1P EFFECT OF MICROINJECTIONS OF VIGABATRIN IN THE CENTRAL PIRIFORM CORTEX ON THE DEVELOPMENT OF AMYGDALA KINDLING IN RATS

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A small part in the anterior part of the posterior primary olfactory cortex or piriform cortex (central PC) seems to be involved in the development and propagation of forebrain (limbic type) seizures. In kindling, a model of complex focal seizures with secondary generalisation in which repeated electrical stimulation of a limbic brain region induces the development of progressively stronger focal epileptic seizures (Löscher & Schmidt, 1988), a region in the central PC was more sensitive to electrical induction of seizures both before and after the kindling compared to adjacent areas of the PC and the amygdala. In amygdala kindling large unilateral lesions including the central PC increased the threshold for induction of focal seizures and slightly retarded the kindling rate, i.e. the increase in seizure severity to generalised seizures induced by repeated electrical stimulation. In response to amygdala kindling, GABA-immunoreactive neurones were reduced in a discrete area including the central PC.

These results indicate that GABAergic synapses in the central PC are involved in the propagation of seizures in amygdala-kindled rats. We therefore studied the role of GABAergic function in the central PC by repeated bilateral microinjection of Vigabatrin on amygdala kindling in rats. Vigabatrin irreversibly blocks the GABA degenerative enzyme GABA-transaminase.

Female Wistar rats (200-220g) were stereotaxically implanted with a stimulating-recording electrode in the basolateral amygdala and a guide cannula for microinjections in the central PC. The effect of 10 µg Vigabatrin in 0.25 µl saline (n=7) was tested before and during kindling; controls (n=7) received vehicle. Kindling was performed by daily stimulation with 500 µA. Vigabatrin was injected 24 h before determination of the afterdischarge threshold for focal seizures and 6 h before stimulation on days 5 and 10 of stimulation.

After the experiment, the brains of all rats were histologically processed for Nissl staining in order to check the correct placement of the electrode and the injection sites.

Before kindling Vigabatrin decreased the seizure severity and the seizure duration significantly ($p<0.05$). It also increased the afterdischarge threshold from 139 µA to 195 µA (40%), but this effect was not significant. Vigabatrin was able to retard the kindling rate significantly. It increased the number of stimulations to reach the first generalised seizure of maximal severity from 8 to 16 (100%) and the cumulative afterdischarge duration from 304 s to 651 s (114%).

The results indicate that the central PC is part of an epileptic network which is important for the seizure susceptibility of the basolateral amygdala and the development and propagation of amygdala kindling-induced seizure activity. GABAergic neurones in the central PC seem to be critically involved in this process.

Löscher & Schmidt (1988) *Epilepsy Res.* **2**: 145-181

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2P EFFECT OF LOCALLY-APPLIED PENTYLENETETRAZOLE ON NIGRAL SINGLE UNIT ACTIVITY IN A RODENT MODEL OF PAROXYSMAL DYSTONIA

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GABAergic dysfunction could be critically involved in the pathophysiology of primary paroxysmal dystonia. In mutant dystonic hamsters (dt^{sz}), an animal model of primary paroxysmal non-kinesiogenic dystonia that displays attacks of generalized dystonia, i.e. sustained twisting movements and abnormal postures in response to mild stress, previous pharmacological studies demonstrated antidystonic effects of GABAmimetic drugs (Richter & Löscher, 1998). The GABA-impairing drug pentylenetetrazole (PTZ) aggravated dystonia at subconvulsant doses.

As shown by recent experiments, PTZ significantly increased the discharge rate of basal ganglia output neurons, i.e. substantia nigra pars reticulata (SNr) neurons, in dystonic but not in non-dystonic control hamsters after systemic administration of a subconvulsant dose. The fact that the majority of basal ganglia neurons utilize GABA as an inhibitory transmitter complicated the interpretation of systemically applied PTZ. The effects of PTZ on firing rate of SNr neurons after systemic application may thus be caused by direct disinhibition of these cells and/or indirectly by effects on other basal ganglia structures.

The present study therefore aimed to investigate whether the SNr is the structure responsible for the intergroup differences in sensitivity to systemic PTZ as described above. We locally applied PTZ (2, 3 and 5 mM in 0.9% saline) by pressure application through a pulled capillary tube glued to the recording electrode to the SNr during standard extracellular single-unit recordings in anaesthetized dystonic hamsters of both sexes (n = 5 to 8). Age-matched non-dystonic hamsters served as controls (n = 5 to 9).

In agreement with previous findings, no changes in spontaneous discharge rates were found in mutants compared to controls (about 22 spikes/s in both animal groups).

Pressure application of 2 mM PTZ caused no significant changes in discharge rates of SNr neurons in either animal group. However, a significantly increased sensitivity of SNr neurons was found in mutant dystonic hamsters after application of 3 mM PTZ, i.e. the mean discharge rate in % relative to the pre-drug period increased by about 30% within 2 min after PTZ application in mutant hamsters ($p<0.05$, two way ANOVA for repeated measurements, *post-hoc* Bonferroni) and thus differed significantly from the relative discharge rates found in controls in response to 3 mM PTZ (0% increase). In response to 5 mM PTZ, the relative nigral discharge rates significantly increased in both animal groups ($p<0.05$, two way ANOVA) and no intergroup differences were found.

The results reveal that direct actions on the SNr clearly contribute to the effects of systemic PTZ application, but that the SNr is possibly not the only structure responsible for the observed intergroup differences in sensitivity to systemic PTZ.

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3P MEASUREMENT OF [^{3}H]GABA RELEASE FROM SLICES REVEALS ALTERED $GABA_B$ RECEPTOR FUNCTION IN GENETIC ABSENCE EPILEPSY RATS FROM STRASBOURG

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Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are a validated animal model of absence epilepsy (Vergnes et al., 1982). Absence seizures are generated by the cortex and thalamus, and $GABA_B$ receptors have been implicated in the causation of these seizures (Marescaux et al., 1992). Here we report that $GABA_B$ receptor control of [^{3}H]GABA release measured *in vitro* is altered in GAERS compared to control rats.

[^{3}H]GABA release was measured using cross-chopped thalamic slices (250μm x 250μm) prepared from 13-week-old female GAERS and non-epileptic control rats. Slices were suspended in artificial CSF (aCSF) containing 100μM β-alanine and 50μM aminooxyacetic acid and loaded with 45nM [^{3}H]GABA for 20min. After 3 washes, slices were perfused at 0.4ml.min^{-1} with aCSF additionally containing 10μM NNC-711 (1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) (Suzdak et al., 1992). After 36min perfusion, perfusate fractions were collected every 4 minutes. Release was stimulated at 48 (S1) and 88 (S2) minutes by the application of biphasic current (60mA, 4Hz) for 3.5min. The $GABA_B$ agonist (-)Baclofen (Bac) and antagonist CGP52432 (3-[[(3,4-dichlorophenyl)methyl]amino]propyl](diethoxymethyl) phosphinic acid) (Lanza et al., 1993), were included 15 and 30 min before S2, respectively. Release levels were calculated as percent fractional release (FR). Basal FR levels were subtracted from each stimulated FR. The S2/S1 ratio was then calculated. Statistical analysis comparing drug-treated S2/S1 to control S2/S1 was by one-way anova with post-hoc Dunnett's test. Values are given as mean±s.e.mean.

(-)Baclofen (10μM) inhibited thalamic [^{3}H]GABA release from GAERS but not control rats. The inhibition was blocked by the $GABA_B$ antagonist CGP52432. CGP52432 alone did not affect [^{3}H]GABA release. (Table 1).

Table 1 [^{3}H]GABA release from thalamic slices.

