filled with K methylsulphate alone, or additionally containing the non-hydrolysable analogues GTP-γ-S, to permanently activate, or GDP-β-S to permanently de-activate G-protein- coupled mechanisms. I_{ADP} was routinely induced by a +40 mV (7.5 s) voltage step from a -60 mV holding potential. Data are presented as means ± S.E.

The mean resting potential (r.p.) and input resistance (R_{inp}) of cells recorded with normal electrodes (10 min) were -67 ± 1 mV and 212 ± 13 MΩ respectively (n=29). Bath-application of the mAChR agonist oxotremorine-M (OXO-M;10 μM) to control cells resulted in a steady inward current (56 ± 8 pA) and induction of an I_{ADP} (32 ± 5 pA peak amplitude; n=8), both of which reversed upon washout. Cells loaded with 100 μM GTP-γ-S (10 min) had a significantly more negative r.p. (-69 ± 2 mV) and decreased R_{inp} (122 ± 10 MΩ, n=19; P<0.05; t-tests) however I_{ADP} was only induced by GTP-γ-S loading alone in 4/19 cells (18 ± 4 pA amplitude; n=4), even in the presence of 100 μM Ba^{2+} (to reduce background leak conductance). Addition of OXO-M induced an I_{ADP} in 6/9 GTP-γ-S-loaded cells (mean 14 ± 3 pA amplitude; n=6) which was significantly smaller than control (P<0.05, t-test), but persisted for the length of recording (up to 1 hour) even after OXO-M washout. In contrast, cells loaded with 500 μM GDP-β-S (10 min, n=8) showed a normal r.p. (-66 ± 2 mV) and R_{inp} (204 ± 27 MΩ) (not significantly different from control; P> 0.5, t-tests), and OXO-M applied within 2 minutes of achieving whole-cell mode, produced a clear inward current (49 ± 10 pA) and induction of I_{ADP} (38 ± 10 pA amplitude, n=8). However, after 30 min loading, a further application of OXO-M failed to produce an inward current or I_{ADP}. The slow outward current produced in control by application of baclofen ($GABA_B$ receptor agonist; 100 μM) was also abolished by GDP-β-S (n=3), confirming suppression of G-protein activity.

In conclusion, these experiments provide strong evidence that the steady inward mAChR induced current and induction of I_{ADP} in olfactory cortical neurones are both mediated by G-protein activation. (Supported by the Wellcome Trust).

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191P MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION AT CENTRAL SYNAPSES BY GROUP II METABOTROPIC GLUTAMATE RECEPTORS

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Previous studies of the modulatory effects of a group III metabotropic glutamate receptor ($mGluR_4$) on glutamatergic synaptic transmission in layer V of the entorhinal cortex (EC) have shown that it can increase the frequency of spontaneous excitatory postsynaptic currents (sEPSCs), but concurrently decrease the amplitude of stimulus-evoked EPSCs (Evans *et al.*, 2000a,b). Group II mGluRs ($mGlu_2$ and $_3$) have similar presynaptic locations to group III ($mGluR_{4,\ 6,\ 7}$ and $_8$) and common intracellular signaling pathways. We are currently investigating whether differential modulation of glutamate-mediated transmission is a paradigmatic aspect of mGluR function and whether this aspect is synapse specific. Here, we describe the effect of the group II mGluRs on sEPSCs at glutamate synapses in layer V of the EC and at the hippocampal CA3 mossy fibre synapse.

Brain slices comprising EC and hippocampus were prepared from male Wister rats (45-60 g) anaesthetised with ketamine (120 mg/kg i.m.) and xylazine (8 mg/kg i.m.) prior to decapitation. Slices were transferred to an interface recording chamber continuously perfused with oxygenated (95% O_2: 5% CO_2) aCSF containing the NMDA receptor antagonist, MK-801 (10 μM). Whole-cell voltage clamp recordings of sEPSCs were made from neurones voltage clamped at –60 mV using a borosilicate patch pipette (4-6 MΩ open-tip resistance) filled with a solution consisting of (in mM): D-Gluconate (100), HEPES (40), QX-314 (1), EGTA (0.6), NaCl (4), $MgCl_2$ (5), TEA-Cl (1), ATP-Na (4), GTP-Na (0.3), 275 mOsm, pH 7.3.The drugs used were: 2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV; 5 μM) and 2*S*-2-amino-2-(1*S*,2*S*-2-carboxycyclo-pro-1-yl)-3-(xanth-9yl)propanoic acid (LY341495; 20 nM). Data were analysed using Minianalysis software (Synaptosoft, USA). Statistical analysis was performed using the nonparametric Kolmogorov-Smirnoff test. Rejection of the null-hypothesis required $P<0.05$. Figures below refer to mean ± s.e.m.

In layer V neurones, mean inter-event interval (IEI) of sEPSCs was 411.1 ± 16.6 ms, n=8. Perfusion with the group II mGluR agonist, DCG-IV decreased IEI to 333.2 ± 15.2 ms, (P=0.001). Subsequent perfusion with the potent and selective group II antagonist, LY341495, increased the IEI above that seen in control conditions (800.5 ± 54.2 ms, P=0.001). Neither drug had any effect on amplitude (control 11.2 ± 0.2 pA *v* 11.7 ± 0.1 pA in DCG-IV, and 11.0 ± 0.2 pA in LY341495). In hippocampal CA3 pyramidal cells, sEPSC frequency was much higher and DCG-IV had no effect on IEI (control 45.9 ± 1.4 ms *v* 43.9 ± 1.4 ms in DCG-IV; P=0.31; n=5) or amplitude (15.8 ± 0.4 pA *v* 15.1 ± 0.4 pA; P=0.57). However, addition of LY341495 increased IEI (from 33.5 ± 1.2 ms to 57.0 ± 2.0 ms; P=0.001, n=7) without any effect on sEPSC amplitude (34.8 ± 0.7 pA *v* 33.5 ± 0.6 pA, P=0.07).

The data suggest that presynaptic group II mGluRs can increase spontaneous glutamate release in both layer V and CA3. These receptors seem to be tonically activated in CA3 pyramidal cells where glutamate release is high, and to a lesser extent in layer V where release is comparatively low. The results also show that the group II mGluRs may have a similar function to group III mGluRs at excitatory presynaptic terminals.

Evans *et al.*, (2000a) J. Neurophysiol. **83**: 2519-25.
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192P PHARMACOLOGICAL EVIDENCE FOR SUBTYPES OF PRESYNAPTIC $GABA_B$ RECEPTORS CONTROLLING GLUTAMATE AND GABA RELEASE IN THE RAT ENTORINAL CORTEX

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Differences in acute seizure susceptibility between layers II and V of the entorhinal cortex (EC) have led us to compare the characteristics of synaptic transmission in these two layers. A disturbance in the balance between synaptic inhibition and excitation is fundamental to epileptogenesis, and we have been interested in the possibility that differences in the presynaptic control of glutamate and GABA release may contribute to laminar differences in seizure susceptibility.

Presynaptic $GABA_B$ receptors are known to depress both GABA and glutamate release in various areas of the brain. Two $GABA_B$ receptor subunits have been identified, $GABA_BR1$ and $GABA_BR2$, and evidence suggests that these combine as heterodimers to form a functional receptor. Splice variants of the two subtypes have been reported, which suggests a possible heterogeneity of structure and function, and there is pharmacological evidence to support this. In the current experiments, we attempted to discriminate pharmacologically between $GABA_BRs$ in layer II and layer V of the EC, and also to compare the pharmacology of $GABA_B$ receptor function at inhibitory and excitatory terminals.

Horizontal EC slices (400 μm) were prepared from male Wistar rats (45-60g) anaesthetised with ketamine (120 mg/kg i.m.) and xylazine (8 mg/kg i.m) prior to decapitation. Whole cell patch clamp recordings were made from neurones visually identified using differential interference contrast microscopy. Spontaneous glutamate and GABA release were monitored by recording either excitatory (EPSCs) or inhibitory (IPSCs) postsynaptic currents, respectively. Postsynaptic $GABA_BR$ were blocked by inclusion of Cs^+ and QX-314 in the patch pipette solution. TTX (1 μM) was included in the perfusion medium to allow recording of miniature (m) EPSCs and mIPSCs. The effects of the $GABA_B$ receptor agonist, CGP44533 (0.01-25μM), and antagonist, CGP64213 (0.001-10μM), were tested on sEPSCs. CGP44533 (3μM and 10μM) was also tested on sIPSCs. Data were analysed using Minianalysis software (Synaptosoft).

CGP44533 caused a concentration-dependent reduction in the frequency of mEPSCs. IC_{50} values were virtually identical in layer II and layer V (approximately 0.9 μM). However, at concentrations (10-25 μM) which produced a maximal reduction in mEPSC frequency, the effect was greatest in layer II (83 ± 3% *v*70 ± 5% in layer V at 10 μM, $P<0.05$ t-test, n=15 in each case). In contrast, sIPSC frequency was reduced by CGP44533 to a similar extent in both layers (not shown). Comparing glutamate to GABA release, CGP44533 reduced to the frequency of sEPSCs and sIPSCs to a similar extent in layer V (not shown) However, in layer II, the maximal reduction of sEPSC frequency by the agonist (10 μM) was 83 ± 3% compared to a 65 ± 5% reduction in sIPSC frequency ($P<0.05$, n=15 in each case). Finally, the $GABA_BR$ antagonist CGP64213 did not significantly alter mEPSC frequency in either layer (layer II 99 ± 7% of control n= 8, layer V 102 ± 10% of control n= 5).

Thus $GABA_B$ receptors reduce GABA and glutamate release in both layer II and V. Differences in the degree of reduction suggest that there may be different auto and heteroreceptor subtypes of present in the EC and that some of these may also exhibit laminar-specific differences.

193P REDUCED G-PROTEIN COUPLING TO THE $GABA_B$ RECEPTOR IN THE NUCLEUS ACCUMBENS AND THE MEDIAL PREFRONTAL CORTEX OF THE RAT AFTER CHRONIC TREATMENT WITH NICOTINE

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Dopaminergic neurones projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and the prefrontal cortex (PfCx) are selectively activated by nicotine. An increase in dopamine (DA) within the terminal fields of the mesolimbic system appears to underlie the addictive properties of the drug (Di Chiara, 2000). In naïve rats, $GABA_B$ receptor activation inhibits both the firing of VTA DAergic cells (Johnson and North, 1992) and the release of DA in NAc and PfCx (Santiago *et al.*, 1993; Smolders *et al.*, 1995).
In the present study, we have investigated whether chronic administration of nicotine is associated with altered G-protein coupling to the $GABA_B$ receptor in discrete rat brain regions.

Male Wistar rats (180-200g) received daily injections of nicotine (0.4mg kg^{-1}s.c.) or vehicle (0.9% NaCl, 1ml kg^{-1}s.c.) for 14 days. On day 15, animals were sacrificed and the brains dissected and frozen at -80°C. 12μm-thick sections were cut at -20°C, mounted on glass slides, and stored at -80°C. On the day of the experiment, sections were thaw-dried and washed (30min, 25°C) in 50 mM Tris-HCl (+100mM NaCl, 3mM $MgCl_2$, 0.2 mM EGTA, pH 7.4). A 20-min preincubation with 2mM GDP was followed by incubation (2h, 25°C) in buffer containing 40pM [^{35}S]GTPγS, with or without 0.1mM baclofen. In some experiments, the $GABA_B$ antagonist CGP55845 (10μM) was also included. Non-specific binding was determined with 10μM GTPγS. Slides were washed (2×2min) in buffer and, when dry, exposed (72h) to Kodak BioMax MR films along with [^{35}S] brain paste standards. After development, films were analysed by computer-assisted densitometry (MCID-M4). [^{3}H]GABA saturation binding was performed on brain sections as described by Bowery *et al.* (1987). Data were expressed as mean±s.e.mean and compared using two-tailed Student's *t* test.

In control rats (n=3), baclofen-stimulated [^{35}S]GTPγS binding was 54-206% of basal throughout the brain, and values returned to basal levels in the presence of CGP55845. Baclofen-stimulated [^{35}S]GTPγS binding (attmol/mg tissue) in the nicotine-treated rats (n=4) was significantly lower compared to saline-controls in the medial PfCx (209±23 vs 388±14, P<0.01), the NAc core (158±16 vs 230±22, P<0.05) and shell (108±13 vs 168±17, P<0.05), while no difference was found in the VTA, the caudate putamen and the parietal cortex. Basal [^{35}S]GTPγS binding and $GABA_B$ receptor expression and affinity were not significantly altered by the nicotine treatment in any of the regions examined.
Our findings demonstrate that chronic nicotine administration is associated with reduced G-protein coupling to $GABA_B$ receptors in terminal mesocorticolimbic regions. Thus, disinhibition of dopaminergic neurones may underlie their hyperactivity in response to repeated nicotine administration.

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194P FORMATION OF GLUTATHIONE ADDUCT METABOLITES OF MDA IN HUMAN LIVER MICROSOMES IS RELATED TO CYP2D6 ACTIVITY

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Serotonergic neurotoxicity occurs following systemic (O'Shea *et al*, 1998) but not central (Paris *et al*, 1992) administration of 3,4-methylenedioxymethamphetamine (MDMA). MDMA is metabolised in the liver by demethylation to 3,4-methylenedioxyamphetamine (MDA) and can then be demethylenated to produce α-Methyldopamine (α-MeDA) (Cho *et al*, 1990). Many MDMA metabolites have been identified in the urine but none have been shown to reproduce the neurotoxicity of the parent compound (Elayan *et al*, 1992). We have previously reported an adapted synthesis for two glutathione adducts of α-MeDA and identified them as the regiosiomers; 5-(glutathion-*S*-yl)-α-MeDA and 6-(glutathion-*S*-yl)-α-MeDA. This study determined whether the cytochrome P450 2D6 (CYP2D6) isoform in human liver microsomes was involved in the formation of these MDA derived adducts.

MDA (30 μM) was incubated at 37°C in a shaking water bath with human liver microsomes (source: UK Human Tissue Bank, n = 5) and the incubates analysed at 20 min intervals over a 2 h period for glutathione adduct formation using HPLC-ECD. Microsomal CYP2D6 activity was measured using (+/-)-bufuralol-hydroxylase.

The results show that both regioisomers are readily formed in human liver microsomes over the first 100 min of incubation. Variation in microsomal CYP2D6 activity in the ranged between 45 and 225 pmol/min/mg of bufuralol hydroxylation.

Linear regression analysis (Figure 1) shows that CYP2D6 activity is related to the formation of both adducts with livers of higher CYP2D6 activity producing up to eight times more of the adducts than livers of lower activity.

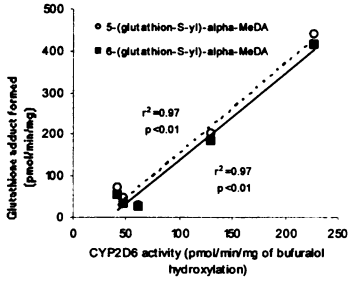

Figure 1. Cytochrome P450 2D6 activity (pmol/min/mg) against amount of glutathione adduct formed (nmol/mg protein) at the 100 minute timepoint in human liver microsomes incubated with MDA (30μM).

The glutathione adducts measured in this study have been shown to be further metabolised to adducts that produce long-term serotonergic neurotoxicity (Miller *et al*, 1995). This is the first report to demonstrate the production of these metabolites by the CYP2D6 phenotype in human liver. It remains to be determined whether human liver can convert these metabolites to the more neurotoxic downstream adducts of α-MeDA and if these compounds are able to cross the BBB.

The authors would like to thank the Wellcome Trust for financial support and the UK Human Tissue bank for provision of the human microsomes.

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195P EFFECTS OF CHRONIC TREATMENT WITH MDMA ON PRE AND POSTJUNCTIONAL RESPONSIVENESS IN THE RAT

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Methylenedioxymethamphetamine (MDMA) is a widely used recreational drug with actions on serotonergic, dopaminergic and adrenergic systems (see McCann et al., 1996). We have recently investigated the actions of MDMA on blood pressure in the anaesthetised rat and found complex pressor and depressor components mediated mainly by $alpha_1$- and $alpha_2$-adrenoceptors (McDaid & Docherty, 2001). Some of these actions may involve direct stimulation of $alpha_2$-adrenoceptors, since MDMA has agonist actions at these receptors (Lavelle et al., 1999). In the present study, we have examined the effects of treatment for 4 days with MDMA on vascular and neuronal responses.