	Control rats	(n)	GAERS	(n)
control S2/S1	1.12±0.17	(4)	1.50±0.28	(4)
3μM (-)Bac	0.90±0.13		0.81±0.03	
control S2/S1	1.30±0.26	(8)	1.42±0.18	(8)
10μM (-)Bac	0.89±0.15		0.72±0.17 **	
control S2/S1	1.48±0.52	(4)	1.34±0.27	(4)
10μM (-)Bac	1.00±0.24		0.45±0.10 *	
10μM CGP52432	1.79±0.38		1.61±0.29	
(-)Bac + CGP52432	2.08±0.71		1.59±0.26	

** P<0.01 and * P<0.05 compared to respective control S2/S1 values

These data suggest that the modulation of GABA release by $GABA_B$ receptors is increased in the thalamus of GAERS, compared to non-epileptic control rats.

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4P CONTROL OF LUMINAL UPTAKE OF L-DOPA AT THE BLOOD-BRAIN BARRIER BY Ca^{2+}/CALMODULIN-MEDIATED PATHWAYS

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RBE 4 cells, an immortalised cell line of rat capillary cerebral endothelial cells (Roux et al., 1994), were demonstrated to take up L-DOPA through the L-type amino acid transporter (Gomes & Soares-da-Silva, 1999). The present study examined the result of manoeuvres that affect molecular mechanisms, namely those concerning protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), protein tyrosine kinase (PTK) and Ca^{2+}/calmodulin mediated pathways, on the uptake of L-DOPA. RBE 4 cells (passages 25-29) were grown in Minimum Essential Medium/Ham's F10 (1:1) supplemented with 300 ng ml^{-1} neomycine, 10% fetal bovine serum, 1 ng ml^{-1} basic fibroblast growth factor, 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B, 100 μg ml^{-1} streptomycin and 25 mM HEPES. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 100 μg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added pargyline (100 μM), tolcapone (1 μM) and benserazide (50 μM). L-DOPA was assayed by h.p.l.c. with electrochemical detection. Results are arithmetic means with s.e.mean, n=4-5. Non-linear analysis of the saturation curves for L-DOPA revealed K_m values (in μM) of 88±14 and V_{max} values (in nmol mg protein^{-1} 6 min^{-1}) of 20±1; uptake of saturating concentrations of L-DOPA at 4°C was less than 10% of that occurring at 37°C. Cyclic AMP (0.5 mM), forskolin (50 μM), isobutylmethylxanthine (1 mM) and cholera toxin (5 μg/ml) failed to affect the accumulation of a non-saturating (2.5 μM) concentration of L-DOPA. Similarly, cyclic GMP (1 mM), zaprinast (30 μM), LY 83583 (6-(phenylamino)-5,8-quinolinedione; 30 μM) and sodium nitroprusside (100 μM) failed to affect the accumulation of L-DOPA (2.5 μM). The PKC activator phorbol 12,13-dibutyrate (PDBu, 0.1 to 1.0 μM), the inactive phorbol ester 4α-phorbol 12,13-didecanoate (PDDC, 0.1 to 1.0 μM) and the PKC inhibitor staurosporine (1 μM) also failed to affect the accumulation of L-DOPA (2.5 μM). However, two other PKC inhibitors, chelerythrine (100 μM) and bisindolylmaleimide (100μM) reduced L-DOPA uptake by 76±5% and 65±3%. The PTK inhibitors genistein and tyrphostin 25 failed to change the accumulation of L-DOPA (2.5 μM). The Ca^{2+}/calmodulin inhibitors calmidazolium and trifluoperazine produced concentration-dependent inhibition of L-DOPA (2.5 μM) uptake with IC_{50}'s of 22.8±1.3 μM and 33.1±2.0 μM, respectively. The inhibitory effect of calmidazolium and trifluoperazine (30 μM) on the accumulation of L-DOPA was of the non-competitive type, as evidenced by the decrease in V_{max} (20±1 vs. 9±1 and 11±1 nmol mg protein^{-1} 6 min^{-1}) but not K_m (88±14 vs. 166±54 and 85±7) values for L-DOPA uptake. It is concluded that L-DOPA uptake in RBE 4 cells is a carrier-mediated system that is temperature dependent and appears to be under the control of Ca^{2+}/calmodulin mediated pathways.

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5P EFFECTS OF BELAPERIDONE IN ACUTE AND CHRONIC ELECTROPHYSIOLOGICAL MODELS: COMPARISON TO RAT CATALEPSY AND PHASE II CLINICAL MOTOR SIDE-EFFECT DATA

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Belaperidone (LU 111995), a D_4/5-HT_{2A} receptor antagonist (Unger, Bialojan, Gross et. al. *this meeting*), is being developed for the treatment of psychosis. In order to characterise its antidopaminergic potential acute and chronic electrophysiological experiments predicting antipsychotic activity and motor side effects were performed. These data are compared to results in the catalepsy model in rats and to the occurrence of extrapyramidal side effects (EPS) in a phase II study.

For acute experiments, in chloralhydrate anaesthetised (400 mg/kg; sc) male Sprague-Dawley (SD) rats, belaperidone was injected in cumulative iv doses (0.128-32.768 mg/kg) to reverse iv quinpirole-induced inhibition of the firing of substantia nigra (A9) dopaminergic (DA) neurons (n=7). In chronic experiments, male SD rats were injected sc with either water, 0.5 mg/kg haloperidol, or belaperidone (0.03-3 mg/kg) daily for 21 days (n=10/group). Then, the number of spontaneously active A9 and A10 (ventral tegmental area) DA cells per electrode track (CpT) under chloralhydrate anaesthesia (400 mg/kg; sc) was determined (Grace 1997). Belaperidone (100 and 215 mg/kg; po) was tested for its potential to induce a cataleptic syndrome in female SD rats according to Wirth et al (1958). In the first phase II study 8 patients received belaperidone (50 – 350 mg/day) and 4 placebo for a total of 35 days. The data from one patient, who stopped treatment after 3 weeks, were omitted from statistical analyses. Patients were rated on the Simpson & Angus scale (1970) for potential EPS.

In the acute experiments the ED_{50} (CL 95%) value for reversal of quinpirole-induced inhibition was 1.66 mg/kg (0.87/3.15). The ED_{50} data for clozapine and haloperidol were 3.58 (1.72/7.44) mg/kg and 0.04 (0.02/0.07) mg/kg, respectively. In the chronic experiments, belaperidone reduced the numbers of A9 as well as A10 DA CpT at 0.3, 1, and 3 mg/kg/day ($p < 0.05$ vs. control). Belaperidone up to 215 mg/kg, po did not induce any catalepsy in rats; 0/16 animals were cataleptic.

In the belaperidone treated patients the mean value of the Simpson-Angus scale declined from 2.7 ± 1.6 (mean ± SEM) at day 0 to 0.3 ± 0.2 at day 35. None of the patients showed an increase in the Simpson-Angus score during the treatment period. In the control group Simpson-Angus score declined from 2.3 ± 0.8 at day 0 to 1.0 ± 1.0 at day 35.

The results from both, the acute and chronic electrophysiological experiments demonstrate antidopaminergic properties of belaperidone. Belaperidone is a potential antipsychotic drug, indicated by its activity in reducing A10 DA CpT. The parallel reduction of A9 DA CpT would predict the propensity to induce EPS for this drug. However, even in the high doses tested no catalepsy was observed. In the phase II study it did not provoke EPS. In summary these data suggest that the decrease in spontaneously active CpT in A9 may not be predictive for EPS in all classes of antipsychotic drugs. Belaperidone is a potential new antipsychotic with a novel mechanism of action and low propensity to induce EPS.