Male Wistar rats (250-350g) were implanted with telemetry probes (Data Sciences International, USA) into the abdominal aorta under pentobarbitone anaesthesia, and were then allowed to recover for 6 days. The telemetry probes allowed the recording of aortic systolic and diastolic blood pressure, temperature and movement. Animals were then injected subcutaneously daily for 4 days with MDMA (20 mg kg^{-1}) or vehicle. The following day, animals were killed by CO_2 overdose, and small mesenteric artery and vas deferens were set up in small vessel myographs, or in organ baths between platinum electrodes, respectively.

In telemetry studies of conscious rats, MDMA (20 mg kg^{-1}) produced significant pressor responses lasting for at least 5 h with a maximum increase in systolic blood pressure of 22.0 ± 1.8mmHg (mean±s.e.m., n=6) and pressor responses were similar on days 1-4. MDMA injection caused a significant transient fall in body temperature on day 1 of 1.9± 0.4°C, recovering to baseline within 2 h, but the effect was virtually absent on day 4. MDMA also increased locomotor activity.

In studies carried out in vitro, MDMA pre-treatment for 4 days did not significantly affect the potency of, or maximum isometric contractile response to phenylephrine in rings of rat small mesenteric artery. However, MDMA pre-treatment significantly reduced the potency of the $alpha_2$-adrenoceptor agonist xylazine at inhibiting isometric contractions evoked by a single stimulus in prostatic portions of rat vas deferens in cumulative concentration response studies (e.g. xylazine 0.1 μM reduced stimulation-evoked contractions to 81.8±5.8% and 48.0±12.5% of control, n=5, in tissues from MDMA and vehicle treated, respectively).

In summary, pre-treatment with MDMA in a dose of 20 mg kg^{-1}, which produces pressor actions in vivo, did not alter response of rat mesenteric artery to phenylephrine but reduced the prejunctional potency of the $alpha_2$-adrenoceptor agonist xylazine.

Supported by the Health Research Board (Ireland)

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196P EFFECT OF AMBIENT TEMPERATURE AND 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) PRETREATMENT ON THE HYPERTHERMIC RESPONSE OF RATS TO A LOW DOSE OF MDMA

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MDMA administration produces an acute hyperthermic response in rats (see Mechan *et al.*, 2002) and can also produce an acute hyperthermic response in humans, which may be fatal (Henry, 1992). Since humans ingesting MDMA recreationally usually do so in crowded situations with high ambient temperature conditions, we have examined the response of rats to a modest dose of MDMA (5 mg kg^{-1}) when housed in both normal and raised ambient conditions, and the effect of a prior neurotoxic dose of MDMA (12.5 mg kg^{-1}; see Mechan *et al.*, 2001) on the response of rats to MDMA (5 mg kg^{-1}) when given to animals housed at high ambient temperature.

Adult male Dark Agouti rats (approx 180 g at start of study) were injected with saline or MDMA (5 mg kg^{-1} i.p.) housed at an ambient temperature of 19°C. Rectal temperature was measured over the next 4 h using a probe. In a further experiment rats were administered either a neurotoxic dose of MDMA (12.5 mg kg^{-1}) or saline when housed at 19°C and 5 weeks later placed in a room kept at an ambient temperature of 30°C. Sixty min later both groups were divided into 2 with the sub-groups injected with saline or MDMA (5 mg kg^{-1} i.p.). The rectal temperature of all 4 groups was measured over the next 4 h. Results are presented as the mean ± s.e. mean (n = 6) of the temperature change following MDMA compared with the mean value of the appropriate saline-injected control group. Statistical analysis was by 2-way ANOVA (BDMP Dynamic).

Administration of MDMA (5 mg kg^{-1}) to rats housed at normal ambient temperature produced a small apparent increase in rectal temperature but which was not significantly different from the control group (Fig). When this dose was given to rats housed at an ambient temperature of 30 °C it produced a significant increase in rectal temperature (Fig). Administration of MDMA (5 mg kg^{-1}) to rats given a neurotoxic dose of MDMA 4 weeks earlier produced a sustained hyperthermic response which was significantly greater than that seen in non-pretreated rats (Fig).

These data extend our findings suggesting that, acutely, MDMA impairs heat loss mechanisms in rats (Mechan *et al.*, 2002) and also support our observation that a neurotoxic dose of MDMA impairs thermoregulation in rats, with an apparent failure of the animals to lose body temperature when this is elevated (Mechan *et al.*, 2001).

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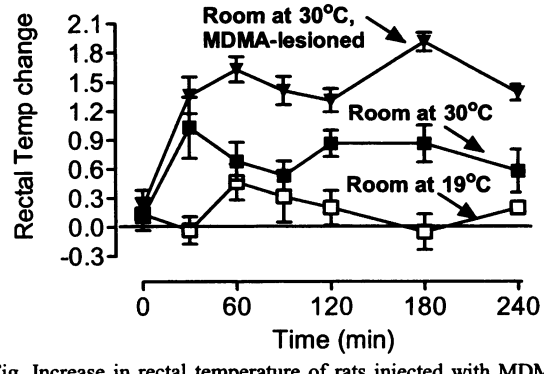

Fig. Increase in rectal temperature of rats injected with MDMA (5 mg kg^{-1}) when at the room ambient temperature shown and a group pretreated with a neurotoxic dose of MDMA (MDMA-lesioned). Lesioned rats different from non-lesioned at high ambient temperature [$F(1,10)=17.40$, $p<0.01$]; non-lesioned rats at high temperature different from those at normal ambient temperature [$F(1,10)=8.18$, $p<0.05$].

197P CEREBROVASCULAR EFFECTS OF ACUTE METHYLENEDIOXYMETHAMPHETAMINE IN RATS PREVIOUSLY EXPOSED TO THE DRUG

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A single dose of 3,4,-methylenedioxymethamphetamine (MDMA) in Dark Agouti (DA) rats induces acute uncoupling of local cerebral blood flow (LCBF) from glucose utilisation (LCMRglu) (Quate *et al.*, 2002). In the longer term the same single dose results in degeneration of 5-HT nerve terminals (O'Shea *et al.*, 1998). In this study we examined whether this persistent neurodegeneration alters the acute cerebrovascular response to subsequent MDMA exposure.

Adult DA rats were pre-treated with either 15mg/kg i.p. MDMA (n=22) or saline (n=22) and three weeks later were exposed to a single acute dose of MDMA (15mg/kg) or saline. LCBF or LCMRglu was measured 25 and 15 minutes post-MDMA in 68 brain areas using [^{14}C]- iodoantipyrine and [^{14}C]-2-deoxyglucose quantitative autoradiography respectively and adjacent sections were used for [^{3}H]-paroxetine binding analysis (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 ($p<0.05$).

[^{3}H]-paroxetine binding was significantly decreased by between 54% in caudal hippocampus and 81% in occipital cortex. The MDMA challenge induced stereotypic motor behaviour and significant increases ($P < 0.05$; t-test) in both temperature (37.31±0.14 to 38.61±0.21°C) and MABP (135±1 to 164±2 mmHg). While MDMA produced significant increases in LCMRglu in 41 brain areas, there was a global trend towards decreased LCBF, with significant decreases recorded in 9 brain areas. However, anatomically random areas of focal hyperaemia were recorded in neocortex (sensorimotor from 174±8 to 201±14; frontal 150±6 to 172±27).

A global analysis of all 68 brain areas revealed a close correlation ($r=0.92$) between LCMRglu and LCBF with an overall ratio of 1.74 in the MDMA pre-treated group. In the groups challenged with a further exposure to MDMA, there was a similar close correlation ($r=0.88$), but, due to the divergence between LCMRglu (increases) and LCBF (decreases), the overall ratio was decreased to 1.14. This ratio is similar to that previously reported (Quate et al, 2002) (saline controls, 1.31; drug naïve animals exposed to MDMA, 1.07).

These results suggest that the acute MDMA-induced cerebrovascular regulatory dysfunction is not diminished by previous exposure, this despite a significant depletion in 5-HT terminals. Moreover, there may be a sub-population in which this dysfunction develops into conditions that might predispose the individual to stroke.

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198P FLUVOXAMINE REDUCES LEVODOPA INDUCED DYSKINESIA IN THE MPTP-TREATED COMMON MARMOSETS

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The selective serotonin reuptake inhibitors (SSRI's) are efficacious antidepressants available to the 40% of Parkinson's disease (PD) patients with depression (Cummings, 1992). When given alone, they exacerbate or have no effect on motor symptoms of PD (Dell'Agnello *et al*, 2001). However, recently it was reported that Citalopram enhances Ldopa induced reversal of bradykinesia in both depressed and non-depressed cases of PD (Rampello *et al*, 2002). While microdialysis findings in hemiparkinsonian rats suggest that SSRI's could reduce Ldopa induced dyskinesia (Kannari *et al*, 2001; Yamoto *et al*, 2001). For this reason, we have investigated the effect of the SSRI, Fluvoxamine (FLUV) (Hiemke *et al*, 1999), on motor complications induced by Ldopa in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets.

Adult common marmosets (*Callithrix Jacchus*; n=2 male & n=2 female: 350-400g) previously treated with MPTP and showing stable motor deficits (Pearce *et al*, 1998), were treated with FLUV maleate (0.05-10 mg/kg po) in 10% sucrose solution. These animals were not levodopa primed. In a separate study, a single high dose of FLUV (10mg/kg po) was administered alone or in combination with Ldopa (12.5mg/kg free base po). MPTP-treated marmosets (n=5-10, total n=13, male: n=6, female: n=7, 338-414g) were treated previously with Ldopa methyl ester (12.5 mg/kg BID for 30 days) to induce severe and stable dyskinesia. Control animals received vehicle (10% sucrose). A modified latin square design was employed with one week between treatments. Locomotor activity was measured in automated photocell cages for 4-hours following treatment. Disability and dyskinesia were assessed during the last 10-minutes of each 30 minute interval by a trained observer blinded to drug treatment. Data was analysed by Friedman's test or Kruskal Wallis followed by the Mann-Whitney test where appropriate.

In MPTP-treated common marmosets, FLUV had no effect on locomotor activity or total motor disability over 4 hours. Therefore, the highest dose of 10 mg/kg was chosen for further study. In dyskinetic MPTP-treated marmosets, Ldopa increased locomotor activity, reduced motor disabilities and induced dyskinesia (Table 1). Sucrose vehicle and FLUV had no effect on these measures. Ldopa/FLUV reduced cumulative dyskinesia scores compared to Ldopa alone, but did not alter the reduction in disability (Table 1). Ldopa/Fluv tended to reduce the increase in locomotor activity induced by Ldopa, although this was not significant (p=0.23, Mann Whitney).

Treatment	N	Locomotor Counts	Total Disability	Total Dyskinesia
Veh/Veh	10	296(9-1242)	99(68-116)	0(0)
Ldopa/Veh	9	6223(2121-11033)*	37(20-80) *	19(5-23)*
Veh/FLUV	5	396(140-796)	102(88-111)	0(0)
Ldopa/FLUV	5	5188(714-9061)*	44(32-72) *	12(1-14)*/++

Table 1: Cumulative locomotor counts, disability and dyskinesia scores over 4h following Ldopa, FLUV or vehicle administration to dyskinetic MPTP-treated marmosets. Data represent the median (range) for each treatment. * p values<0.05 vs. vehicle ++ p<0.05 vs. Ldopa (Mann Whitney-Test).

These data show that FLUV reduced the incidence of Ldopa induced dyskinesia giving support to previous findings (Durif *et al*, 1995). However, it did not abolish Ldopa's antiparkinsonian effects (Yamoto *et al*, 2001). This action could be mediated by enhanced stimulation of presynaptic serotonin 1A receptors leading to reduced striatal dopamine release. In conclusion, FLUV may be both a useful antidepressant and antidyskinetic adjunct to Ldopa treatment in PD patients suffering from motor complications.

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199P ECSTASY (3,4-METHYLENEDIOXYMETHAMPHETAMINE) INHIBITS DYSKINESIA EXPRESSION AND NORMALISES LOCOMOTOR ACTIVITY IN PARKINSONIAN PRIMATES

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To date, the most effective treatment for Parkinson's disease (PD) is dopamine replacement using L-DOPA. However, during long-term therapy, involuntary movements (dyskinesia) appear and increase in intensity, affecting approximately up to 40% of all patients (Ahlskog and Muenter, 2001). These involuntary movements reduce the effectiveness of L-DOPA as a treatment for advanced cases of PD and become treatment limiting. Consequently, therapeutic strategies that maintain the effectiveness of L-DOPA by reducing motor disability without inducing dyskinesia are being sought. A recent anecdotal report has suggested that the recreational drug ecstasy (MDMA) is able to alter motor functions in a patient with PD (www.bbc.co.uk/science/horizon/ecstasyagony_transcript.shtml). Our objective is to investigate the effect of ecstasy (MDMA) on parkinsonian motor disability, and L-DOPA induced dyskinesia in L-DOPA primed animals.

6 adult common marmosets of either sex were treated with 5 consecutive daily doses of 2 mg.kg^{-1} MPTP. Animals were primed for dyskinesia by daily doses of 12.5 mg.kg^{-1} L-DOPA/12.5 mg.kg^{-1} carbidopa (p.o.) on a daily basis until development of stable dyskinesia was achieved. Locomotor activity, motor disability and dyskinesia (chorea and dystonia) was measured using automated activity monitoring or observer rating scale respectively on a weekly basis either in the presence or absence of an acute L-DOPA/carbidopa or in combination with varying doses of MDMA (3-12 mg.kg^{-1}). Motor disability, chorea, dystonia and overall dyskinesia was analysed using a Freidman's test followed by Dunn's multiple comparison test.

We now show that the locomotor activity shown by MPTP treated marmosets following administration of L-DOPA and 12 mg.kg^{-1} MDMA was not significantly different from the locomotor activity of naïve animals (naïve: 1344 ± 389 counts /4h, MPTP+LD+12 mg.kg^{-1} MDMA: 1081 ± 370 counts / 4h, P>0.05). Furthermore, MDMA did not modify L-DOPA's ability to reduce motor disability (L-DOPA, mean disability/4h: 54 ± 3.8; L-DOPA + 12 mg.kg^{-1} MDMA: 52 ± 6.5, P>0.05, fig1a) but dose dependently reduced the mean intensity of chorea from 18.3 ± 3.3 to 9.3 ± 2.0 and 4.0 ± 1.3 respectively (P<0.001). L-DOPA induced dystonia was only significantly reduced at the highest dose of MDMA tested (mean total dystonia/4h: L-DOPA, 14.2 ± 3.3, L-DOPA + MDMA, 9.6 ± 3.7; P<0.05). Overall, MDMA dose dependently reduced the total dyskinesia (total dyskinesia/4h, L-DOPA: 19 ±1.5; L-DOPA + 3 mg.kg^{-1} MDMA: 13.9 ± 1.9; L-DOPA + 12 mg.kg^{-1} MDMA: 7.8 ± 2.9; P<0.001).

We conclude that the ability of MDMA to inhibit dyskinesia results from its broad spectrum of action on 5-HT system. Serotoninergic system may play an important modulatory role in L-DOPA induced dyskinesia. This study may provide a framework for the use of serotoninergic agents in treatment of L-DOPA induced dyskinesia. ***Fig1a(disability), b(dyskinesia).***

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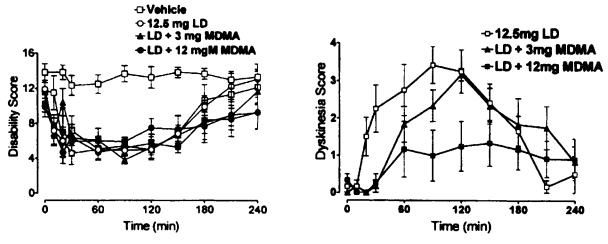

200P CHRONIC ADMINISTRATION OF BTS 74 398 DOES NOT INDUCE ABNORMAL MOVEMENTS IN 6-OHDA LESIONED RATS

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L-DOPA (LD) induces a well characterised circling response (CR) in 6-hydroxydopamine (6-OHDA)-lesioned rats that becomes sensitised on prolonged administration (Papa *et al.*, 1994). More recently, additional abnormal movements (AIM's) have been identified in this model (Henry *et al.*, 1998). Both responses are proposed to reflect motor complications of LD therapy in Parkinson's disease. BTS 74 398 (BTS), a monoamine uptake inhibitor, reverses the motor and behavioural deficits in MPTP-treated marmosets without provoking established dyskinesias (Smith *et al.*, 1998). This study examines the effects of repeated administration of LD and BTS on the CR and the production of AIMS in the 6-OHDA-lesioned rat.