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6P PHENCYCLIDINE-INDUCED INCREASE OF NUCLEUS ACCUMBENS DOPAMINE EFFLUX AND LOCOMOTOR ACTIVITY IS ENHANCED BY THE 5-$HT_{2C/2B}$ RECEPTOR ANTAGONIST SB221284

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The NMDA receptor antagonist phencyclidine (PCP) induces symptoms in man which closely resemble those observed in schizophrenia. In rodents, n. accumbens dopamine (DA) efflux and locomotor activity are increased by PCP, effects which are attenuated by the selective 5-HT_{2A} receptor antagonist MDL 100907 (Murray et al 1998). In the present study we have determined the effect of (1H-indole-1-carboxamide,2,3-dihydro-5-(methylthio)-N-3-pyridinyl -6-(trifluoro-methyl)), SB221284 a 5-$HT_{2B/2C}$ receptor antagonist (Bromidge et al 1998), on the increase of n. accumbens DA efflux and locomotor activity induced by PCP.

Male Sprague-Dawley rats (250-200g, B&K) were implanted under isoflurane anaesthesia with a 2mm concentric dialysis probe (Hospal membrane) in the n. accumbens (Paxinos & Watson, 1982: AP +2.2 mm from bregma, L 1.5 mm, H -7.5 mm from dura, incisor bar –3.5 mm). 18h later, the probe was perfused with Ringer solution, at 1µl/min. Samples were collected at 20min intervals and analysed for DA by HPLC with electrochemical detection (Hutson et al., 1991). Rats (n=6-12/group) were injected with either vehicle (8% cyclodextrin in 25mM citric acid, 1ml/kg, i.p.), or SB221284 (1mg/kg, i.p.) 20 min prior to administration of saline (1ml/kg,s.c.) or PCP (5mg/kg, s.c.). DA efflux area under curve, A.U.C. (mean± s.e.mean, fmol/15ul/min) was calculated from the percent change of basal DA efflux for the 180 min following saline or PCP injection.

To assess locomotor activity, rats were habituated to individual activity cages equipped with 2 infra-red beams to record cage crossings. Rats (n=7-8/group) were pre-treated with either vehicle (8% cyclodextrin in 25mM citric acid, 2ml/kg, i.p.), or SB221284 (1mg/kg, i.p.) 20 min prior to injection of saline (1ml/kg,s.c.) or PCP (2.5mg/kg, s.c.). Photocell beam breaks were monitored for 150 min. All data were subjected to two-way analysis of variance followed by Tukey's test. *P<0.05 and †P<0.05 compared with veh/sal and veh/PCP groups respectively.

PCP (5mg/kg, s.c.) increased n. accumbens DA efflux 40-60 min after injection (AUC=0.41±0.07*) compared with saline administration (AUC=0.18±0.05). SB221284 (1mg/kg, i.p.) did not affect basal DA efflux *per se* (AUC=0.17±0.05), but significantly enhanced PCP induced DA efflux (AUC=0.65± 0.12*†). Similarly, locomotor activity was increased by PCP (2.5mg/kg s.c.) (mean ± s.e.mean total cage crosses: veh/sal = 11±2; veh/PCP=129±46*). However, pre-treatment of rats with SB221284 (1mg/kg i.p.) at a dose which did not affect locomotor activity *per se* (21±4), significantly enhanced (289± 49*†) PCP induced locomotor activity.

In contrast to previous findings that blockade of 5-HT_{2A} receptors attenuated the neurochemical and behavioural effects of PCP (Murray et al., 1998) results in the present study demonstrate that SB221284 a 5-$HT_{2C/2B}$ receptor antagonist markedly enhanced n. accumbens DA efflux and locomotor activity induced by PCP.

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7P VISUALISATION AND CHARACTERISATION OF FUNCTIONAL 5-HT RECEPTORS IN THE HUMAN HIPPOCAMPUS BY [^{35}S]GTPγS AUTORADIOGRAPHY

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Agonist-stimulated activation of some G-protein-coupled receptors (GPCRs) can be detected in membrane preparations by measuring changes in [^{35}S]GTPγS binding in the presence of excess GDP (Lorenzen et al., 1993). This technique can also be applied to tissue sections, where agonist-stimulated increases in [^{35}S]GTPγS binding are detected by autoradiography, enabling anatomical resolution of receptor function (Sim *et al.*, 1997).

In the present study, we have used [^{35}S]GTPγS autoradiography to study 5-HT receptor activation in the human hippocampus. Briefly, un-fixed sections were pre-incubated in 50mM Tris-HCl (pH7.4), 3mM $MgCl_2$, 0.2mM EGTA, 100mM NaCl, 0.3mM GDP, 1mM DTT, 10μM pargyline and 0.5mM ascorbic acid for 30min, followed by incubation in the presence of 0.1nM [^{35}S]GTPγS and a range of 5-HT concentrations (0-100μM) for 2h. Sections were then washed, dried and apposed to phosphor screen or x-ray film, alongside ^{35}S-brain paste standards. Signal intensity was measured using NIH image software and converted to nCi/g using the ^{35}S standard curve.

We found that 5-HT caused the greatest increases in [^{35}S]GTPγS binding over basal levels in the CA1 region and in superficial layers of the subiculum and entorhinal cortex (see Figure 1). 5-HT concentration-effect curves, based on signal intensity measurements taken in the CA1 region, resulted in a mean pEC_{50} of 7.2 ± 0.2 (n=3 donors). The selective 5-HT1A receptor antagonist, WAY100635 (30nM) surmountably antagonised the response to 5-HT with an apparent pK_B of 9.1 ± 0.2 (n=3 donors), consistent with the reported affinity of WAY100635 for human 5-HT1A receptors (Burnet *et al.*, 1997).

The marked inhibition of 5-HT-stimulated [^{35}S]GTPγS binding by WAY100635 suggests that 5-HT-stimulated receptor activation in the CA1 region is mediated predominantly via 5-HT1A receptors. Furthermore, the pattern of 5-HT-stimulated [^{35}S]GTPγS binding in the hippocampus corresponds closely with that seen using direct binding of [^{3}H]WAY100635 to human hippocampal sections (Burnet *et al.*, 1997). Our findings support and extend those of Elliott and Reynolds (1999) who demonstrated 5-HT1A receptor-stimulated [^{35}S]GTPγS binding in human hippocampal membranes. In addition, we demonstrate the feasibility of using agonist-stimulated [^{35}S]GTPγS binding to obtain quantitative data on the regional activation of functional GPCRs in human tissue.

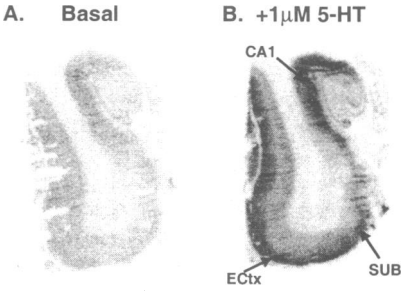

Figure 1: [^{35}S]GTPγS binding in human hippocampal sections in the absence (A) and presence (B) of 1μM 5-HT (phosphor screen detection). CA1, CA1 field of the hippocampus; SUB, subiculum; ECtx, entorhinal cortex.

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8P LONG-TERM CHANGE IN SOCIAL INTERACTION WITHOUT ACCOMPANYING SEROTONERGIC NEUROTOXICITY FOLLOWING MDMA TREATMENT OF YOUNG ADULT RATS

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3,4-methylenedioxymetamphetamine (MDMA, ecstasy) is a popular drug of abuse which has been reported to produce acute hyperthermia and a characteristic serotonin syndrome in rats (Green *et al.*, 1995). Administration of a single dose of MDMA also produces serotonergic neurodegeneration in adult rats, although animals under post-natal day (PND) 35 appear to be protected from this effect (Aguire *et al.*, 1997). The aim of the current study was to establish whether MDMA could induce long-term behavioural alterations without accompanying serotonergic neurotoxicity when given to immature rats.