Male Wistar rats (220-250g) were lesioned, under halothane anaesthesia, by unilateral injection of 6-OHDA (8µg in 4µl) into the left median forebrain bundle (AP: -2.2, ML: +1.5, DV:-8mm from bregma (Paxinos and Watson, 1986). Six weeks post surgery rats were given vehicle (0.9% saline, 1ml/kg ip), LD (12.3 mg/kg plus carbidopa 12.5 mg/kg ip) or BTS (4.6 mg/kg ip) for 21 days. The CR was recorded on days 1, 3, 4, 7, 8, 10, 11, 14, 15, 17, 18, 21 for 6 h using an automated Rotorat system. AIMs in 4 categories (axial, limb, orolingual and locomotor) were scored every 30 min on day 21 with a severity rating (1-4) of the presence of behaviours over each observation period (based on Lundblad *et al.*, 2002). In each category the maximum score was 48, with a total of 192.

	Saline	LD	BTS
Day 1	36 ± 11	1069 ± 84**	1282 ± 381**
Day 10	11 ± 32	3100 ± 764**$^{\nabla\nabla}$	1152 ± 472*
Day 21	21 ± 12	3227 ± 1034**$^{\nabla}$	871 ± 235

Table 1: Full turns induced by saline (ipsilateral), LD (contralateral) and BTS (ipsilateral) over 6 h, *p<0.05, **p<0.01 BTS or LD vs saline, $^{\nabla}$p<0.05 $^{\nabla\nabla}$p<0.01 LD vs BTS (two-way ANOVA, Neuman Keuls post test, n=6)

Both LD and BTS produced a CR greater than saline on day 1. By day 10 the response to LD had increased over BTS and this enhancement was maintained at day 21 (table 1). The levels of axial, limb and orolingual AIMs were greater in the LD group compared to the BTS group, that was reflected in the total dyskinesia score (fig 1).

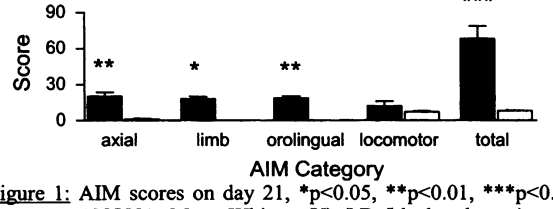

Figure 1: AIM scores on day 21, *p<0.05, **p<0.01, ***p<0.001 (two-way ANOVA, Mann-Whitney U), LD (black columns) versus BTS (white columns).

In this model, LD produces a sensitised circling response and abnormal movements that have been likened to the side-effects observed in patients undergoing longterm LD therapy (Papa *et al.*, 1994, Lundblad *et al.*, 2002). BTS produces no such effects in the 6-OHDA rat and these findings support data from the LD primed MPTP-treated marmoset that shows BTS has antiparkinsonian potential without evoking established dyskinetic responses (Smith *et al*, 1998).

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201P OSTEOPONTIN AND TYROSINE HYDROXYLASE EXPRESSION FOLLOWING INTRANIGRAL INJECTION OF LIPOPOLYSACCHARIDE

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Parkinson's disease (PD) is characterised by loss of dopamine neurones in the substantia nigra pars compacta (SNc) accompanied by a reactive microgliosis. The cause of PD remains unknown, but may be the result of inflammatory processes and oxidative stress (Le et al., 2001). Osteopontin (OPN) is a glycosylated phosphoprotein, found constitutively in bone and kidney, that is induced in response to inflammation and oxidative stress (Denhardt and Guo, 1993). OPN down-regulates hydrogen peroxide production and regulates transcription of inducible nitric oxide synthase, both of which are linked to cell death in PD (Le et al, 2001). Whether OPN plays a role in neurodegeneration in PD is not known. We now report the effects of intranigral injection of the bacterial endotoxin lipopolysaccharide (LPS) on OPN immunoreactivity in the rat basal ganglia.

Male Wistar rats (250-280g, n=6) were treated with LPS (6μg in 2μl) or vehicle ((sham) PBS, n=6) into the SN under halothane anaesthesia (AP: -4.8mm, L: +2.0mm, V:-8.0mm; Paxinos and Watson, 1986). Twenty-four hours after surgery, animals were anaesthetised with sodium pentobarbitone (60mg/kg i.p.), perfused-fixed and the brains removed and cryoprotected. Tissue from untreated animals was used as a control. Cryosections (30μm) were cut throughout the basal ganglia and incubated overnight in mouse anti-rat OPN (1:500), mouse anti-rat OX-42 (1:500) or rabbit anti-rat tyrosine hydroxylase (TH) (1:500) antibodies. Peroxidase immunohistochemistry was used to visualise the immunoreactive products. OPN and TH positive cells were counted in a blinded manner and the results were analysed by one-way ANOVA followed by Neuman-Keuls post-hoc test.

In untreated rats, OPN positive staining was observed in the SN pars reticulata (SNr), with weaker immunoreactivity in the globus pallidus and subthalamic nucleus. Staining was absent in the striatum. Following LPS injection there was an increase in the number of OPN positive cells throughout the SN, particularly surrounding the injection tract, accompanied by an increase in degenerating dopaminergic neurones as shown by the loss of TH positive cells (Figure 1) and a reactive microgliosis as shown by an increase in OX-42 staining.

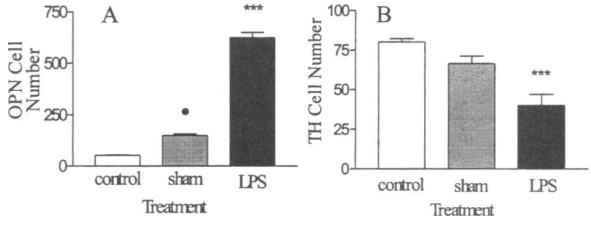

Fig.1. Effect of LPS on the number of OPN (A) and TH (B) positive cells. *** $p<0.001$ compared to sham and control, •$p<0.05$ compared to control

This is the first study to show the presence of OPN protein in the normal rat brain although the cell type at basal levels still needs to be elucidated. Intranigral LPS treatment produces an increase in OPN expression corresponding to both an increase in gliosis and dopaminergic neuronal degeneration 24 h after LPS treatment. Whether these responses are linked has not yet been established and further work into the role of OPN in the brain is required.

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202P IMMUNOHISTOCHEMICAL AND BEHAVIOURAL EFFECTS OF UNILATERAL SUPRANIGRAL LIPOPOLYSACCHARIDE ADMINISTRATION IN RATS

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There is increasing evidence that inflammation plays an important role in the parkinsonian degeneration of the dopaminergic neurons (Hirsch, 1998). We have already shown that supranigral administration of lipopolysacchride (LPS) results in the loss of nigral dopaminergic neurones (Iravani et al., 2002). In the present investigation we have studied the effects of a single supranigral LPS administration in rats, and 30 days later, we have assessed the locomotor asymmetry and tyrosine hydroxylase together with astrocyte and microglia markers in the substantia nigra and the striatum.

Fourteen adult male Wistar rats (200-300g) were administered with either 2μl saline (n=7) or 10μg/2μl LPS (n=7) supranigrally, under halothane anaethesia, using a 10 μl Hamilton syringe with a gauge 22 needle. The supranigral co-ordinates were AP: -4.80mm, L: -2.0mm and V:-7.6mm according to the atlas of Paxinos and Watson (1986). Thirty days after the surgery, we tested the locomotor asymmetry of these animals in response to 5 $mg.kg^{-1}$ (+)-amphetamine (i.p.) for 180 min. Shortly thereafter (2-3 hrs), all animals were killed by pentobarbitone overdose (100 $mg.kg^{-1}$, i.p.). Animals were perfused with cold 0.1M PBS, followed by 4% paraformaldehyde. After fixation and cryoprotection, striatal and nigral tissue were sectioned and processed for tyrosine hydroxylase (TH), OX-42 (microglia), GFAP (astrocytes), 3-nitrotyrosine (3-NT) and inducible nitric oxide synthase (iNOS) using avidin-biotin technique (Iravani et al., 2002). Nigral TH-positive neurones were counted at the level of the the 3rd nerve. The relative optical density of striatal TH-immunoreactivity was quantified dorsomedially, dorsolaterally, ventromedially and ventrolaterally, at sections

In all cases the differences in treatments were compared using a one-way ANOVA followed by a Newman-Keuls post test.
In 5 out of 7 animals, (+)-amphetamine evoked a modest ipsiversive rotation in LPS-treated rats but not in controls. Immunohistochemical analysis of the brain sections revealed a marked loss of TH-positive neurons in the LPS-treated nigra and a moderate loss of TH in the terminal regions compared to the saline-treated animals (table 1). In the LPS group little or no 3NT+ve cells, but some iNOS+ve cells were observed in the nigra as well as in the striatum. Moderate numbers of GFAP and OX-42 +ve were observed only in the LPS group only.

	Control	LPS-treated	significance
Mean ipsiversive rotation/min	-0.17±0.88	39±6.3	*P<0.0001*
SN TH cell counts	178±13	39±16	*P≤0.0006*
DM-Str ROD	0.26±0.005	0.14±0.005	*P<0.001*
DL-Str	0.22±0.007	0.04±0.002	*P<0.001*
VM-Str	0.25±0.006	0.15±0.007	*P<0.001*
VL Str	0.21±0.005	0.09±0.005	*P<0.001*

Table 1: comparison of changes (mean±sem) between saline-treated (control) and LPS-treated rats. ROD, relative optical density.

The results of this study show that supranigral administration of the inflammogen, LPS can lead to the loss of dopaminergic neurons and terminals and can lead to motor abnormalities in rat. This study highlights the utility of nigral administration of LPS as a useful experimental model of inflammation in Parkinson's disease.

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Supported by The Wellcome Trust

203P EFFECT OF THE MITOCHONDRIAL COMPLEX I INHIBITOR MPP^+ AND THE PROTEASOMAL INHIBITOR LACTACYSTIN ON SH-SY5Y CELLS

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The molecular pathogenesis of Parkinson's Disease (PD) remains unknown, however recent evidence suggests that dysfunction of the proteasome, an enzyme responsible for the majority of intracellular proteolysis, may be implicated in the disease process (McNaught and Jenner, 2001). The neurotoxin 1-methyl-4-phenyl pyridinium (MPP^+) causes a parkinsonian syndrome in humans, non-human primates and mice *via* inhibition of complex I of the mitochondrial electron transport chain. Whether inhibition of proteasomal function alters the vulnerability of neurones to MPP^+ is not known. For this reason, we used the catecholaminergic SH-SY5Y cell line to determine whether proteasomal inhibition renders SH-SY5Y cells more susceptible to the effects of MPP^+.

SH-SY5Y cells were maintained at 37°C, 5% CO_2, in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 2mM glutamine, 20U/ml penicillin, 20µg/ml streptomycin and 100µg/ml kanamycin. SH-SY5Y cells were treated with a range of concentrations of MPP^+ (10, 100, 500, 1000µM) for up to 144h (n=5). Lastly, cells were treated with 1mM MPP^+ or 1µM lactacystin, alone or in combination, for 48h (n=4). Cell viability was measured by the 3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. Briefly, MTT was added to cells (5mg/ml) and incubated for 2 hours at 37°C. MTT was then removed and replaced with 100µl dimethyl sulfoxide. Optical density was measured at 595nm in a microtiter plate reader. Data was analysed by 2 WAY ANOVA or 1 way ANOVA followed by Dunnett's or Neuman-Keul's posthoc tests.

MPP^+ (10-1000µM) produced a concentration- and time-dependent decrease in mitochondrial activity in SH-SY5Y cells ($p<0.05$, 2 way ANOVA) whereby at 72h, 1mM MPP^+ caused a decrease in mitochondrial activity ($p<0.05$). After 72h, there was a decrease in mitochondrial activity at 500µM ($p<0.05$) and 1mM MPP^+ ($p<0.01$). Untreated cells and cells treated with 10µM MPP^+ showed an increase in mitochondrial activity after 96h ($p<0.05$), reflecting cell proliferation. To investigate the effect of proteasomal inhibition on MPP^+ treatment, we chose a concentration of 1mM MPP^+ for 48h, the highest dose at which there is no effect on mitochondrial activity. Lactacystin was used at a concentration of 1µM, leading to partial proteasomal inhibition (Schwarz *et al.*, 2000). In the combination study, neither lactacystin (1µM) or MPP^+ (1mM) altered mitochondrial activity, however in combination MTT reduction was reduced compared to control or either treatment alone (Figure 1, $p<0.05$, 1 way ANOVA with Neuman-Keuls).

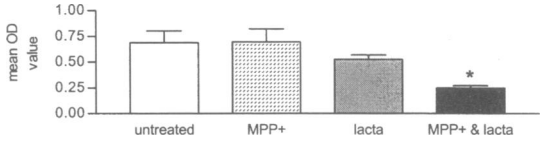

Figure 1: Effect of 1mM MPP^+ or 1µM lactacystin, alone or in combination, for 48h on total MTT reduction in SH-SY5Y cells

These data suggest that MPP^+ (1mM) inhibits mitochondrial function, but does not cause cell death in SH-SY5Y cells, but rather inhibits cell proliferation. In addition, these data show that a low, non-toxic dose of lactacystin increased the vulnerability of SH-SY5Y cells to MPP^+, suggesting that proteasomal inhibition may contribute to cell death associated with toxic insults.

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204P PYRUVATE RESCUES OLIGODENDROCYTE PROGENITOR CELLS FROM ZINC TOXIC INSULTS

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Chelatable Zinc (Zn^{2+}) is reported to be sequestered within glutamatergic synaptic vesicles and released upon neuronal depolarisation. During acute CNS injury (e.g. ischaemia), toxic levels of extracellular Zn^{2+} are reported to accumulate and induce neuronal degeneration (Weiss *et al.*, 2000). Interestingly, recent data suggest that Zn^{2+}-induced cortical neuronal toxicity may be reversed by pyruvate (Sheline *et al.*, 2001). However, it is unclear whether brain oligodendrocytes (macroglia responsible for CNS axonal myelination) are also vulnerable to Zn^{2+} toxic insults.

Oligodendrocyte progenitor cells (OPCs) were purified from mixed glial cell cultures prepared from neonatal Wistar rat (mixed sex) cerebral cortices and maintained in Bottenstein-Sato N2 medium, supplemented with basic fibroblast growth factor (10 ng ml^{-1}) and platelet derived growth factor-AA (10 ng ml^{-1}). OPC identity was confirmed via indirect immunofluorescence using a monoclonal anti-A_2B_5 antibody (Kelland and Toms, 2001). Assessment of OPC viability was performed via standard fluorescein diacetate and propidium iodide fluorescence microscopy or via 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrozolium bromide (MTT) reduction. Generation of cellular reactive oxygen species (ROS) was examined via 2',7'-dichlorofluorescein fluorescence. ATP levels were quantified using ATP Bioluminescence Assay Kit (Roche). Statistical analyses were performed using one-way ANOVA (Dunnett or Tukey-Kramer post-test).