Male hooded Lister rats housed by litter in groups of six from weaning were given food and water *ad libitum* and received MDMA (2 x 20 mg kg^{-1} i.p. on PND 28 and 29) or saline (1 ml kg^{-1} i.p. n = 12 each). Thirty mins after the second injection rectal temperature was recorded and 15 min later locomotor activity was monitored in infra-red activity boxes (40 x 25 x 25 cm) for 30 mins and the serotonin syndrome scored (0 = absent to 3 = present all the time) on 5 x 30s occasions by an observer screened from the rat. On PND 39 social interaction (10 mins, 60 x 60cm arena, bright light unfamiliar conditions) between weight and treatment matched rats and on PND 42 elevated plus maze behaviour (5 min) were recorded prior to removal of hippocampus and frontal cortex to evaluate 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acide (5-HIAA) levels by HPLC with electrochemical detection. In addition, [^{3}H]5-HT uptake (1nM 15 min, 20°C) was examined in cortical synaptosomes using norfluoxetine (10^{-5}M) to define non-specific uptake. All results are expressed as mean ± s.e. mean and Student's *t*-test (or Mann Whitney for the serotonin syndrome) was used for statistical analysis.

Acute MDMA significantly ($P < 0.05$) elevated rectal temperature (38.3 ± 0.2 compared to 37.6 ± 0.1°C in saline), increased locomotor activity ($P < 0.001$), lateral head weaving ($P < 0.01$) and reciprocal forepaw treading ($P < 0.01$) compared with that in saline controls. Ten days later MDMA treated rats spent significantly less time in passive (16 ± 1 compared with 54 ± 10 s, $P < 0.001$) and exploratory (17 ± 3 and 47 ± 9s, $P < 0.01$) behaviour resulting in a dramatic reduction in total interaction (33 ± 2 compared with 102 ± 11 s, $P < 0.001$) in the social interaction test. In contrast, MDMA had no effect on behaviour in the elevated plus maze, 5-HT content, or 5-HIAA/5-HT ratio in the hippocampus and frontal cortex or cortical [^{3}H]5-HT uptake compared with saline controls.

MDMA produced the expected acute hyperthermic and behavioural effects consistent with increased 5-HT release and inhibition of re-uptake when given to 28-29 day old rats, but it failed to produce any alteration in neuronal 5-HT levels or turnover measured on PND 42. This is consistent with evidence that young rats are protected from MDMA-induced neurotoxicity (Aguire *et al.* 1997). Nonetheless, MDMA produced a profound decrease in social interaction without altering elevated plus maze behaviour, suggesting that it caused a long-lasting alteration in specific anxiety-related behaviour by a mechanism independent of serotonergic neurotoxicity.

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9P THE ANTIAPOPTOTIC EFFECT OF THE NMDA-RECEPTOR ANTAGONIST MK-801 IN THE KAINATE MODEL OF TEMPORAL LOBE EPILEPSY

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Kainate (KA) treated rats develop seizures similar to that in human temporal lobe epilepsy (TLE). We and others previously showed that neurodegeneration which occurs mainly in parts of the limbic system after KA-induced status epilepticus (SE) is due to apoptosis, at least to some extent (Charriaut-Marlangue *et al.*, 1996; Pollard *et al.*, 1994, a,b). We detected a specific expression pattern of the two apoptosis-specific proteins Bax (inducer) and Bcl-2 (inhibitor). Further, we found TUNEL-stained neurones and DNA-laddering in brain regions strongly affected by neurodegeneration, indicating involvement of apoptosis. Electron microscopic studies showed neurones with apoptosis-specific morphological changes in the piriform cortex. We now investigated whether the NMDA receptor antagonist MK-801 exhibits a neuroprotective effect in rats with KA-induced SE.

Adult female Wistar rats were treated with 10 mg/kg KA intraperitoneally (i.p.). MK-801 (0.1 mg/kg) was administered i.p. 90 min after the development of a SE. Rats without MK-801 following KA-induced SE served as controls. SE was terminated after 100 min by an i.p. injection of diazepam in both groups.

The rats were perfused 48h after the first generalised seizure. The brains underwent immunohistochemical processing for the detection of Bax and Bcl-2 by polyclonal antibodies. Further brain sections were treated with the TUNEL-staining method. The extent of lesions which occurred in the piriform cortex (PC; Figure 1) and the amygdala after KA-induced SE was markedly reduced in MK-801 treated rats ($p<0.05$; t-test). A significant reduction of Bax and Bcl-2 stained neurones could be detected in the PC and the amygdala ($p<0.05$, rank sum test). In addition, TUNEL-stained neurones were decreased in the PC in rats treated with MK-801 ($p<0.05$, t-test; Figure 2).

From these results we conclude that MK-801 develops a neuroprotective effect in the KA model of TLE when administered after the development of a status epilepticus. The reduction of neurodegeneration might play an important role in the prevention of spontaneous recurrent seizures.

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Supported by the BMBF (AZ: 0310972)

Figure 1

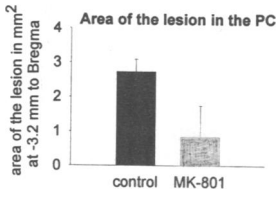
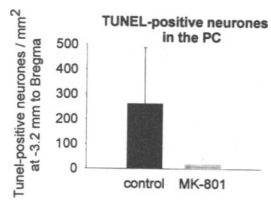


Figure 2



10P EFFECTS OF KYNURENINE-3-HYDROXYLASE INHIBITION ON EXTRACELLULAR KYNURENIC ACID AND *N*-METHYL-D-ASPARTATE-INDUCED DEPOLARISATION IN THE RAT BRAIN

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Inhibition of kynurenine-3-hydroxylase suppresses the synthesis of quinolinic acid (QA) and, therefore, shunts all kynurenine metabolism toward kynurenic acid (KYNA) synthesis. This may be a pertinent anti-excitotoxic strategy because QA is an *N*-methyl-D-aspartate (NMDA)-receptor agonist, whereas KYNA is an antagonist of all ionotropic glutamate-receptors with preferential affinity for the NMDA-receptor glycine site. We have examined, *in vivo*, whether the kynurenine-3-hydroxylase inhibitor, 3,4-dimethoxy-*N*-[4-(3-nitrophenyl)thiazol-2-yl]-benzene-sulfonamide (Ro-61-8048) (Röver *et al.*, 1997) increases extracellular KYNA sufficiently to control excessive NMDA-receptor function.

Microdialysis probes incorporating an electrode (Obrenovitch *et al.*, 1994) were implanted into the striatum (series **1**) or frontal cortex (series **2**) of adult, male Lister Hooded rats anaesthetised with halothane (concentration reduced to 1.5 % in 2:1 N_2O:O_2 after surgery; spontaneous breathing throughout). In series **1**, repeated NMDA-stimuli consisted in 2-min perfusion of 200 µM NMDA through the probe, each followed with 28-min recovery, and the resulting depolarisations were recorded with the microdialysis electrode. Ro-61-8048 (100 mg/kg) was administered i.p., and resulting changes in extracellular KYNA were examined by HPLC analysis of consecutive 30-min dialysate samples (Röver *et al.*, 1997). In series **2**, exogenous KYNA (0.03 to 1 mM) was co-perfused with 150 µM NMDA through the microdialysis probe, starting 10 min before NMDA application.

In series **1**, Ro-61-8048 increased the dialysate levels of KYNA from 3.0 ± 1.1 to 30.5 ± 5.8 nM 4 h post-injection (mean ± s.e.m., $n = 6$; $P < 0.01$, Student's paired *t*-test) (Fig. 1), but did not reduce the responses to NMDA (data not shown). With regard to exogenous KYNA (series **2**), the estimated IC_{50} (i.e. concentration in the perfusion medium) for inhibition of the NMDA-responses was 180.7 ± 9.0 µM ($n = 6$).

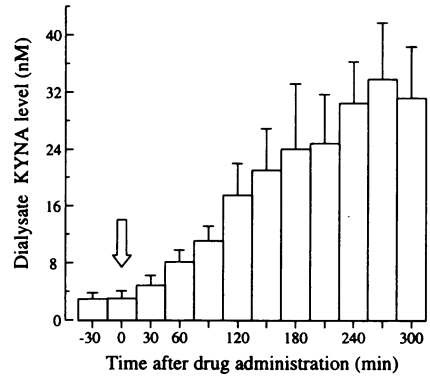

Figure 1. Changes in dialysate level of KYNA in the rat striatum after inhibition of kynurenine-3-hydroxylase with Ro-61-8048 (100 mg/kg i.p.).

These data challenge the suitability of kynurenine-3-hydroxylase inhibition as a neuroprotective strategy against acute excitoxicity. Indeed, the increase in extracellular KYNA concentration that could be achieved in this way was still far below that necessary for attenuation of NMDA-receptor function.

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11P CHARACTERISATION OF PRE- AND POST-SYNAPTIC α-ADRENOCEPTOR SUBTYPES IN RAT HIPPOCAMPAL NEURONS

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In the rat hippocampus, transcripts for α_{1B}, α_{1D}, α_{2C}, α_{2D}, β_1, and β_2 subtypes of adrenoceptors have been found, and the α_{1D} and α_{2C} receptors are expressed at the highest levels (Nicholas et al, 1996). Noradrenaline (NA) has been suggested to cause presynaptic inhibition of glutamatergic transmission via α_1-adrenoceptors (Scanziani et al, 1993). This study characterises pre- and postsynaptic α-adrenoceptor subtypes in hippocampal neurons by using single neurons isolated in glial microcultures which form synapses onto themselves, so-called autapses.

Autaptic currents and voltage-activated Ca^{2+} currents were recorded from microculture neurons, and some additional Ca^{2+} currents were determined in mass cultures, as described (see Boehm and Betz, 1997). When Ca^{2+} currents were recorded, the bathing solution (in mM: NaCl 140, KCl 6, $CaCl_2$ 3, $MgCl_2$ 2, glucose 20, HEPES 10, adjusted to pH 7.4 with NaOH) contained 1 μM tetrodotoxin. Pipettes were filled with a KCl-based solution to record autaptic currents and with a CsCl-based solution to measure Ca^{2+} currents. Agonists and antagonists were applied to the cells under investigation via a DAD-12 device (Adams & List, Westbury, NY, USA).

NA (1 μM) reduced glutamatergic (38.6 ± 6.8 % of control; n = 13), but not GABAergic (100.4 ± 1.5 % of control; n = 5), autaptic currents, and did not alter currents evoked by the direct application of glutamate, indicating that glutamate receptors were not affected. The α_2-adrenergic agonists clonidine and bromoxidine also reduced glutamate release, the IC_{50} values being 114 ± 63 nM (NA), 76 ± 23 nM (clonidine), and 83 ± 36 nM (bromoxidine). NA and bromoxidine were equieffective and caused a maximum inhibition of 65.0 ± 8.7 % and 59 ± 7.0 %, respectively; clonidine was less effective yielding only 37 ± 4.8 % maximal inhibition. Isoprenaline and methoxamine did not alter glutamatergic autaptic currents. The inhibition by 1 μM NA (61.1 ± 12.5%; n=5) was attenuated by yohimbine (7.2 ± 5.3 % inhibition; n=5), but not by prazosine, urapidil, or propranolol (all at 1 μM). Concentration-response curves for the NA-induced inhibition of glutamate release were shifted to the right by phentolamine (0.3 μM) by a factor of 37 and by rauwolscine (0.3 μM) by a factor of 9, but not by prazosine (1 μM). Pretreatment with pertussis toxin (PTX; 100 ng/ml for 24 h) abolished the inhibitory action of NA on glutamate release.

At the somata of glutamatergic microisland neurons (n=5), NA inhibited voltage-activated Ca^{2+} currents by 25.4 ± 4.3%, an action not observed in GABAergic neurons (n=4; 4.6 ± 4.3 % inhibition). In mass culture neurons, NA, bromoxidine and clonidine were equipotent in inhibiting Ca^{2+} currents with half-maximal effects at 0.17 ± 0.05, 0.12 ± 0.03, and 0.12 ± 0.08 μM, respectively. Again, clonidine displayed a smaller maximum of inhibition than NA and bromoxidine. The NA-induced reduction of Ca^{2+} currents was abolished after treatment with PTX and antagonized by yohimbine, but not by urapidil or propranolol; the inhibition by NA (1μM) was not altered by prazosine, but reduced by rauwolscine by 41.8 ± 6.8 % and by phentolamine by 94.6 ± 3.3% (0.3 μM; n=5).

These results show that glutamatergic, but not GABAergic, hippocampal neurons express pre- as well as postsynaptic α_2-adrenoceptors which display pharmacological characteristics of the α_{2D} subtpye (Supported by FWF, P12997-MED).

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12P ACTIVATION OF BOTH THE ADENOSINE A1 AND A3 RECEPTORS INHIBITS SYNAPTIC TRANSMISSION IN RAT CORTICAL PYRAMIDAL CELLS

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The purine nucleoside adenosine influences numerous physiological processes, including effects on neuronal communication within the central nervous system. These observations suggest that adenosine may play a role as a neurotransmitter or neuromodulator. Four G protein-coupled adenosine receptors have been cloned an designated A1, A2a, A2b and A3 subtypes. The stimulation of A1 receptors may counteract excitotoxic glutaminergic stimulation at multiple stages. However, the physiological role of the A3 receptor is mostly unexplored. This study was done to investigate the role of these receptors on the synaptic neurotransmission on pyramidal cells of the prefrontal cortex, an area which is essential for higher order cognitive functions, in particular for learning tasks requiring a change in strategy.

Electrophysiological investigations (n=32) in brain slices of male Wistar rats (own breed: 150-200g) containing the prelimbic area of the prefrontal cortex were performed by means of the intracellular recording technique. Monophasic, depolarizing synaptic potentials (PSPs) were evoked in layer V pyramidal cells by electrical stimulation of layer I.

In a first series of experiments, N^6-cyclopentyladenosine (CPA), a selcetive A1 agonist and 8-cyclopentyl-2,3-dipropylxanthine (DPCPX), a selective A1 antagonist were used. CPA (10μM, 100μM) inhibited the amplitudes of the PSPs by 48% and 51%, respectively. The effect was reversed by washout. DPCPX (0.1μM) did not influence the PSPs, when applied alone. Superfusion with CPA (100μM) failed to inhibit the amplitudes of PSPs in the presence of DPCPX (0.1μM). In further investigations effects of the selective A3 agonist N^6-(3-iodobenzyl)-adenosine-5′-N-methylcarboxamide (IB-MECA) and the selective A3 antagonist 9-chloro-2-(2-furanyl)-5-[(phenyl-acetyl)amino]1,2,4-triazolo[1,5-c]quinazoline (MRS-1220) were examined. IB-MECA at 10μM but not at 1μM inhibited significantly ($P<0.05$, Student's t-test) the amplitudes of the PSPs by 32%. The effect of IB-MECA was reversed by washout but it was not antagonized by the A1 receptor antagonist DPCPX (0.1μM). The A3 receptor antagonist MRS-1220 (1μM) itself decreased the amplitudes of the PSPs significantly by 23%. The application of IB-MECA (10μM), in the presence of MRS-1220 led to no further inhibition of the PSPs. When the experiments were done in the presence of the adenosine A1 receptor antagonist DPCPX (0.1μM) no inhibitory effect of MRS-1220 was found. Furthermore, in the presence of DPCPX, MRS-1220 (1μM) completely inhibited the effect of IB-MECA (10μM).