Zn^{2+} induced widespread OPC death (pEC_{50}= 4.1 ± 0.1), with significant ($p < 0.01$) death occurring after either a 3 hr exposure to 300 µM Zn^{2+} or a 24 hr exposure to 100 µM Zn^{2+}. No significant synergy was found to occur between kainate and Zn^{2+} toxic insults. Additionally, Zn^{2+} induced toxicity was unaffected by either 10 µM Evans Blue (an AMPA receptor antagonist) or 1 µM nicardipine (a L-type voltage-gated Ca^{2+} channel inhibitor). Neither the antioxidants Trolox (100 µM), N,N'-diphenyl-1,4-phenylenediamine (1 µM) nor the spin-trap reagent N-tert-butyl-α-phenylnitrone (100 µM) afforded significant protection from 100 µM Zn^{2+}-induced toxicity. When tested at either 100 µM Zn^{2+} or 300 µM concentrations, Zn^{2+} failed to induce any significant increase in ROS production, in contrast to the H_2O_2 control (200 µM). When examined over a 24 hr period, Zn^{2+}-induced marked cellular ATP depletion. Furthermore, when tested at either 5 mM ($p < 0.01$) or 10 mM ($p < 0.001$), pyruvate was found to afford significant OPC protection from 100 µM Zn^{2+} insults.

These data suggest that OPCs are vulnerable to Zn^{2+} insults, possibly via a mechanism involving the inhibition of OPC glycolysis.

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Funded by the MRC and the Wellcome Trust.

205P CHANGES IN ALPHA- AND BETA- SECRETASE IN ALZHEIMER'S DISEASE

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Alzheimer's disease is characterised by deposition of beta-amyloid (Aβ) to form amyloid plaques. Aβ is cleaved from Amyloid Precursor Protein (APP) sequentially by β- and then γ-secretase. However, more typically APP is cleaved first by α-secretase, thus precluding Aβ production. The enzymes responsible for the majority of α- and β-secretase cleavage have been identified as tumour necrosis factor-converting enzyme (TACE) (Buxbaum *et al.*, 1999) and BACE (β-APP Site Cleaving Enzyme) (Vasser *et al.*, 1999) respectively. Increased Aβ is responsible for the neuropathology in early Alzheimer's disease. However the only proven risk factor for sporadic Alzheimer's disease is the presence of the apolipoprotein E4 isoform (Saunders *et al.*, 1993), which is also associated with decreased cholinergic function in Alzheimer brain (Poirier *et al.*, 1995). This study describes the measurement of activity of α- and β-secretase, cholinergic function and the relationship between them in sporadic Alzheimer patients.

α- and β-secretase activity was measured in brain tissue (South West Brain Bank) from control (n = 16) and Alzheimer temporal cortex (n=15) using synthetic peptides spanning the specific APP cleavage sites with EDANS (5-[(2-aminoethyl) amino]-napthalene-1-sulphonic acid) and DABCYL (4-(4-dimethylaminophenyl azo) benzoic acid) as reporter molecules (R&D systems). Choline acetyltransferase (ChAT) activity was measured in the same samples (Allen *et al.*, 1997) and *Apoε* genotype was determined (Wenham *et al.*, 1991). β-secretase activity was increased to 185% of normal (28.1±15.0) in sporadic Alzheimer patients (51.9±23.7) (p=0.003). Mean ± SD is given, comparison of means determined using the t-test, with Welch's correction where appropriate. By contrast, mean α-secretase activity in Alzheimer samples (1.40± 0.38) was reduced to 81% of normal (1.73±0.41; p=0.029). Mean ChAT activity in the Alzheimer cortex (11.9±6.4) was reduced to 13% of normal (90.5±26.3; p=0.0001). β-secretase activity negatively correlated (p=0.005) and α-secretase positively correlated with ChAT activity (p=0.047). In addition α-secretase activity was significantly lower in normal samples (62% p=0.002) and in all samples (73% p=0.001) from cases with at least one apoE4 allele present. Our findings that 80% of Alzheimer brains examined had an increase in β-secretase, a decrease in α-secretase, or both; may account for the means by which the majority of people develop Alzheimer's disease.

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206P EVIDENCE THAT 17BETA ESTRADIOL (17B) REDUCES CYTOCHROME C TRANSLOCATION AND MINIMIZES HIPPOCAMPAL DAMAGE CAUSED BY TRANSIENT GLOBAL BRAIN ISCHEMIA IN RAT

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A large body of evidence has been accumulated demonstrating that 17B confers neuroprotection to male and female rats exposed to focal cerebral ischemia (see Garcia-Segura *et al.*, 2001). At variance with these data, a few, inconclusive, studies have been carried out to address the question as to whether 17B may also be neuroprotective under conditions of transient global brain ischemia (4VO) (see Garcia-Segura et al. 2001). Here we report the original observation that early cytochrome c (cyt-c) translocation and delayed hippocampal damage caused by 4VO is minimized by systemic administration of 17B in male rats. The experimental protocol was in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992). Adult male Wistar rats (250-300 g) were subjected to 5 min 4-VO as previously described (see Pulsinelli & Buchan, 1988). After 6 h reperfusion the rat (n=3-4 per group) hippocampi have been dissected out, processed for subcellular fractionation (Corasaniti *et al.*, 2001) and cyt-c translocation was assessed in the cytosolic fraction by western blotting using a monoclonal antibody (1:2000 dilution; Pharmingen, CA, USA). Densitometric analysis of autoradiographic bands corresponding to cyt-c signal was according to Corasaniti *et al.* (2001) and the results expressed as mean±sem arbitrary units normalized to the densitometric signal of actin. After 72 h reperfusion, damage to hippocampal subsectors has been evaluated by bilateral counting the cells (mean±sem cells/100μm) in cresyl fast violet stained brain coronal (7-10 μm) sections (n=6 per rat; n=4 rats per group) of wax embedded brains as previously described (Sancesario *et al.*, 1994). Five min 4VO caused cyt-c translocation (2.01±0.28 vs sham 0.82±0.02, P<0.05, Student's t test) and this was minimized by 17B (0.2 mg/kg i.p.) given 1h before ischemia (0.91±0.27; P<0.05 vs 4VO); the latter effect has been partially reversed (1.32±0.41; P>0.05 vs 4VO) by prior administration of tamoxifen (TX; 0.25 mg/kg i.p. 1h before 17B). Delayed hippocampal damage typically caused by 4VO was abolished by 17B (0.2 mg/kg given i.p. once daily) and this was partially reversed by TX (0.25 mg/kg given i.p. once daily) (Table 1).

Table 1. *Systemic administration of 17B prevents hippocampal damage caused by 5 min 4VO: partial reversal by TX.*

Hippocampal area	Sham	4VO	17B+4VO	TX+17B +4VO
CA1/CA2	30.3±0.0	12.8±0.3*	29.7±0.7#	28.5±0.2$
CA3	32.8±0.1	16.6±0.1*	34.2±1.5#	28.4±0.8§
CA4	31.6±0.1	12.9±0.7*	36.4±0.4#	35.3±0.8$

The mean cell numbers have been evaluated statistically for differences by ANOVA followed by Tukey-Kramer test for multiple comparisons. *P<0.001 vs sham; #P<0.001 vs 4VO; $P>0.05 vs 17B+4VO; §P<0.01 vs 17B+4VO.

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207P ACTIONS OF (RS)-3,4-DICARBOXYPHENYLGLYCINE AND ITS (R)- AND (S)-ENANTIOMERS ON [^{3}H]D-ASPARTATE RELEASE IN THE RAT CEREBRAL CORTEX *IN VITRO*

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The phenylgylcine nucleus has recently provided a novel template for the synthesis of new ligands acting at ionotropic and metabotropic glutamate (mGlu) receptors. In particular, (RS)-3,4-dicarboxyphenylglycine (RS-DCPG) and its separate (R)- and (S)-enantiomers have been shown to be effective new anticonvulsant agents in a rodent model of reflex epilepsy, with the racemate proving notably more potent than either isomer alone (Moldrich et al., 2001). In an attempt to further elucidate the mechanism of action of these agents, the present study investigated the ability of the dicarboxyphenylglycines to modulate depolarisation-evoked efflux of pre-accumulated [^{3}H]D-aspartate ([^{3}H]D-Asp) from superfused rat cerebrocortical minislices.

Minislices were prepared from male Sprague-Dawley rats (300-350g) as previously described (Palmer & Reiter, 1994). [^{3}H]D-Asp release was evoked by rapidly superfusing the tissue with Krebs buffer containing 35mM or 50mM KCl for two 1 min periods (S1 and S2) and responses were quantified as maximum percent increases in tritium efflux over basal, as determined by liquid scintillation spectrophotometry. Drugs were applied 4 min prior to S2 and their effects expressed as mean percentage of control S2/S1 ratios. Significance of differences between control and treated groups were evaluated using a two-tailed Student's t-test for matched pairs.

(R)-DCPG (1-300μM) caused a concentration-dependent decrease in 50mM KCl-evoked [^{3}H]D-Asp release with maximal inhibition ($P<0.05$) seen at 100μM (% control S2/S1 ratios, 1μM: 96.0 ±6.7; 10μM: 89.3 ±4.4; 100μM: 82.2 ±6.4; 300μM: 84.9 ±5.7; n=6-7). Similar inhibitory activity was observed with (RS)-DCPG (1-300μM) (% control S2/S1 ratios, 1μM: 96.3 ±6.8; 10μM: 91.2 ±8.5; 100μM: 93.3 ±6.1; 300μM: 81.6 ±3.3; n=6-7; $P<0.01$ at 300μM). (R)- and (RS)-DCPG also caused significant ($P<0.05$) and comparable reductions in 35mM KCl-evoked [^{3}H]D-Asp release with maximal responses of 79.3 ±5.2% (n=7) and 82.4 ±5.3% (n=7), respectively. In contrast, (S)-DCPG (10nM-100μM) showed no significant effects ($P>0.05$) on 35mM or 50mM KCl-evoked [^{3}H]D-Asp release.

These data demonstrate that DCPG inhibits cortical excitatory amino acid release *in vitro* but unlike its anticonvulsant profile, this modulatory response appears to be wholly dependent on the (R)-enantiomer of the molecule. Inhibition of cortical excitatory transmitter release may therefore contribute to, but is unlikely to represent the sole mechanism of the anticonvulsant action of DCPG. As (S)-DCPG is an mGlu8 receptor-selective agonist, its lack of effect on KCl-evoked tritium efflux suggests that mGlu8 receptors do not play a role in modulating cortical glutamate release.

Supported by The Sir Jules Thorn Charitable Trust.

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208P *IN VIVO* EVIDENCE THAT THE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST 2-METHYL-6-(PHENYLETHYNYL)-PYRIDINE (MPEP) ACTS AS AN INHIBITOR OF THE NOREPINEPHRINE TRANSPORTER

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MPEP has been recently shown to have benzodiazepine-like anxiolytic properties (Brodkin et al., 2002) and desipramine-like effects in animal models of depression (Pilc et al., 2002). The mechanisms through which MPEP action results in anxiolytic and antidepressant effects are currently unknown, but likely systems involved are norepinephrine (NE) neurotransmission and the amygdala (AMY). In the present study, we therefore hypothesised that the anxiolytic-like and antidepressant-like profile of MPEP may be mediated by inhibition of the NE transporter (NET).

First, we examined the potency of MPEP to bind to, or inhibit uptake at 50 receptors and transporters. The only significant effect of MPEP was a 56% inhibition at the human NET at 1 μM. We then examined the simultaneous *in vivo* effects of MPEP (30 mg/kg i.p.) and desipramine (10 mg/kg i.p.) on both NE outflow in the AMY and cell firing in the locus coeruleus (LC) by means of differential pulse voltammetry (DPV) coupled with electrophysiology (Crespi, 2002). Anaesthetised adult male CD rats were implanted with carbon fibre microelectrodes (mCFE) into the AMY for DPV measurements and into the LC for electrophysiological analysis. DPV measurements (scans) were performed every 10 min, each scan lasting 20 sec. In between DPV measurements, continuous electrophysiological analysis was performed in the LC. After 60 min of concomitant measurements of basal extracellular NE levels in the AMY and cell firing in the LC the animals received MPEP, desipramine or saline.

In vivo DPV data revealed that MPEP significantly increased extracellular NE levels in the AMY ($p<0.01$). The increase reached a maximum level of approximately 150±19% of basal levels within 80 min post-treatment. Successively, the NE signal tended to return toward control levels. The concomitant cell firing monitored from the mCFE in the LC decreased to approximately 80% of basal levels within 3 min after MPEP treatment. Cell firing was then rapidly and significantly ($p<0.01$) reduced to approximately 10% of control levels 6 to 9 min later and remained at this level for the next 100 min. Treatment with desipramine resulted in a significant increase in extracellular NE in the AMY ($p<0.01$) up to 133±19% of basal levels within 10 min post-treatment. The increase reached a maximum level of approximately 142±16% of baseline and was observed 50 min post-treatment. Basal levels were recovered 60 to 90 min after treatment. Concomitantly, desipramine progressively and significantly decreased ($p<0.01$) cell firing in the LC to approximately 20% of basal levels within 18 min. This decrease persisted for the following 80 min of recording.

Thus, MPEP produced similar effects to those of desipramine both in the LC (cell firing) and at the terminal region (NE outflow in the AMY). Collectively, the results of the present studies provide potential new mechanisms through which MPEP exerts its anxiolytic and antidepressant effects.

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209P THE EFFECTS OF THE NMDA RECEPTOR ANTAGONISTS CERESTAT AND MEMANTINE ON THE HUMAN NICOTINIC α7 RECEPTOR EXPRESSED IN *XENOPUS* OOCYTES

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The α7 nicotinic acetylcholine receptor (nAChR) is widely expressed in the central nervous system and being located mainly at nerve terminals, is believed to regulate the release of neurotransmitters such as GABA and glutamate. It has also been implicated in neuroprotection and the pathogenesis of various neurological and psychiatric disorders such as Alzheimer's disease, Schizophrenia, Parkinson's disease and Tourette's syndrome. Previous studies have shown that N-methyl-D-aspartate (NMDA) receptor antagonists such as memantine, ketamine and MK-801 act as open channel blockers of the human α4β2 nAChR (Buisson and Bertrand, 1998; Coates and Flood, 2001). To further characterise the effect of NMDA antagonists on nAChR we have investigated the effect of cerestat and memantine on the human α7 nAChR using electrophysiological and radioligand binding techniques.

Mature female *Xenopus* frogs were sacrificed and the ovary lobes removed. Oocytes were manually defolliculated and microinjected with 50 ng *in vitro* transcribed human α7 nAChR RNA and maintained in Barth's medium until ready to use. Electrophysiological recordings were made 3 days post injection using standard two-microelectrode voltage-clamp methods.

^{125}I-α-bungarotoxin (^{125}I-αBgTx) binding studies were carried out using standard radioligand procedures on oocytes 4 days after RNA injection. Apool of approx. 100 oocytes was injected to provide sufficient material for binding studies.

In oocytes expressing the recombinant human α7 nAChR, ACh induces an inward current (EC_{50} = 100 μM). Following 1-2 minutes of preincubation, memantine or cerestat caused a reversible, concentration-dependent inhibition of the response to ACh (100 μM) with comparable potency (memantine IC_{50} = 5.4 ± 1.2 μM, mean ± S.E.M. n = 4; cerestat IC_{50} = 3.0 ± 1.1 μM, n = 4). Full concentration-response curves to ACh indicated that the inhibition was insurmountable, indicating non-competitive antagonism. Current-voltage curves constructed with 100 μM ACh in the presence or absence of IC_{50} concentrations of either memantine or cerestat showed that their inhibition was not voltage dependent (n = 6). Radioligand binding studies showed that neither memantine nor cerestat displaced more than 10 % of ^{125}I-α-BgTx binding to α7 nAChR, even at concentrations as high as 100 μM (n = 3).

These results indicate that both memantine and cerestat inhibit human α7 nAChR in a non-competitive and voltage-independent manner, indicative of allosteric inhibition.

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210P INHIBITION OF [^{3}H]-MK-801 BINDING BY NMDA CHANNEL BLOCKERS IN THE PRESENCE OF ALLOSTERIC MODULATORS.

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The N-methyl-D-aspartate (NMDA) receptor is a highly Ca^{2+}- and Na^{2+}-permeable ligand-gated ion channel with several distinct binding domains that can regulate opening of the cationic channel. [^{3}H]-MK-801 binds within the channel and blocks the transit of ions. The apparent affinity of this binding is modulated by the endogenous agonists L-glutamate, glycine and spermidine (Ransom & Stec, 1988). Some NMDA channel blockers are reported to be 'well-tolerated' (amantadine and memantine), whilst others are psychotomimetic (PCP and ketamine), (Chen *et al.*, 1992). The mechanism for this is not well described. We have tested 'well-tolerated' and 'poorly-tolerated' compounds to investigate molecular correlates of tolerability.

[^{3}H]-MK-801 radioligand receptor binding assays were carried out in extensively washed rat (male Sprague-Dawley) cortical membranes (Ransom & Stec, 1988). MK-801, amantadine and PCP (10^{-10} M to 10^{-3} M) competition concentration response experiments were performed in the presence of various allosteric modulators: 50 μM L-glutamate, 30 μM glycine and 50 μM spermidine and combinations of these. Following 60 min incubation at 21^{0}C, assays were terminated by rapid filtration through Whatman GF/B filters using a Packard Filtermate 196. The radioactivity was assessed with a Packard TopCount NXT microplate scintillation counter.

Table 1: Inhibition of [^{3}H]-MK-801 binding by MK-801, amantadine and PCP. The data are the means ± s.e.m. from three experiments for each condition listed.

Condition	Inhibition of [^{3}H]-MK-801 binding (EC_{50})		
	MK-801 (nM)	Amantadine (μM)	PCP (nM)
Control	6.4 ± 0.9	22.7 ± 2.8	61.7 ± 15.0
L-glutamate	4.6 ± 0.3	36.7 ± 5.0	84.3 ± 0.2
Glycine	5.0 ± 0.2	35.9 ± 0.7	94.3 ± 0.3
Spermidine	2.7 ± 0.1	72.3± 8.9	103.9 ± 20.2
L-glutamate + glycine	4.0 ± 0.5	48.4 ± 4.9	145.5 ± 9.8
L-glutamate + spermidine	3.1 ± 0.2	120.0 ± 0.9	177.7 ± 15.0
Glycine + spermidine	2.4 ± 0.2	92.4 ± 11.4	158.2 ± 41.8
L-glutamate + glycine + spermidine	3.0 ± 0.3	127.2 ± 11.4	174.1 ± 21.9

L-glutamate, glycine and spermidine all decreased EC_{50} values for MK-801 as a displacer, this increase in affinity has been reported previously (Ransom & Stec, 1988). The potency of amantadine and PCP as displacers of [^{3}H]-MK-801 was decreased in the presence of allosteric modulators, (table 1). This could be explained by the increase in apparent affinity of [^{3}H]-MK-801 or a change in the kinetic properties of the assay. The profile of modulation seen with 'well-tolerated' compounds and 'poorly-tolerated' compounds was not therefore dissimilar. Tolerability might well therefore reside in a dynamic feature of channel blockade such as use-dependence, voltage-dependence or dissociation rate.

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Ransom, R.W. & Stec, N.L. (1988) J. Neurochem., 51, (3) 830-836.

211P SYSTEMIC ADMINISTRATION OF BERGAMOT ESSENTIAL OIL (BEO) CAUSES Ca^{2+}-DEPENDENT ELEVATION OF HIPPOCAMPAL AMINO ACIDS NEUROTRANSMITTERS IN RAT

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Experimental data show that components of bergamot (*Citrus Bergamia* Risso) essential oil (BEO) interferes with fundamental cellular mechanisms such as K^+ and Ca^{2+} currents (During *et al.*, 2000; Occhiuto & Circosta, 1997) suggesting that it might affect finely regulated mechanisms such as exocytotic release and reuptake of neurotransmitters. Accordingly, the present work was carried out to characterise the effects of BEO on extracellular levels of amino acids (AAs) using the brain microdialysis technique in the rat. Male Wistar rats (250-300g) were anaesthetised with chloral hydrate (400 mg/kg i.p.) and a concentric microdialysis probe (2 mm) was implanted in the hippocampus (P, 5.2; L, 4.8; V, 8 mm, relative to bregma). After 24 h the probe was perfused with artificial CSF (flow-rate=1µl/min) and samples collected at 20 min intervals for 3 h, stored at -80°C and the AAs analysed by HPLC with fluorimetric detection (Richards *et al.*, 2000). The BEO, provided by Bergamot Consortium (Reggio Calabria, Italy; lot n° 41-FG-CM), was diluted 10:1 in 90% ethanol and was administered (after the third sample has been collected) in a final dose of 100 µl/kg (i.p.). As reported in table 1, this treatment (n=10 rats) showed a trend towards an increase of all of the AAs studied as compared to basal levels defined as the mean of the last 3 samples prior to BEO administration. However, statistical significance was achieved by aspartate and glycine only and this concerned the first sample after treatment; per se ethanol (100 µl/kg i.p.; n=3), 90% in double distilled H_2O, yielded no significant changes ($P>0.05$) as compared to basal levels of aspartate (0.22+0.09 vs 0.19±0.09), glutamate (4.17±1.72 vs 3.22±1.4), glycine (2.08±0.55 vs 2.07±0.5) and GABA (0.06±0.01). The observed increases in aspartate and glycine are Ca^{2+}-dependent since experiments (n=5) carried out in Ca^{2+}-free CSF yielded no significant changes (table 1) versus basal levels (µM, aspartate=0.28±0.17; glycine=0.97±0.30).

Table 1. Effect of i.p. administration of BEO on AAs extracellular levels (mean±sem) in the hippocampus of rat.

	Basal levels	Effect of BEO	
	(µM)	Normal CSF	Ca^{2+}-free CSF*
Aspartate	0.27±0.06	0.46±0.08**	0.11±0.02
Glutamate	2.76±0.73	3.95±0.86	ND
Glycine	1.60±0.18	2.48±0.16**	0.82±0.19
GABA	0.06±0.00	0.10±0.04	ND

*see basal levels in the text. **denote $P<0.001$ compared to basal, repeated measures ANOVA with post-hoc Dunnet's test. ND= not determined.

In conclusion, our present data suggest that BEO is endowed with properties enhancing discrete amino acid neurotransmission in the hippocampus of rat.

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Occhiuto F. & Circosta C. (1997) *Phytother Res* **11**, 450-453.
Richards D.A. *et al.* (2000) *Neurosci Lett* **293**, 183-186.

212P INDOMETHACIN, NAPROXEN AND CELECOXIB REVERSE MECHANICAL HYPERALGESIA IN A RAT MODEL OF POST-SURGICAL PAIN

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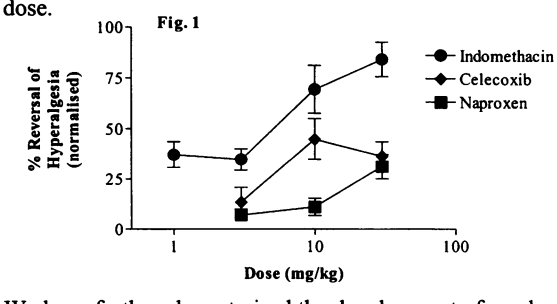
A rat model of post-surgical pain has recently been described (Brennan, 1996). Here we investigate the effectiveness of clinically relevant non-steroidal anti-inflammatory drugs (NSAIDs) in this model using the paw pressure assay of mechanical hyperalgesia.

Male Sprague-Dawley rats (180-200g) were used (Taconic, USA). A baseline paw withdrawal threshold (PWT) was measured (Walker *et al.*, 2000) and then rats were anaesthetised with. isoflurane/O_2 and the skin and muscle of the plantar surface of the left hind-paw was incised, the wound closed and the animals allowed to recover as previously described (Brennan, 1996). A time course of PWT measurements was made 1, 2, 3, 4, 7, 9 and 14 days post surgery. The effects of NSAIDs were measured 1 day post surgery. A pre-drug PWT was measured and compounds administered orally (p.o.) in 0.5% methylcellulose at 1, 3, 10 or 30 $mgkg^{-1}$ (volume = 10 $mlkg^{-1}$). Further PWT were measured 1, 3, 5 and 24 hours post dosing. Data is presented as mean PWT and percent reversal (± standard error of the mean [SEM]) according to the equation {[(PWT at 1 hour – Pre-drug PWT)/(Pre-injury PWT – Pre-drug PWT)]100}. Statistical significance was assessed by ANOVA followed by Student's *t* test ($P < 0.05$).

One day following surgery the mean PWT fell from 146 ± 19 g to 48 ± 6 g. On day 14 the incision group displayed a mean PWT of 119.4 ± 5.9 g which was significantly lower than that of the naïve control (158.3 ± 13.3 g; $P < 0.05$; n = 9/group). Indomethacin, significantly reversed PWT in the incised paw from 52 ± 8 g to 145 ± 19 g (30 $mgkg^{-1}$; 1 h). Celecoxib significantly reversed PWT in the incised paw from 42 ± 6 g to 77 ± 9 g (30 $mgkg^{-1}$, 1 h). Naproxen significantly reversed PWT in the incised paw from 42 ± 8 g to 75 ± 9 g (30 $mgkg^{-1}$; 1 h). All experiments included n = 9 rats/group ($P < 0.5$). Figure 1 shows percent reversal of hyperalgesia 1 hour post-dose.



We have further characterised the development of mechanical hyperalgesia in a previously described rat model of post-surgical pain. Here we demonstrate the efficacy of clinically effective NSAIDs. The relative order of potency for indomethacin, celecoxib and naproxen in this rat model is in accordance with their respective *in vitro* potencies at human cyclo-oxygenase I (Warner *et al.* 1996).

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213P BLOCKADE OF N-TYPE Ca^{2+} CHANNELS IN THE CENTRAL NUCLEUS OF THE AMYGDALA MODULATES NOCICEPTIVE BEHAVIOUR IN THE FORMALIN TEST IN RATS

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The amygdala is believed to mediate affective-motivational responses to noxious stimuli. The central nucleus of the amygdala (CEN) is an important component of the spino-(trigemino)-ponto-amygdaloid nociceptive pathway both receiving and projecting nociceptive information (Bernard and Besson, 1990; Bernard *et* al., 1992). The present study investigated the effect of intra-CEN administration of the irreversible N-Type Ca^{2+} channel blocker ω-conotoxin GVIA on formalin-induced nociceptive behaviour in rats with a view to understanding the contribution of the CEN in this model of tonic persistent pain.

Male Sprague-Dawley rats (200-250g, Charles River UK) were anaesthetised (isoflurane) and stereotaxically implanted with unilateral guide cannulae aimed at the CEN (AP -2.5 mm, ML -4.6 mm, DV -7.3 mm relative to Bregma, Paxinos and Watson, 1986). The experiment began 6-7 days post-surgery with rats receiving a single intra-CEN micro-injection of vehicle (250 nl artificial CSF) or ω-conotoxin GVIA (0.2 μg in 250nl vehicle) 10 min prior to intraplantar injection of formalin (50μl, 2.5% in 0.9% saline) or saline vehicle into the right hind paw. Using Ethovision tracking software, nociceptive behaviour was scored for 60 min post-formalin according to the CPS-$WST_{0,1,2}$ method (Watson *et al.*, 1997). Data were analysed by one- or two-way ANOVA followed by Bonferroni-Dunn post-hoc test.

Formalin-evoked nociceptive behaviour was biphasic with two peaks of intensity occurring within the first 5-10 min and 30-35 min post-formalin in vehicle treated rats (Figure 1).

Two-way ANOVA revealed that intra-CEN administration of ω-conotoxin GVIA had a significant effect on nociceptive behaviour over the 60 min trial ($F_{1, 11} = 2.445$, $P = 0.012$). Post-hoc tests revealed that administration of ω-conotoxin GVIA did not significantly effect the magnitude of the CPS but did result in earlier onset of the nociceptive response as evidenced by the left-ward shift in the curve (Figure 1). The early and late phase peaks in nociceptive response were observed 5 minutes earlier in ω-conotoxin GVIA-treated rats compared with vehicle-treated controls. Furthermore, ω-conotoxin GVIA-treated rats spent significantly more time licking/biting/flinching the injected paw during the first 5 minutes of the trial compared with vehicle-treated controls (89.1 ± 20.5 secs vs 19.3 ± 1.8 secs; $P < 0.05$).

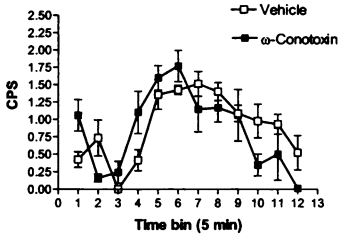

Figure 1. Effect of intra-CEN administration of ω-conotoxin GVIA (0.2 μg/250 nl) on the composite pain score (CPS) of formalin-induced nociceptive behaviour in rats. Data are means ± s.e. mean ($n = 4$).

These data demonstrate that functional N-Type Ca^{2+} channels in the CEN are involved in modulating the nociceptive response to tonic persistent pain. The results further support a role for the CEN in the integration of nociceptive processing.

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214P Δ^9-TETRAHYDROCANNABINOL ATTENUATES SENSORY NEUROTRANSMISSION IN THE RAT ISOLATED MESENTERIC ARTERIAL BED

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Capsaicin-sensitive sensory nerves are widely distributed in the cardiovascular system (Maggi & Meli, 1988). Activation of sensory nerves in the rat isolated mesenteric arterial bed releases calcitonin gene-related peptide (CGRP) resulting in vasodilatation (Kawasaki *et al.*, 1988). The psychoactive plant cannabinoid Δ^9-tetrahydrocannabinol (THC) acts as a partial agonist at CB_1 and CB_2 receptors (Pertwee, 1999). HU210, an analogue of THC, attenuates sensory neurotransmission in the rat isolated mesenteric arterial bed via a non CB_1/CB_2 mechanism (Ralevic & Kendall, 2001) In this study, we investigated if THC attenuates sensory neurotransmission in the rat isolated mesenteric arterial bed.

Male Wistar rats (250-300g) were killed by exposure to CO_2 and decapitation. Mesenteric beds were isolated and perfused with oxygenated Krebs' solution containing guanethidine (5μM) to block sympathetic neurotransmission (Ralevic & Kendall, 2001). After 30 min equilibration, preparations were preconstricted with methoxamine (10-100μM) and three consecutive frequency response curves to electrical field stimulation (EFS, 1-12Hz, 60V, 0.1ms, 30s) (EFS control, EFSI and EFSII) were constructed in each preparation. THC or vehicle (0.01% ethanol) was added after EFS control, 15 min before EFS I. Antagonists were added at the start of the equilibration period. In separate preparations, dose response curves were constructed to CGRP and capsaicin (0.05 pmol – 0.5 nmol) in the presence of THC (1μM) and ethanol (0.01%). Data are expressed as mean±s.e.m. and analysed by ANOVA with Tukey's post hoc test or by Student's unpaired t test.

EFS produced frequency-dependent relaxation (1-12Hz) of the rat mesenteric bed. THC (0.1 and 1μM) attenuated sensory neurogenic relaxation evoked during EFS I and EFS II compared with EFS control in a concentration-dependent manner. In the presence of 1μM THC the response at a submaximal frequency of 8Hz was reduced from 53.1±5.0%, EFS control, to 11.1±1.6%, EFSII (n=4, P<0.001).

The selective CB_1 receptor antagonist SR141716A (1μM) failed to block inhibition of the relaxation response by 1μM THC (8Hz, EFS control, 71.8±6.9% to EFSII, 27.9±5.6%, n=5, P<0.001). The CB_2 receptor antagonist SR144528 (1μM) also had no effect on THC-mediated inhibition (8Hz, EFS control, 52.08±4.7% to EFSII, 24.0±7.1%, n=8, P<0.01). There was no significant difference between EFS control, EFSI and EFSII generated in the presence of 0.01% ethanol. THC failed to affect the response to CGRP (pD_2 =10.49±0.1 and 10.12±0.1 in the presence and absence respectively of 1μM THC; P>0.05, unpaired t test), or capsaicin (pD_2 =10.08±0.1 and 10.11±0.2 in the presence and absence respectively of 1μM THC; P>0.05, unpaired t test)

These data show that THC attenuates sensory neurogenic relaxation in the rat isolated mesenteric arterial bed. The inhibitory actions of THC cannot be blocked by CB_1 and CB_2 antagonists possibly indicating that its actions are mediated by a novel receptor subtype. THC was found to have no inhibitory actions on vasorelaxation produced by exogenous CGRP or capsaicin indicating that its site of action is prejunctional and is not VR1 mediated.