Our results suggest the existence of both the adenosine A1 and A3 receptors on pyramidal cells of the rat prefrontal cortex. Moreover, the present data indicate that the selective activation of both the A1 and A3 receptors inhibits synaptic transmission. Based on these results, we hypothesize that not only the A1 receptor but also the A3 receptor may play a role in the pathophysiology of neurologic discorders and may represent a novel target for drug development.

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13P EVIDENCE FOR CANNABINOID RECEPTOR-MEDIATED INHIBITION OF VOLTAGE-ACTIVATED Ca^{2+} CURRENTS IN NEONATAL RAT CULTURED DAG NEURONES

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Cannabinoid receptor ligands have been found to be antinociceptive and part of this effect may relate to direct modulation of sensory neurone activity. The aim of this study was to evaluate the expression of CB_1 cannabinoid receptors in cultured sensory neurones using fluorescence-activated cell sorting (FACS) analysis and immunohistochemistry and to investigate the inhibition by cannabinoids of high voltage-activated Ca^{2+} currents in these neurones. The binding of rabbit polyclonal antibodies raised against the first 77 amino acids of rat CB_1 cannabinoid receptors was also observed. Immunolabelling of CB_1 receptors was visualized on both the cell bodies and processes of cultured DRG neurones using Cy^3-conjugated goat anti rabbit secondary antibody and confocal microscopy. Additionally, FACS analysis using antibodies against CB_1 receptors provided further evidence that these cannabinoid receptors are expressed on populations of cultured DRG neurones. Cultured dorsal root ganglion (DRG) neurones bathed in choline chloride-based recording medium which contained tetrodotoxin (2.5 μM), tetraethylammonium (25 mM) and 0.01% ethanol were voltage clamped at -90 mV. Whole cell recordings were made with patch pipettes containing CsCl-based patch pipette solution and high voltage-activated Ca^{2+} currents were evoked with 100 ms voltage step commands to 0 mV. 0.01% ethanol was constantly present in the extracellular environment during the experiments and was used as a carrier of the cannabinoid receptor agonist (+)-WIN55212 *{(R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl) methanone}*, the inactive enantiomer (−)-WIN55212 and the CB_1 selective receptor antagonist SR141716A *[N-(piperidin-1-yl)-5-(4-chlorophenyl) -1-(2,4-di-chlorophenyl)4-methyl-1H-pyrazole-3-carboxamine hydrochloride*]. Results are expressed as the mean ± standard error of the mean, with statistical significance determined using the Student's *t* test. Acute (5 min) and chronic (30 min to 3 h) application of 0.01% ethanol had no effect on Ca^{2+} current amplitude (n=15). The CB receptor agonist (+)-WIN55212 (10 & 100 nM) inhibited the mean Ca^{2+} current by 21 ± 3% (n=4) and 30 ± 4% (n=6) respectively. (+)-WIN55212 was applied by low pressure ejection for 3-5 minutes and in some neurones partial recovery was observed. In contrast (−)-WIN55212 (10 & 100 nM) produced significantly less inhibition of 6 ± 2% (n=4, $P<0.05$) and 10 ± 2% (n=4, $P<0.01$) respectively. The CB_1 receptor antagonist SR141716A (100nM) applied alone for 1-2 minutes enhanced the peak Ca^{2+} current by 30 ± 14% (n=6, $P<0.05$). Following 3 minutes application of SR141716A alone, simultaneous application of SR141716A (100nM) and (+)-WIN55212 (100nM) resulted in significantly less Ca^{2+} current inhibition, 8 ± 6% (n=5; $P<0.02$) compared with the inhibition obtained with (+)-WIN55212 alone. The electrophysiology showed a degree of stereoselectivity for the receptor agonist, clear sensitivity to the CB_1 receptor antagonist and suggests a possible tonic modulation of Ca^{2+} channels involving CB_1 receptors. Cultured DRG neurones express a variety of functional receptors, some of which *in vivo* are only expressed peripherally or on presynaptic terminals. Therefore the actions of cannabinoid receptor ligands on the cell bodies of cultured DRG neurones may reflect events which occur on the presynaptic membrane such as presynaptic inhibition and autoregulation of transmitter release. However, CB_1 receptors may be expressed on the cell bodies of DRG neurones and have a direct bearing on physiological events within ganglia *in vivo*.

The authors thank The Wellcome Trust for support and Dr Ken Mackie for the generous gift of the antibodies.

14P SPINAL ADMINISTRATION OF HU210, A CANNABINOID AGONIST, ATTENUATES NOCICEPTIVE TRANSMISSION IN THE CARRAGEENAN MODEL OF INFLAMMATION

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Recent studies implicate a role of spinal cannabinoid receptors in nociceptive processing. Spinal administration of the cannabinoid agonist WIN55,212-2 inhibits noxious heat evoked responses of spinal neurones (Hohmann, *et al.*, 1998). Here the effect of spinal administration of (-)-11-OH-Δ^8-THC-dimethylheptyl (HU210), a cannabinoid agonist, on noxious versus innocuous evoked responses of dorsal horn neurones of rats with a local carrageenin inflammation was studied.

Extracellular recordings of convergent dorsal horn neurones were made in anaesthetised (1.5% halothane in 66% N_2O / 33% O_2) Sprague Dawley rats, n = 11, (Chapman *et al.*, 1994). Neuronal responses to transcutaneous electrical stimulation (3 x C-fibre threshold, trains of 16 stimuli at 0.5Hz) of the receptive field were recorded, post-stimulus histograms were constructed. Responses were separated and quantified on the basis of post stimulus latencies: Aβ-fibre 0-20ms; Aδ-fibre 20-90ms; C-fibre 90-300ms and post discharge 300-800ms. λ-carrageenin (100μl, 2% in saline) was injected into the plantar region of the paw, electrical evoked neuronal responses were recorded for three hours. The last three recordings were taken as controls and HU210 (0.5-500ng / 50μl, $2.5x\ 10^{-8}$-$2.5x10^{-5}$M) was applied to the spinal cord. Drug effects were followed every 10 minutes, for 60 minutes per dose. Antagonism of the effect of HU210 by the CB_1 receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716A) was also studied. A separate group of rats (n=4) received spinal SR141716A (0.01μg/50μl, $4.2x\ 10^{-7}$M) three hours after intraplantar injection of carrageenin, prior to the spinal administration of HU210. Data are presented as mean maximal effects; statistical analysis was performed with ANOVA.

The mean depth of the neurones studied (n = 11) was 637±28μm (mean ± s.e.m.), the mean C-fibre threshold and latency of the neuronal responses were 1.6±0.1mA and 186±13 ms, respectively. The control Aβ-fibre, Aδ-fibre, C-fibre evoked responses of the spinal neurones were 97±10, 87±13 and 445±40 action potentials respectively. The control post-discharge response, a measure of neuronal excitability following repetitive C-fibre stimulation, was 336±30 action potentials. HU210 (0.5, 5, 50, 500ng / 50μl) dose-relatedly and significantly reduced the post-discharge responses of the dorsal horn neurones to 72±10%, 63±9%, 52±9% ($p<0.01$), and 52±6% ($p<0.01$) of control, respectively. Maximal effects of the highest concentration of HU210 were observed at 23±8 min post-drug administration. The inhibitory effect of HU210 (500ng) on the post-discharge was reduced (80±3% of control, n= 4, mean depth 610±144μm) in the presence of spinal SR141716A. The highest concentration of HU210 studied did not significantly influence the C-fibre (68±4% of control), Aδ-fibre (51±9% of control) or Aβ-fibre (67±5% of control) evoked responses of the dorsal horn neurones.

HU210 reduced the post-discharge responses of dorsal horn neurones, a measure of neuronal excitability mediated by N-methyl-D-aspartate receptor activation, of rats with a carrageenin inflammation. This study has shown that activation of spinal cannabinoid receptors reduces inflammatory nociceptive transmission. A functional role of the spinal cannabinoid receptor system in the control of dorsal horn neuronal hyperexcitability following repetitive C-fibre activation is implicated.

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15P EXPERIMENTALBRAIN INJURY IN RATS: TIME COURSE OF HIPPOCAMPAL CELL DAMAGE AND ACTIVATION OF MICROGLIA

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Traumatic brain injury (TBI) is one of the main causes of morbidity and mortality in modern society. Clinical experience suggests that traumatic brain injury can be classified into primary damage, which occurs at impact, and secondary damage distant to the impact. We used an experimental contusion trauma in order to investigate the time course of cell damage in the hippocampal formation of rat brain.

During narcosis with pentobarbital (40 mg/kg) a 20g weight was guided from a definite height onto a footplate resting upon the surface of the dura (4.5 mm diameter, 1.5 mm posterior and 2.5 mm lateral to the bregma of the right hemisphere). Cresyl violet-stained coronal sections were prepared in order to assess the number of neurons in different hippocampal regions at various time points after trauma.

For each experimental group, 7-9 (7 week-old Wistar) rats were analysed. The site of impact was characterized by damage of cortical neurons and extension of the extracellular space 1 h after trauma. Changes in the hippocampus were restricted to the ipsilateral side, whereas the contralateral hippocampus remained unaffected in comparison with control animals. The most vulnerable part appeared to be the CA3 region.

Two days after trauma, the number of neurons in the CA3 was decreased by 65%, in the CA4 subfield by 23% and in the CA1 region by 11% in comparison to the contralateral side. This cell loss is at least partly due to apoptosis, since we found TUNEL positive cells during the first 8 h after trauma not only in the cortical focus but also in the dentate gyrus of the ipsilateral hippocampus. Additionally, these regions showed a strong increase in c-fos expression 1 h after trauma, as shown by *in situ* hybridization. Surprisingly, the loss of pyramidal cells seven days after trauma was less pronounced than that two days after trauma (the number of neurons decreased by 36% in CA3, 12% in CA4 and 12% in the CA1 subfield.

Immunohistochemistry showed a strong activation of glial cells near to the most visible zone of cell loss. Labelling experiments with bromodeoxyuridine for mitotically active cells revealed a threefold incrtease of newly generated granule cells in the ipsilateral dentate gyrus.

We speculate that both findings – the activation of glia and the increase of newly generated granule cells – reflect possible mechanisms of ongoing regeneration, which in future might be a target for pharmacological treatment.

16P REACTIVE OXYGEN SPECIES GENERATION BY NON-IMMUNOLOGICAL MAST CELL ACTIVATION: MODULATION BY THE SYNTHETIC CANNABINOID CP55,940

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In addition to their effects upon the central nervous system, the family of cannabinoid compounds has clearly been shown to have immunomodulatory effects (Kaminski, 1998) mediated by the CB_2 receptor. Rat mast cells express this receptor (Facci *et al.*, 1995), and respond to cannabinoids *in vivo* (Mazzari *et al.*, 1996). Nerve growth factor (NGF) and substance P have previously been shown to cause a significant generation of intracellular reactive oxygen species (ROS) at physiologically relevant concentrations in purified rat peritoneal mast cells (Brooks *et al.*, 1998). The aim of the present study was to examine the effects of the synthetic cannabinoid receptor agonist, CP55,940, upon NGF and substance P induced intracellular ROS generation in rat peritoneal mast cells.

Mast cells were harvested from male Wistar rats (200-400g) by peritoneal lavage with HEPES-buffered Locke's solution and purified by centrifugation (450g, 4°C, 15 minutes) over a 38% w/v bovine serum albumin gradient. Following purification, mast cells (>95% purity) were resuspended at 10^6 cells ml^{-1} in phosphate buffered saline supplemented with $CaCl_2$ (1mM). Mast cells were then incubated for a total of 20 minutes at 37°C with the synthetic cannabinoid receptor agonist, CP55,940 (10-1000nM final concentrations). After 5 minutes dichlorofluoroscein diacetate (1.25μM final concentration in 0.01% methanol) was added to the mast cell suspensions. Mast cells were then treated with vehicle alone, NGF (10^{-4}μg ml^{-1}), or substance P (50μM). ROS were measured as the change in dichlorofluoroscin fluorescence from basal levels following activation, and expressed as percentage change in fluorescence. The effects of CP55,940 were expressed as percentage inhibition of control. The effects of the specific CB_1 receptor antagonist SR141716A and the specific CB_2 receptor antagonist SR144528 on ROS modulation by CP55,940 were also examined. Statistical analysis was performed using an unpaired Student's t-test, and values were considered significantly different if $P<0.05$.

Mast cells produced a low basal generation of ROS. CP55,940 caused a significant ($P<0.05$) inhibition of basal ROS generation with maximal inhibition of 81 ± 8% at 100 nM CP55,940. Substance P (50μM) caused a 53 ±0.5% generation of ROS above the basal, which was significantly inhibited by CP55,940 (maximal inhibition of 81 ±17% at 100nM). NGF (10^{-4} μg ml^{-1}) caused a 95 ±0.6% generation of ROS above the basal. CP55,940 caused a concentration dependent inhibition of NGF induced ROS generation with maximal inhibition of 85 ±4% at 1μM CP55,940. The CB_1 receptor antagonist, SR14176A reduced CP55,940 (100nM) inhibition of ROS generation from 40 ±8% to 18 ±7%. The CB_2 receptor antagonist, SR144528 caused a slight, but not significant effect ($P=0.43$) upon CP55,940 (100nM) inhibition of ROS generation.

Our data show that rat peritoneal mast cells generate ROS in response to NGF and substance P as described previously (Brooks *et al.*,1998), and that the synthetic cannabinoid agonist, CP55,940, causes a significant reduction in the basal rate of ROS generation, as well as ROS generation in response to NGF and substance P. The data also show that the CB_1 receptor antagonist SR141716A causes a marked decrease in the ability of CP55,940 to inhibit ROS generation at the basal level. This is contrast to previously reported findings which suggest that the CB_2 receptor family is involved in the modulation of mast cell activation by cannabinoids (Facci *et al.*, 1995), and further work may be necessary to confirm our findings. In conclusion we speculate that endogenous cannabinoids may be important in the modulation of mast cell function under physiological and pathological conditions, particularly those involving increases in localised NGF and substance P.

We would like to thank Sanofi Recherche for their gifts of SR141716A and SR144528.

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17P INCREASE OF ARGINASE IN RAT ALVEOLAR MACROPHAGES BY LIPOPOLYSACCHARIDES OCCURS INDEPENDENT OF THE CONCOMITANT iNOS INDUCTION

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L-Arginine is the substrate of nitric oxide synthase (NOS) and arginase. In macrophages (MΦ), including alveolar MΦ (AMΦ), both enzymes can be coexpressed and compete for the common substrate, eventually resulting in a limitation of L-arginine utilization by either pathway (Hecker *et al.*, 1995; Hey *et al.*, 1995). As little is known about the regulation of the expression of the two arginase isoforms in MΦ, the effect of lipopolysaccharides (LPS), a potent inductor of iNOS in AMΦ, was studied.

Rat AMΦ (10^6 cells well^{-1}) were cultured for 20 h in DME-F12 medium containing 5 % FCS in the absence or presence of LPS (see Hey *et al.*, 1995). Thereafter, arginase activity was determined in cell lysates by measuring the formation of urea during 1 h incubation in the presence of 0.5 M L-arginine (Corraliza *et al.*, 1995). In parallel experiments, AMΦ cultured for various times were used to isolate total RNA and to extract cellular proteins for the use in semi-quantitative RT-PCR and immunoblotting of arginase I and II.