We are grateful to Servier for financial support.

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215P EFFECT OF SELECTIVE ACTIVATION OF SPINAL CB_1 RECEPTORS ON MECHANICAL EVOKED RESPONSES OF SPINAL NEURONS IN RATS WITH SPINAL NERVE LIGATION AND SHAM RATS

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Mixed CB_1/CB_2 cannabinoid agoinsts have antinociceptive effects in animal models of neuropathic pain (Fox *et al.*, 2001). Arachidonyl-2-chloroethylamide (ACEA), a synthetic derivative of anandamide, is selective for the CB_1 receptor (Hillard *et al.*, 1999). In the present study effects of spinal administration of ACEA on mechanical evoked responses of spinal neurons in rats with L5 and L6 spinal nerve ligation and sham operated rats were investigated.

Spinal nerve ligated (SNL) and sham operated Sprague-Dawley rats (120-140g) were prepared under halothane anaesthesia (Chapman *et al.*, 1998). Development of allodynia was assessed with von Frey hair stimulation (8g). Extracellular recordings of convergent dorsal horn neurons (L4-L6) ipsilateral to SNL (n=8) or sham (n=7) procedure were made in anaesthetised (1% halothane in 66% N_2O / 33% O_2) rats two weeks post-surgery. Neuronal responses to Von Frey stimulation (12 and 80g for 10secs) of the receptive field were recorded. Responses were quantified as neuronal firing rate (Hz) during a 10sec stimulus duration: Control responses were determined and the effects of spinal administration of ACEA (0.5-500ng/50µl) applied to the spinal cord on evoked responses of spinal neurons was followed for 60 minutes. Data are presented as mean maximal effects and s.e.m; statistical analysis was performed using repeated measures and 2-way ANOVA and Dunnett's *post hoc* test. Mean depths of neurons were 618 ± 27µm (SNL), 649 ± 40 µm (sham).

Mechanical punctate stimulation of peripheral receptive fields with von Frey filaments evoked an incremental increase in spinal neuronal firing.

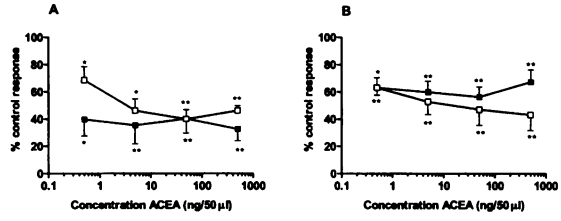

Fig 1. Effect of ACEA on innocuous (A; 12g) and noxious (B; 80g) mechanical punctate-evoked responses of spinal neurons in SNL (filled squares) and sham rats (open squares). *p<0.05, **p<0.001.

Spinal ACEA significantly reduced the 12 and 80g evoked responses in SNL and sham operated rats (Fig 1A,B). In SNL rats, spinal ACEA inhibited 12g evoked responses to a greater degree than 80g evoked responses (p<0.01) (Fig 1A,B). Whereas, in sham rats, 12 and 80g evoked responses were inhibited to a similar degree (Fig 1A,B).

Our data demonstrate that following SNL, innocuous transmission (A-fibres) is more sensitive to CB_1 mediated inhibition than noxious transmission (C-fibres) at the level of the spinal cord. These results indicate that the CB_1 receptor may be a potential target for the treatment of allodynia following nerve injury.

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216P INHIBITORY EFFECTS OF MORPHINE ON MECHANICAL PUNCTATE EVOKED RESPONSES OF POSTERIOR THALAMIC NEURONS IN THE ANAESTHETISED RAT

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One of the major pain pathways is the spinothalamic tract which terminates at various nuclei in the thalamus (Dostrovsky, 2000) including the posterior (Po) thalamic nuclear group (Dado *et al*, 1994). Systemic administration of morphine inhibits nociceptive activity in ventral posterolateral nucleus of the thalamus (Martin *et al.*, 1996). The aim of this study was to investigate the effect of systemic morphine on the mechanical punctate evoked responses of Po neurons.

In *vivo* electrophysiological studies were carried out on fifteen male Sprague-Dawley rats (220-280g weight). Extracellular recordings were made with stereotactically manipulated eight-channel microwire electrode arrays (NB Labs, Texas USA) connected to a Plexon Multichannel Acquisition Processor system (Jagger *et al.*, 1999). Multiple single-unit activity of nociceptive Po neurons (n=40) were recorded in isoflurane-N_2O:O_2 anaesthetised rats. Spontaneous and evoked neuronal responses to a range of calibrated (von Frey) monofilaments 7g, 14g, 21g, 60g and 80g were measured. Spontaneous activity was substracted from evoked response. Effects of intravenous morphine (0.5 mg/kg) and naloxone (0.2 mg/kg) administered 30 minutes following morphine were studied. Recording sites in the thalamus were determined histologically. Data was analysed using NeuroExplorer (Plexon Inc., Texas USA) and Prism v 3.03. Data are presented as mean maximal inhibition ± s.e.m. Statistical analysis of drug effects compared to pre-drug control values used Student's t-test, p<0.05.

Spontaneous activity of Po neurons was 13.2 ± 1.7 Hz. Control mechanical (7g, 14g, 21g, 60g and 80g) evoked responses of Po neurons were 2.4 ± 0.6 Hz , 4.4 ± 0.7 Hz, 4.5 ± 0.9 Hz ; 7.0 ± 1.4 Hz and 7.7 ± 1.0 Hz respectively. Spontaneous activity of the Po neurons was not altered by morphine, or naloxone. Morphine inhibited mechanical (7g, 14g, 21g, 60g and 80g) evoked responses 85 ± 1% (p<0.05), 91 ± 0.2% (p<0.001), 85 ± 0.4% (p<0.01), 89 ± 0.2% (p<0.01) and 99 ± 0.2% (p<0.001) respectively. Maximum inhibitory effects of morphine were observed at 30 minutes post administration. Inhibitory effects of morphine on mechanical punctate (14g, 21g, 60g and 80g) evoked responses of Po neurons were significantly reversed by naloxone 69 ± 1% (p<0.05), 59 ± 1% (p<0.05), 48 ± 1% (p<0.05) and 50 ± 1% (p<0.05) respectively.

Inhibitory effects of morphine on mechanical evoked responses reported in this study are similar to those described for ventral posterolateral thalamic nucleus (Martin *et al.*, 1996) Naloxone partially blocked the inhibitory effects of morphine, demonstrating the role of mu opioid receptor in mediating the antinociceptive effect of morphine in the posterior thalamic nuclear group of the thalamus.

CBAA is supported by UNIVERSITI SAINS MALAYSIA

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217P MEASUREMENT OF THE NOXIOUS HEAT THRESHOLD: A NOVEL APPROACH TO STUDY HEAT HYPERALGESIA AND THE ANTINOCICEPTIVE EFFECTS OF DRUGS

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In the conventional hot plate, tail flick and paw withdrawal tests latency of nocifensive behavioural reactions to heat stimuli of constant suprathreshold intensity is measured. These tests are largely insensitive to the antinociceptive effect of cyclooxygenase inhibitors.

In this study a novel approach to study thermonociception was established using a newly developed increasing-temperature hot plate to determine the noxious heat threshold temperature of conscious unrestrained rats or mice. The temperature of the plate was linearly (6 or 12°C/min) increased until the animal exhibited nocifensive behaviour i.e. licking or lifting of the hindpaw; the corresponding plate temperature was considered as the noxious heat threshold.

The heat threshold of female Wistar rats (120-180g) was reproducible upon 5 repeated determinations at intervals of 5 min, 30 min or 1 day (ANOVA followed by Newmann-Keuls test, n=8). Morphine, diclofenac and paracetamol caused an elevation of the noxious heat threshold following a 30 min i.p. pretreatment, the minimum effective doses being 3, 10, and 200 mg kg^{-1}, respectively (n=8, t-test for paired samples for comparison of pre-drug and post-drug values). Intraplantar (i.pl.) injection of resiniferatoxin (RTX, 0.05 nmol), a potent agonist at the capsaicin VR1/TRPV1 receptor (Gunthorpe *et al.*, 2002), induced a profound drop of heat threshold to the innocuous range with a maximal effect (10.0±1.1°C drop, n=16) 5 min after RTX administration and a complete recovery by 25 min. This heat allodynia was inhibited by i.pl. pretreatment for 5 min with the VR1/TRPV1 receptor antagonist iodo-resiniferatoxin (Wahl *et al.*, 2001) (I-RTX, 0.05 nmol) to 49% (n=8) and by i.p. pretreatment for 25 min with morphine, diclofenac and paracetamol, the minimum effective doses being 1, 1, and 100 mg kg^{-1}, respectively (n=12, t-test for unpaired samples for comparison of threshold drops at 5 min in drug- and solvent-treated animals). I-RTX (0.1 or 1 nmol/paw, n=10) failed to alter the heat threshold either acutely (5-30 min) or on the long-term (1-3 days). α-β-methylene-ATP (0.3 μmol/paw), a metabolically stable analogue of ATP, also induced a drop of the heat threshold (5.2±1.4°C at 5 min, n=8) which was inhibited to 17% by a 5 min pretreatment with the P2 purinergic receptor antagonist PPADS (0.15 μmol/paw, n=6) but not I-RTX (0.05 nmol/paw, n=8). Bradykinin (3 nmol/paw) also induced a decrease in the noxious heat threshold (3.8±0.6°C at 5 min, n=8). Painting the plantar skin with xylene (10-50%) induced nociception but failed to alter the heat threshold. The heat threshold of VR1/TRPV1 knock-out female C57Bl6 mice (Davis *et al.*, 2001) weighing 10-30g was not different from that of the wild-type animals (45.6±0.5 vs. 45.3±0.4°C, n=10, t-test for unpaired samples). The VR1/TRPV1 receptor is unlikely to be involved in the detection of the noxious heat threshold of peripheral nociceptors.

It is concluded that the RTX-induced massive drop of heat threshold measured by the increasing-temperature hot plate is a novel heat allodynia model exhibiting a high sensitivity to various analgesics including a cyclooxygenase inhibitor. I-RTX proved to be a potent and selective VR1/TRPV1 receptor antagonist. RTX, α-β-methylene-ATP and bradykinin appeared to have different efficacies regarding the drop of heat threshold whereas xylene was ineffective.

G. Pethő was granted by the Bolyai János Research Fellowship.

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218P ANALGESIC EFFECT OF TT-232, A HEPTAPEPTIDE SOMATOSTATIN ANALOGUE, IN ACUTE AND CHRONIC PAIN MODELS OF THE RAT

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Somatostatin is released from capsaicin-sensitive sensory nerve terminals and exerts systemic anti-inflammatory and anti-nociceptive actions (Szolcsányi et al., 1998). TT-232 is a selective and stable heptapeptide somatostatin analogue (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2), which inhibits in-flammatory responses and neuropeptide release in rat and mouse models (Helyes et al., 2001; Pintér et al., 2002). In the present study the effect of this analogue was examined on formalin-induced acute pain, heat threshold and thermal hyperalgesia, Freund's adjuvant-induced chronic inflammatory pain and neuropathic mechanical hyperalgesia.

Subplantar (s.pl.) formalin injection (50 μl, 2.5%) was applied into the left hindpaw of male Wistar rats (200-220g, n=12/ group). Nocifensive behaviour was examined in the intervals of 0-5 min (phase 1) and 25-45 min (phase 2). TT-232 (20-160 μgkg^{-1}) was administered i.p. 10 min before formalin. Composite pain score (CPS) was calculated: (1 x number of paw elevations + 2 x duration of paw lickings in sec)/ test duration. Thermal anti-nociceptive action of TT-232 (5-200 μgkg^{-1} i.p.10 min earlier) was measured on female Wistar rats (140-180g, n=8/ group) on increasing temperature hot plate heated up from 30°C (6°C/min) until paw licking or shaking was observed. In the heat allodynia test the VR1 agonist resiniferatoxin (RTX, 0.05 nmol) was injected in the same way 10 min after TT-232 to induce drop of heat threshold (10-12°C) for 20 min. Arthritis of the left tibio-tarsal joint of male Lewis rats (180-220g, n=10/ group) was induced by 0.1 ml complete Freund's adjuvant. Hindpaw volume was measured by plethysmometry and mechano-nociceptive threshold by Randall-Selitto test before and every day throughout the experimental period of 18 days. TT-232 (50-400 μgkg^{-1}) was administered i.p twice a day. Neuropathic hyperalgesia of male Sprague-Dawley rats (180-200g, n=8/ group) was induced by partial ligation of unilateral sciatic nerve. TT-232 (2.5-20 μgkg^{-1}) was injected i.p. on the 7th postoperative day 10 min before measuring mechanonociceptive thresholds. Solvent of TT-232 was given to control rats. ANOVA followed by Bonferroni's modified t-test was used for statistical evaluation.

CPS in phase 2 of the formalin test was inhibited by 43.3±2.1% (mean ± S.E.M) and 72.5±2.9% after 40 and 80 μgkg^{-1} TT-232, respectively. TT-232 (100 and 200 μg/kg) induced 0.9±0.2 and 1.5±0.4°C increase of heat threshold. RTX-evoked heat allodynia was diminished by 60.0±3.2 and 63.2±5.1% after 10 and 20 μgkg^{-1} TT-232. Adjuvant-induced mechanical hyperalgesia (22.9±2.9%) was converted to 18.3±1.8% and 14.1±1.4% hypoalgesia by 100 and 400 μgkg^{-1} TT-232 on the 14th day. TT-232 (10 and 20 μgkg^{-1}) reversed neuropathic hyperalgesia to 13.7±1.9 and 26.3±2.8% hypoalgesia.

Our results demonstrate that TT-232 inhibits acute chemical nociception, thermal hyperalgesia, chronic inflammatory/ neuropathic pain, therefore it can open future perspectives in the treatment of several pain syndromes. Zs.

Helyes, G. Pethő were supported by J. Bolyai Fellowship.

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219P EFFECT OF VANILLOID RECEPTOR ANTAGONIST IODO-RESINIFERATOXIN ON SPINAL NOCICEPTIVE TRANSMISSION IN ANAESTHETISED RATS

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Vanilloid receptors (VRs) have been shown to be present on nociceptive C- and Aδ-fibres in the dorsal root ganglia and on primary afferent neurones (Guo *et al.*,1999). Previously, we have shown that capsazepine, the VR1 antagonist, inhibits C- and Aδ-fibre evoked responses of spinal neurones (Kelly and Chapman, 2002). Iodo-resiniferatoxin (I-RTX) is at least 40-fold more potent than capsazepine at VR1 (Wahl *et al., 2001).* This study investigates the effect of I-RTX on evoked responses of spinal neurones *in vivo*.

Extracellular recordings of convergent dorsal horn neurones were made in anaesthetised (1% halothane in 66% N_2O / 33% O_2) male Sprague Dawley rats (230-300g) (Kelly and Chapman, 2002). Neuronal responses to a train of 16 transcutaneous electrical stimuli at 0.5Hz (3 x C-fibre threshold) of the receptive field were recorded. Responses were quantified: Aβ-fibre 0-20ms; Aδ-fibre 20-90ms; C-fibre 90-300ms and post-discharge 300-800ms. Effects of spinal application of I-RTX (0.05-10μM / 50μl) on evoked neuronal responses were measured. Data are presented as mean maximal effects and standard error of mean; statistical analysis was performed using repeated measures ANOVA and Dunnett's *post hoc* test.

Spinal application of I-RTX did not significantly alter Aβ-evoked responses of spinal neurones, however Aδ-fibre evoked responses were significantly inhibited by I-RTX in a dose related manner (fig. 1A). Similarly, evoked C-fibre responses and post-discharge responses of spinal neurones were dose relatedly reduced (fig. 1B).

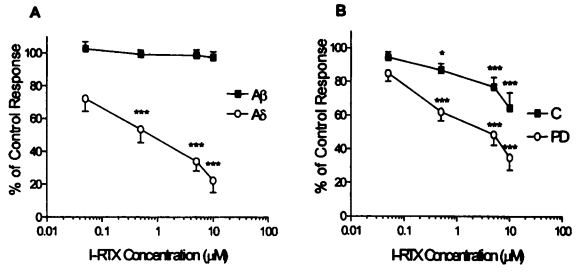

Fig. 1. Effect of spinal I-RTX (0.05-10μM / 50μl) on (A) A-fibre and (B) C-fibre evoked responses of dorsal horn neurons (n=7). $^{*}P< 0.05$, $^{***}P< 0.001$.

Spinal application of I-RTX selectively inhibited noxious Aδ- and C-fibre evoked responses of dorsal horn neurones. These results further support the contribution of VR1 to nociceptive processing. Our data demonstrate that I-RTX is a more potent antagonist than the previously reported capsazepine compound (Kelly and Chapman, 2002) at inhibiting nociceptive responses *in vivo*.

This study was supported jointly by the University of Nottingham and GlaxoSmithKline.

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220P DIFFERENCES IN THE AFFINITY OF CAPSAZEPINE AT RECOMBINANT RAT AND HUMAN VR1 RECEPTORS

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Vanilloid (VR1) receptors are members of the TRP family and act as ligand gated, Ca^{2+} permeable ion channels (Gunthorpe *et al.*, 2002). Capsazepine is the standard antagonist used in studies of VR1 activity despite a wide range of apparent antagonist potency values (e.g., pK_B from 5.1 to 7.5) being reported (Belvisi *et al.*, 1992; Jerman *et al.*, 2000). In this study we have compared the effects of this antagonist at recombinant human and rat VR1 receptors expressed in HEK293 cells at 22 and 37°C.

HEKhVR1 and HEKrVR1 cells were maintained in minimum essential medium supplemented with 10% foetal calf serum, 0.2 mM L-glutamine, 2.5 μg/ml fungizone, 50 IU/ml penicillin and 50 μg/ml streptomycin, at 37°C in 5% CO_2/air. Monolayer cultures were detached, washed twice with and suspended in Krebs-HEPES buffer. Resulting suspensions were loaded with 5 μM Fura-2AM for 30 min at 37°C, followed by 20 min de-esterification at room temperature in the dark. Loaded cells were stored on ice until use. 200 μl of cell suspension was added to 1.8 ml of Krebs-HEPES buffer in a cuvette and allowed to equilibrate to 22°C or 37°C. Fluorescence emission was measured at 510 nm with excitation at 340 nm and 380 nm using a Perkin-Elmer LS50B fluorimeter. Intracellular Ca^{2+} was calculated using temperature corrected Fura2 K_d values as described (Sprague *et al.*, 2001). Full concentration response curves (crc) were constructed to capsaicin in the absence and presence of 30 μM capsazepine (preincubated for 10mins). Following basal subtraction and normalisation to the maximum control capsaicin response paired pEC_{50} values were obtained using GRAPHPAD PRISM (V3.0) and pK_B values estimated using the Gaddum-Schild equation.

Capsaicin produced a concentration dependent increase in $[Ca^{2+}]_i$ in HEK cells expressing either human or rat VR1 receptors and at both temperatures (pEC_{50} values ~200 nM to 1 μM). There was a significant increase in potency at the lower temperature in HEKrVR1. Capsazepine produced a rightward shift in the crc to capsaicin yielding pK_B values shown in Table 1. Capsazepine was ~6 fold more potent at hVR1.

Table 1. Comparison of pK_B values for capsazepine at human and rat VR1 receptors. Data are mean±s.e.mean (n=3-4) at 22 and 37°C. *p<0.05 compared to rVR1, unpaired t-test.

	pK_B 22°C	pK_B 37°C
rVR1	5.98±0.09	6.02±0.10
hVR1	6.76±0.25*	6.75±0.04*
rVR1/hVR1	6.0	5.4

In this study we have used a classical rightward shift approach in a cuvette based system to determine competitive antagonism of capsazepine. We have shown that capsazepine is a more potent antagonist at hVR1 receptors.

Funded by a project grant from *The British Journal of Anaesthesia/ Royal College of Anaesthetists.*

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221P FUNCTIONAL COUPLING OF THE NOCICEPTIN RECEPTOR NATIVELY EXPRESSED IN DOG BRAIN MEMBRANES

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Nociceptin (N/OFQ) is the endogenous ligand for the $G_{i/o}$ protein coupled nociceptin receptor, NOP (Mogil *et al.*, 2001). We have previously reported that dog brain membranes express low levels (~36fmol/mgp) of NOP (Johnson & Lambert, 2002). In this study we have examined the effects of N/OFQ in a series of [^{3}H]N/OFQ and GTPγ^{35}S binding assays to assess functional activity of dog NOP.

Dog brain (Beagle, either sex, 9-12 months old and 9-14kg) membranes were prepared essentially as described in Okawa *et al.* (1999). Displacement assays were performed in 0.5ml 50mM Tris-HCl, 5mM $MgSO_4$, pH7.4 with 0.5% BSA, 120mM NaCl, 100μM GTPγS and 10μM amastatin, bestatin, captopril and phosphoramidon in various combinations. 200μg of membrane protein was incubated with ~0.2nM [*leucyl*-^{3}H]N/OFQ and N/OFQ at different concentrations. Non-specific binding (NSB) was determined with 1μM N/OFQ. GTPγ^{35}S binding experiments were performed essentially as described by Berger *et al.* (2000). 20μg of membrane protein was incubated in 0.5ml 50mM Tris-HCl, 100mM NaCl, 1mM $MgCl_2$ 0.2mM EGTA pH 7.4 containing 100μM GDP, 0.15mM bacitracin, 1mg/ml BSA, peptidase inhibitors (as above) and ~150pM GTPγ^{35}S with various concentrations of N/OFQ. NSB was defined with 10μM unlabelled GTPγS.

N/OFQ displaced [^{3}H]N/OFQ with pK_i and slope values shown in table 1. These data were modelled to two sites using GraphPad PRIZM V3. Addition of NaCl/GTPγS produced a steepening of the curve.

N/OFQ produced a consistent although small stimulation of GTPγ^{35}S binding with pEC_{50} and E_{max} (stimulation factor) values of 8.32±0.36 and 1.11±0.02 (n=5), respectively.

Table 1. One and two site analysis of [^{3}H]N/OFQ displacement curves in the absence and presence of NaCl/GTPγS (H represents high and L low affinity sites). Data are mean±s.e.mean (n=3).

	- NaCl/GTPγS	+ NaCl/GTPγS
One site - pK_i	9.62±0.07	9.58±0.24
Slope	0.38±0.05	0.95±0.06
Two site - pK_i H	11.64±0.37	Not present
% H	33	Not present
pK_i L	9.44±0.15	9.68±0.30
% L	67	100

Despite relatively low receptor density we were able to detect functional activity of native dog NOP using a series of "GTP-shift" studies and N/OFQ stimulated GTPγ^{35}S binding.

We thank Dr R. Guerrini (Ferrara, Italy) for N/OFQ. E.E. Johnson is in receipt of a Pfizer sponsored studentship.

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222P PHARMACOLOGICAL CHARACTERIZATION OF [^{3}H]UFP-101, A NOVEL RADIOLIGAND SELECTIVE FOR THE NOCICEPTIN/ORPHANIN FQ RECEPTOR

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Currently there are no radiolabelled antagonists available for the nociceptin/orphanin FQ (N/OFQ) receptor (NOP) despite the fact that these would possess several advantages over radiolabelled agonists, in that they are not influenced by G-protein coupling. In the present study using membranes prepared from rat cerebrocortex and Chinese hamster ovary cells expressing the human NOP (CHO_{hNOP}) , we characterised a [^{3}H]labelled form of the novel NOP selective antagonist UFP-101 ([Nphe[1],Arg[14],Lys[15]]N/OFQ-NH_2 (Calo' et al 2002)).

Binding of [^{3}H]N/OFQ (specific activity ~140Ci/mmol) and [^{3}H]UFP-101 (specific activity 22.6 Ci/mmol) was performed in 0.5ml of 50mM Tris-HCl, 5mM $MgSO_4$ buffer plus 10μM amastatin, bestatin, captopril and phosphoramidon and 150-300μg of rat (Female Wistar 200-250g) cerebrocortical or 15-30μg of CHO_{hNOP} membrane protein (Hashiba et al., 2002). Saturation studies (~0.01-2nM [^{3}H]N/OFQ, 0.01-4nM [^{3}H]UFP-101) were used to determine receptor density (B_{max}) and radioligand equilibrium dissociation constant (K_D). In competition studies a fixed concentration of either radioligand (~0.2nM [^{3}H]N/OFQ, 0.4nM [^{3}H]UFP-101) was incubated with increasing concentrations of unlabelled NOP ligands. In all studies non-specific binding was defined in the presence of 1μM N/OFQ.

The binding of both [^{3}H]N/OFQ and [^{3}H]UFP-101 was concentration dependent and saturable. B_{max} and pK_D values (n=6) in rat were 88±3 and 65±4 fmol/mg protein and 10.34±0.04 and 10.12±0.04 respectively. B_{max} and pK_D values (n=6) in CHO_{hNOP} were 580±100 and 561±91 fmol/mg protein and 10.19±0.08 and 9.79±0.08 respectively. pK_i values for a range of NOP ligands in CHO_{hNOP} are shown in Table 1. Similar data were obtained in rat.

Table 1. pK_i values for a range of peptide and non-peptide NOP ligands estimated in competition binding assays with [^{3}H]N/OFQ and [^{3}H]UFP-101 in CHO_{hNOP}.

	[^{3}H]-N/OFQ	[^{3}H]-UFP-101
N/OFQ	9.91±0.16	9.70±0.04
N/OFQ(1-13)NH_2	10.18±0.06	9.67±0.17
[Nphe[1]] N/OFQ(1-13)NH_2	8.48±0.07	8.19±0.19
N/OFQ(1-9)NH_2	7.95±0.13	7.97±0.36
[pF(phe[4])]N/OFQ(1-13)NH_2	10.57±0.08	9.86±0.30
[Arg[14],Lys[15]]N/OFQ	10.63±0.02	10.24±0.09
UFP-101	9.62±0.09	9.40±0.09
J 113397	8.83±0.14	8.43±0.15
Naloxone	<<5	<<5

Data are mean±s.e.mean of 4-5 separate experiments.

In this study we report the binding characteristics of the first radiolabelled peptide antagonist and suggest that this may be a useful alternative to the traditional radiolabelled N/OFQ agonist.

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223P PHARMACOLOGICAL PROFILE OF THE CYCLIC NOCICEPTIN/ORPHANIN FQ ANALOGUES c[$Cys^{10,14}$]N/OFQ(1-14)NH_2 AND c[$Nphe^1$,$Cys^{10,14}$]N/OFQ(1-14)NH_2

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Nociceptin/Orphanin FQ (N/OFQ) elicits various biological actions via activation of a specific receptor named N/OFQ peptide receptor (NOP). N/OFQ(1-13)NH_2 (1-13) and [$Nphe^1$]N/OFQ(1-13)NH_2 behaved, at NOP receptors, as full agonist and pure antagonist, respectively (Calo' et al, 2000). We have produced cyclic analogues of these two peptides by introducing Cys residues in positions 10 and 14 (Ambo et al, 2001). c[$Cys^{10,14}$]N/OFQ(1-14)NH_2 ([$Cys^{10,14}$]) and c[$Nphe^1$,$Cys^{10,14}$]N/OFQ(1-14)NH_2 ([$Nphe^1$,$Cys^{10,14}$]) have been characterized in receptor binding, GTPγ^{35}S binding, and cAMP accumulation experiments performed with Chinese hamster ovary cells expressing the human NOP(CHO_{hNOP}).

Binding of [*leucyl*-^{3}H]N/OFQ was performed in 0.5ml of 50mM Tris-HCl, 5mM $MgSO_4$ buffer plus 10μM amastatin, bestatin, captopril and phosphoramidon with 10μg of CHO_{hNOP} membranes (Hashiba et al., 2002). GTPγ^{35}S binding was performed in buffer containing 50mM Tris-HCl, 100mM NaCl, 1mM $MgCl_2$, peptidase inhibitors (as above), 150μM bacitracin, 100μM GDP, ~150pM GTPγ^{35}S and 20μg CHO_{hNOP} membranes (McDonald et al., 2002). Inhibition of forskolin stimulated cAMP formation was performed with whole cells in Krebs/HEPES buffer and assayed using a competitive protein binding assay (McDonald et al, 2002). 1-13, [$Cys^{10,14}$] and [$Nphe^1$,$Cys^{10,14}$] were included at various concentrations and in various combinations.

All ligands displaced [^{3}H]N/OFQ binding with a rank order pK_i of 1-13>[$Cys^{10,14}$]>>[$Nphe^1$,$Cys^{10,14}$], Table 1. Both 1-13 and [$Cys^{10,14}$] stimulated GTPγ^{35}S binding and inhibited forskolin stimulated cAMP formation with similar maximal effects, Table 1. In GTPγ^{35}S binding (but not cAMP) assays [$Nphe^1$,$Cys^{10,14}$] was inactive as an agonist but antagonised the effects of 1-13 with a pK_B value as in Table 1.

Table 1. Effects of 1-13, [$Cys^{10,14}$] and [$Nphe^1$,$Cys^{10,14}$] at human recombinant NOP receptor expressed in CHO cells

	1-13	[$Cys^{10,14}$]	[$Nphe^1$,$Cys^{10,14}$]
pK_i	10.81±0.07	10.23±0.16	8.47±0.10
GTPγ^{35}S	8.57±0.09[a]	8.29±0.08[a]	7.05±0.05[b]
cAMP	10.19±0.14[a]	9.29±0.05[a]	Agonist

Data are mean±s.e.mean (n=3-7). [a]pEC_{50} values, [b]pK_B values (based on 10μM [$Nphe^1$,$Cys^{10,14}$]).

Cyclic[$Cys^{10,14}$] modification produces a small decrease in affinity/potency. [$Nphe^1$,$Cys^{10,14}$] is likely to act as a partial agonist. *In vivo* studies of these novel cyclic peptides are required to determine their actual value as pharmacological tools.

Supported in part by a grant from the BJA/RCA.

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224P MODULATION OF HUMAN SEROTONIN TRANSPORTER ACTIVITY BY HUMAN NK_1 RECEPTOR: EVIDENCE FOR AN INTRACELLULAR CROSS-TALK MECHANISM

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Recently, antagonists at the neurokinin 1 (NK_1) receptor, the preferred receptor for Substance P (SP), have been reported to have antidepressant activity in man and in pre-clinical animal models. However, little is known regarding their molecular mechanism of action. An interaction between the NK_1 receptor and serotoninergic systems has been reported (Froger N. *et al*, 2001). The aim of this study was to investigate further whether the NK_1 receptor is able to interact with the human serotonin transporter (hSERT) through an intracellular cross-talk mechanism. A report from Ramamoorthy *et al.* (1998) showing that SERT activity can be regulated through protein kinase C (PKC)-mediated phosphorylation has suggested that the transporter activity could be regulated through a Gq/PKC-dependent pathway. To test the hypothesis that the NK_1 receptor is able to regulate SERT activity through the NK_1-preferred Gq/PKC signal transduction pathway, we developed a co-expression cell system consisting of the transient expression of a hSERT-containing plasmid in Chinese Hamster Ovary (CHO) cells stably expressing the human NK_1 receptor (CHO- hNK_1). Porcine Renal Proximal Tubule (LLC-PK_1) cells stably expressing hSERT were used as a control.

The pharmacological properties of the hNK_1 receptor and hSERT were assessed by measuring intracellular cytosolic calcium and serotonin (5-HT) uptake levels, respectively. The determination of intracellular calcium levels was performed using a fluorometric imaging plate reader (FLIPR). Cells were pre-labelled with Fluo-4 at 37 °C and non-cumulative concentration-response curves (CRC) were performed with SP. When appropriate, cells were preincubated with the NK_1 receptor antagonist GR205171 (Gardner C. J. *et al*, 1996) for 30 min at 37 °C. Response signals were expressed as a percentage of the maximal signal observed with 300 nM SP. In the 5-HT uptake assay, cells were incubated for 10 min in the absence (total uptake) or presence (non specific uptake) of 1 μM paroxetine. Uptake was initiated by addition of 50 nM [^{3}H]5-HT, and terminated after 10 min by rapid washing. The intracellular [^{3}H]5-HT levels were measured by scintillation counting. The inhibitory effect of paroxetine was determined by preincubating the cells for 10 min at room temperature. Data were expressed as mean ± s.e.m. unless stated otherwise. Statistical analysis was performed using the Tukey test.

SP (5 pM-300 nM) increased cytosolic calcium release in a concentration dependent manner with a similar pEC50 value in CHO-hNK_1 cells transiently expressing hSERT (10.26 ± 0.08, n=3) and in mock-transfected cells (10.09 ± 0.07, n=3).

Moreover, GR205171 (0.3-3 nM) produced a similar rightward shift of the SP-CRC and a concentration dependent decrease of the agonist maximum response with an apparent pK_B value of 10.70 ± 0.06 (n=3) in control CHO-hNK_1 cells and of 10.70 ± 0.07 (n=3) in hSERT expressing cells, as determined with 1 nM GR205171.

A good level of functional expression of hSERT by transient transfection was evidenced in the 5-HT uptake assay where [^{3}H]5-HT total uptake was 7.0 ± 0.4 % (n=6). Paroxetine (10 pM-1 μM) inhibited 5-HT uptake in a concentration-dependent manner with a pIC50 value of 8.64 ± 0.11 (n=6), in line with previously obtained data in LLC-PK_1 cells stably expressing hSERT (8.21 ± 0.03, n=6).

To verify that hSERT can be modulated by PKC-dependent phosphorylation, we studied the effect of 1 μM of the PKC activator phorbol 12-myristate 13-acetate (ß-PMA). ß-PMA produced a time-dependent decrease of 5-HT uptake with a maximal reduction of 62.2 % (n=2) after 60 min of treatment, confirming previously reported data in other cell system (Quian Y. *et al*, 1997).

We then tested the effect of NK_1 receptor activation on hSERT activity co-expressed in CHO-hNK1 cells. Cell treatment with 1, 10 and 100 nM SP resulted in a concentration-dependent decrease of 5-HT uptake after a 60 minutes of preincubation. A significant reduction of 13.7 ± 2.1% (n=4, $p<0.01$) and 18.3 ± 0.8% (n=5, $p<0.0001$) was obtained preincubating the cells with 10 nM and 100nM of SP respectively. This effect was completely abolished by addition of 100 nM GR205171.

Taken together, these results provide the first evidence that SP can inhibit 5-HT uptake through a NK_1 receptor-mediated cross–talk mechanism which may involve a Gq/PKC-dependent phosphorylation of hSERT. Further studies are ongoing to understand the post-receptor intracellular pathways involved in such cross-talk mechanism.

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225P THE ANALGESIC EFFECT OF PARACETAMOL IN MICE IS EXPLAINED BY THE INHIBITION OF CYCLO-OXYGENASE ACTIVITY IN THE CENTRAL NERVOUS SYSTEM

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Paracetamol is a widely used over-the-counter analgesic and anti-pyretic drug with weak anti-inflammatory effects. Although the pharmacological mechanism of the analgesic effects of paracetamol have not been fully elucidated, it has been shown to reduce the biosynthesis of prostaglandins (PGs) in central nervous tissues (Flower *et al*, 1972; Muth-Selbach *et al*, 1999). Therefore it has been theorised that it inhibits activity of cyclo-oxygenase (COX) enzymes in the central nervous system (CNS) which are involved in mediating the nociceptive signal in peripheral and central tissues. The present studies investigated whether the analgesic effects of paracetamol in mice is dependent on inhibition of the activity of COX isoenzymes and whether this actions occur in peripheral and/or central tissues.

A "writhing" response was elicited in female C57BL/6 mice (21.3±0.25g) by intraperitoneal administration of either acetic acid (0.1ml/ 10g body mass of 0.6%v/v acetic acid in 0.9% saline) or iloprost (0.25µg/10g body mass in 0.1ml saline). The degree of pain experienced was assessed by counting the number of writhes over a 20 minute period. For assessment of analgesic activity, animals were pre-treated by subcutaneous injection of either paracetamol (50-400 mg/kg) or diclofenac (10-100 mg/kg) with 5 animals per group, 30 minutes prior to the irritant. . Whole brain and spinal cord tissues were then removed and peritoneal wash-outs obtained by lavage with 1 ml of saline. Brain and spinal cord tissues were snap frozen and pulverised using a nitrogen bomb, resuspended in 15% v/v aqueous ethanol and prostaglandins purified using C18 Sep-Pak columns.

Using enzyme immunoassays, PGE_2 and PGI_2 were measured in the peritoneal fluid and PGE_2, PGD_2 and $PGF_{2\alpha}$ measured in brain and spinal cord. Data was analysed by ANOVA followed by *post-hoc* Dunnett's test to compare vehicle with drug treated groups and expressed as mean ± standard error of mean (s.e.m). A $P \leq 0.05$ was considered to be significant.

Both paracetamol (ED_{50} = 172mg/kg) and diclofenac (ED_{50} = 17mg/kg) inhibited the acetic acid-induced writhing in a dose-dependent, statistically significant manner. Paracetamol similarly inhibited the iloprost-induced writhing dose-dependently (ED_{50} = 108mg/kg), whereas diclofenac required elevated doses (compared to the acetic acid induced model) to exhibit analgesic activity (ED_{50} = 53mg/kg). In the acetic acid model, diclofenac dose-dependently reduced the amount of PGI_2 and PGE_2 in the peritoneal lavages, but paracetamol did not. On the other hand, both diclofenac and paracetamol reduced the levels of PGs in spinal cord and brain tissues in both the acetic acid and iloprost-induced writhing tests ($P \leq 0.05$).

Paracetamol's analgesic effects in the writhing model may be dependent on the reduction of the PG levels in central tissues, whereas the analgesic effect of diclofenac may comprise of reductions in PG levels in both peripheral and central tissues. However, the relative reduction in analgesic effect for diclofenac in the iloprost induced-writhing model (which is independent of PGs derived from COX in peripheral tissues), despite the reduction in PG levels in central tissues, indicates that the analgesic effect of diclofenac is largely mediated via the inhibition of COX enzymes in the peripheral, rather than central, tissues. The potent analgesic effect of paracetamol in the iloprost-induced writhing along with reductions in PGs in central tissues is further support that the analgesic effect of paracetamol may be dependent on inhibition of COX enzymes primarily in central tissues.

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226P INTRAVENTRICULAR AND INTRAPALLIDIAL INJECTIONS OF THE GROUP III METABOTROPIC GLUTAMATE RECEPTOR AGONIST L-SERINE-O-PHOSPHATE (L-SOP) ALLEVIATE RESERPINE-INDUCED AKINESIA IN THE RAT

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Loss of striatal dopaminergic innervation in Parkinson's disease (PD) leads to downstream changes in glutamate and GABA transmission in the basal ganglia motor loop leading to symptoms such as akinesia. We have previously shown that activation of $5\text{-}HT_{1B}$ autoreceptors on striatopallidal terminals in the globus pallidus (GP) alleviates PD-like akinesia in reserpine-treated rats by reducing the excess GABA release in this region (Chadha *et al.*, 2000). Group III metabotropic glutamate (mGlu III) heteroreceptors also reside on striatopallidal terminals within the GP (Bradley *et al.*, 1999) hence the present study examined the ability of mGlu III receptor activation to similarly reverse akinesia.

Under general anaesthesia (Halothane), male Sprague Dawley rats (270-300g) were stereotaxically implanted with a 23 gauge guide cannulae above the GP or third ventricle. Animals were rendered akinetic three days later by the injection of reserpine (5 mg kg^{-1} s.c.). Eighteen hours later unilateral injections of the group III mGlu receptor agonist LSOP (1500 – 2500 nmoles in 2.5µl Phosphate Buffered Saline; pH 7.4) were made and locomotor activity assessed. For intracerebroventricular (i.c.v) injections, activity was measured over 60 min in Arbitrary Locomotor Units (ALUs; where 1ALU = rats front paws crossing a 5cm square gridline on the base of the locomotor cage). Following intrapallidal injections, full contraversive rotations over 120 min were assessed as an index of reversal of akinesia. In both cases, the effects of LSOP were assessed using a 1-way ANOVA with a Dunnett's post-hoc test.

Intrapallidal injection of LSOP produced a dose-dependent increase in contraversive rotations compared to vehicle ($P<0.05$; Figure 1a), whilst i.c.v. injection produced a bell-shaped response with a significant increase in ALUs observed with 2250 nmoles compared to vehicle ($p<0.05$; figure 1b).

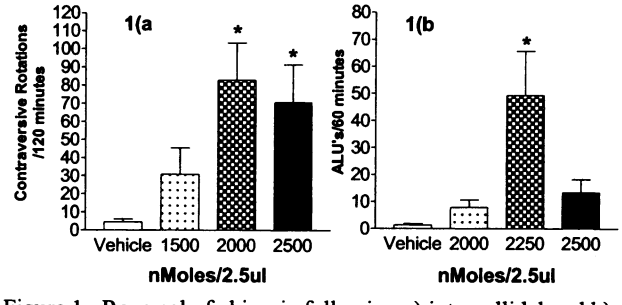

Figure 1. Reversal of akinesia following a) intrapallidal and b) i.c.v. injection of LSOP. Values are mean ± S.E.M (n=6-8). *Indicates a significant difference to vehicle ($p<0.05$).

In conclusion, LSOP reverses reserpine-induced akinesia following intrapallidal or i.c.v. injection. Whether this response results from an heteroreceptor-mediated reduction in GABA release in the GP remains to be examined. However, these data suggest that mGlu III receptor agonists warrant further investigation as potential antiparkinsonian agents.

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Funded by Merck Sharp & Dohme and The Wellcome Trust.

227P AT_2 RECEPTOR-MEDIATED VASODILATION IN HUMAN AND RAT CORONARY ARTERIES. ROLE OF NITRIC OXIDE

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Angiotensin (Ang) II type 2 (AT_2) receptor stimulation results in coronary vasodilation in the rat heart (Schuijt *et al.*, 2001). In contrast, the AT_2 receptor-mediated vasodilation could not be observed in large human coronary arteries (MaassenVanDenBrink *et al.*, 1999). Thus, it is possible that such a vasodilation occurs in microarteries rather than in large conductance vessels. In this investigation, we investigated AT_2 receptor-mediated vasodilation in human coronary microarteries (HCMAs). We also studied the possibility that nitric oxide (NO) mediates such vasodilation in both humans and rats.

HCMAs (diameter 200-500 μm), obtained from 14 heart valve donors (17-65 years; 9 men, 5 women) were mounted in Mulvany myographs. Ang II constricted HCMAs in a concentration-dependent manner (pEC_{50} 7.9±0.3; E_{max} 64±13 % of the contraction to 100 mmol/L K^+). The Ang II type 1 receptor antagonist irbesartan (1μM) prevented this vasoconstriction, whereas the AT_2 receptor antagonist PD123319 (1μM) shifted the Ang II concentration-response curve ≈10-fold to the left (pEC_{50} 8.5±0.2; P<0.01) and increased E_{max} to 84±6.2% (P=0.052). This PD123319-induced potentiation was not observed in the presence of the NO synthase inhibitor L-NAME. Ang II relaxed U46619-preconstricted HCMAs in the presence of irbesartan by maximally 61±23%.

In 6 pentobarbitone anaesthetised artificially ventilated male Wistar rats (body weight 260-280 g) treated with vehicle, Ang II (100 and 300 ng/kg/min) increased arterial pressure (MAP) from 102±5 to maximally 139±11 mm Hg (P<0.01), whereas heart rate (366±11 vs. 386±13 beats/min) and cardiac output (82±9 vs. 79±8 mL/min) were not significantly altered. Pretreatment with indomethacin (5 mg/kg; n=3) affected neither the baseline haemodynamic parameters, nor the Ang II-induced systemic haemodynamic effects. Pretreatment with L-NAME (10 mg/kg; n=3) + sodium nitroprusside (SNP; 4 μg/kg, to restore MAP to pre-NOS blockade level), also did not affect baseline haemodynamic parameters, or the Ang II-induced effects on MAP and heart rate. In the presence of L-NAME+SNP, however, Ang II did reduce cardiac output from 62±2 to 38±3 mL/min (P<0.01). Finally, in vehicle-treated rats, Ang II increased myocardial vascular conductance (myocardial blood flow/MAP), measured with the radiolabelled microsphere technique (Schuijt *et al.*, 2001), from 42±4.1 to maximally 51±3.3 μl/min/mm Hg per g, in agreement with the concept that Ang II causes coronary vasodilation. This increase was unaffected by indomethacin and reversed into a decrease (from 43±10 to 36±4.5 μl/min/mm Hg per g; P=0.06 vs. vehicle) following pretreatment with L-NAME+SNP.

In conclusion, AT_2 receptor-mediated vasodilation in the heart is most likely limited to coronary microarteries and is mediated by NO.

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228P SUPEROXIDE: A DIRECT OR AN INDIRECT VASOCONSTRICTOR?

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Increased vascular superoxide (O_2^-) production may contribute to endothelial dysfunction and hypertension (Berry *et al.*, 2000). Moreover, angiotensin (Ang) II has been demonstrated to stimulate O_2^- production via NAD(P)H oxidase (Griendling *et al.*, 1994; Kawazoe *et al.*, 2000). To investigate whether O_2^- production results in vasoconstriction in a direct or indirect (through inactivation of the vasodilatory nitric oxide (NO)) manner, concentration response curves (CRCs) were constructed in 36 femoral and 6 coronary arteries of 3-month old pigs (Yorkshire x Landrace, weight 10-15 kg) to Ang II, and the NO-donors sodium nitroprusside (SNP) and S-nitroso-N-acetyl-penicillamine (SNAP). Extracellular O_2^- generation in porcine femoral artery rings was measured by reduction of ferricytochrome c in the absence and presence of 200 U/ml superoxide dismutase (SOD).

Following preconstriction with 3 nM of the thromboxane A_2 analogue, U46619, SNP and SNAP concentration-dependently relaxed femoral and coronary vessels (pEC_{50} 6.31±0.10 and 6.63±0.06; mean±s.e.m, n=7 and 6, respectively). The superoxide dismutase inhibitor diethyldithiocarbamate (DETCA) (100μM) reduced the vasodilatation of 10 μM SNP by 22±10 % and of 1 μM SNAP by 73±6.3% (P<0.05), whereas the NAD(P)H oxidase inhibitor diphenylene iodonium (DPI) (10μM) shifted the SNAP CRC in porcine coronary arteries to the left (pEC_{50} 6.86±0.05; P<0.05).

Ang II constricted porcine femoral arteries in a concentration-dependent manner (pEC_{50} 8.86±0.10; E_{max} 50±5.5% of the response to 100 mM K^+). DETCA reduced the Ang II-mediated vasoconstriction (pEC_{50} 8.59±0.05; E_{max} 19.6±5.6%; n=5), while DPI, superoxide dismutase (SOD) and the membrane-permeable SOD mimetic agent tempol (1mM) were without effect. DETCA also induced a rightward shift of CRCs constructed to U46619 (pEC_{50} 7.92±0.08 vs. 7.37±0.04; P<0.05 and E_{max} 130±12.7% vs. 117±11.8%; n=4), indicating that its inhibitory effect towards Ang II is non-specific.

Incubation of coronary artery rings (~25 mg; n=6) with Ang II (10 nM) did not result in detectable O_2^- generation whereas xanthine oxidase (XO; 5 U/ml) with xanthine (X; 100 μM) did (see table 1).

Table 1: Generated ferrocytochrome c (nmol/segment/15 min)

vehicle		Ang II	XO + X
without SOD	0.45±0.10	0.43±0.08	2.23±0.45
with SOD	0.33±0.08	0.32±0.10	0.53±0.69
O_2^- generation	0.12±0.05	0.11±0.09	1.70±0.42

In conclusion, in porcine arteries O_2^- indirectly induces vasoconstriction, through inactivation of NO, but it does not mediate Ang II-induced vasoconstriction.

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