Arginase activity in rat AMΦ, cultured under control conditions amounted to 38 ± 5 mU (10^6 cells)$^{-1}$, (n=9) (means ± s.e.mean of n experiments). After culture in the presence of LPS it was increased to 85 ± 7 mU (10^6 cells)$^{-1}$ (n=9) ($p < 0.01$ vs controls, *t* test). Presence of dexamethasone (10 μM) during the culture period decreased arginase activity by 35 ± 4 % and prevented its LPS induced increase (each n=9). Presence of the NO synthase inhibitors N-monomethyl-L-arginine (L-NMMA, 100 μM) or S-ethyl-isothiourea (100 μM) during the culture period (largely suppressing LPS induced nitrite accumulation) had no effect on the subsequently determined arginase activity, but enhanced the LPS induced increase by 73 ±11 and 52 ± 6 % respectively.

LPS caused an induction of mRNA of arginase I, an effect seen after 5 h. This was followed by an increase in protein levels, determined after 20 h culture in the presence of LPS. Dexamethasone (10 μM) inhibited the LPS mediated induction of arginase I, shown at the level of mRNA and protein. In contrast to observations on arginase I, dexamethasone did not affect the initial (5 h) increase of iNOS mRNA, but opposed the LPS induced increase of iNOS mRNA seen after 20 h. Rat AMΦ also express arginase II, although at a lower level, and LPS caused an increase in mRNA and protein of arginase II. Time course experiments indicated that LPS may slow down a decline of arginase II mRNA seen during the *in vitro* culture period.

In conclusion, in rat AMΦ induction of iNOS by LPS is accompanied by an increase in arginase activity, and this effect appears to involve an induction of arginase I by mechanisms independent of the enhanced NO production and/or enhanced L-arginine consumption by iNOS.

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18P EVIDENCE FOR A LIPOPOLYSACCHARIDE (LPS) INDUCIBLE L-ARGININE SELECTIVE CATIONIC AMINO ACID TRANSPORTER IN RAT ALVEOLAR MACROPHAGES (AMΦ) LINKED TO iNOS

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NO synthesis in AMΦ, after induction of iNOS, largely depends on the cellular uptake of L-arginine (L-Arg) (Hammermann *et al.*, 1998). The cellular uptake of L-Arg is carried out by specific cationic amino acid transporters (CATs). Here, it was tested whether induction of iNOS, a condition of enhanced need of L-Arg, affects transport of L-Arg in AMΦ.

Rat AMΦ (10^6 cells well^{-1}) were cultured for 20 h in DME-F12 medium containing 5 % FCS in the absence or presence of LPS (Hey *et al.*, 1995). Thereafter, L-Arg uptake was studied by measuring the cellular radioactivity after 2 min and 5 min of incubation with ^{3}H-L-Arg (37 kBq, 0.1 or 100 μM, respectively) (Racké *et al.*, 1998). In parallel experiments, cultured cells were incubated for 6 h in amino acid-free Krebs solution to which L-Arg (30 or 100 μM) alone or in combination with L-ornithine was added. Nitrite accumulated in incubation media was determined as a measure of NO synthesis.

Under control conditions, rat AMΦ incubated with 0.1 or 100 μM ^{3}H-L-Arg accumulated 5.5 ± 0.6 (n=21) and 1 439 ± 168 (n=21) pmol ^{3}H-L-Arg per 10^6 cells, respectively (means ± s.e.mean of n experiments). After culture in the presence of LPS rat AMΦ incubated with 0.1 or 100 μM ^{3}H-L-Arg accumulated 11.1 ± 1.4** (n=21) and 5 053 ± 346** (n=21) pmol ^{3}H-L-Arg per 10^6 cells, respectively (* $p < 0.05$, ** $p < 0.01$ vs respective value without LPS treatment, *t* test). The uptake of 0.1 μM ^{3}H-L-Arg in AMΦ, cultured in the absence of LPS, was inhibited by L-ornithine (30, 100, 300, 1000 μM) by 28 ± 1, 44 ± 2, 67 ± 3 and 84 ± 1 % and that in AMΦ, cultured in the presence of LPS, by 8 ± 5*, 18 ± 3**, 48 ± 4* and 50 ± 2** %, respectively (each n=9). Uptake of 100 μM ^{3}H-L-Arg was inhibited by L-ornithine (100, 300 and 1000 μM) by 15 ± 5, 39 ± 3 and 77 ± 3 % in control AMΦ and by 3 ± 5, 18 ± 5** 33 ± 5** % in LPS treated AMΦ, respectively (each n=9). Similarly, L-lysine inhibited ^{3}H-L-Arg uptake in a concentration dependent manner and the concentration response curve was shifted to the right in LPS treated AMΦ.

AMΦ cultured in the presence of LPS and thereafter incubated in medium containing 0, 30 or 100 μM L-Arg accumulated 4.7 ± 0.6, 10.0 ± 0.5 and 16.4 ± 0.4 pmol (6 h)$^{-1}$ nitrite respectively. Nitrite accumulation under either condition was not affected by the additional presence of L-ornithine (30, 100, 300 and 1000 μM) in the incubation medium.

In conclusion, in rat AMΦ induction of iNOS by LPS is accompanied by an increase in L-arginine transport activity which displays a reduced sensitivity toward inhibitory effects by other cationic amino acids. Moreover, L-ornithine, in concentrations reducing L-Arg uptake, failed to inhibit L-Arg dependent NO synthesis, suggesting a particular, L-ornithine insensitive L-Arg transport mechanism linked to iNOS.

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19P THE PROSTACYCLIN ANALOGUE CICAPROST REVERSES THE INCREASE IN GM-CSF PRODUCTION CAUSED BY NSAID TREATMENT OF HUMAN GASTRIC BIOPSIES

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Non-steroidal anti-inflammatory drugs (NSAIDs), a diverse group which act through inhibition of the enzyme cyclo-oxygenase (COX), are the most widely used drugs for the treatment of arthritis. Beneficial effects of these drugs are attributed to inhibition of inducible COX-2 while inhibition of prostacyclin production via constitutive COX-1 may underlie their side effects, including gastric ulceration (Vane *et al.*, 1998). For this reason COX-2 inhibitors have been proposed to be safer. NSAIDs stimulate the release of granulocyte macrophage colony stimulating factor (GMCSF) by cytokine treated vascular smooth muscle cells (Mitchell *et al.*, 1998). Since damage caused by NSAIDs is associated with neutrophil activity (Wallace *et al.*, 1990), and GMCSF stimulates maturation, activation and survival of leukocytes, including neutrophils, we have investigated the effects of COX-1 (indomethacin) and COX-2 (L-745,337; Chan *et al.*, 1995: DFP; Gottesdiener *et al.*, 1998) selective NSAIDs on GMCSF production by human gastric (antral) tissue. Antral biopsies, from routine endoscopy were equilibrated at (37°C, 5% CO_2) in DMEM supplemented with 2mM glutamine and antibiotics for 30min prior to addition of NSAIDs (10μM) in combination with the prostacyclin analogue, cicaprost (10μM, Schering AG, Germany) or vehicle. There was a trend towards greater PGE_2 production, a measure of COX activity determined by radioimmunoassay, tissue from patients with gastritis and *Helicobacter pylori* (Hp) infection (22.2±4.8ng/mg protein, n=24) or gastritis alone (20.7±1.9ng/mg n=23) than controls (9.1±3.2ng/mg, n=16). GMCSF production, measured by ELISA, was also elevated in patients with gastritis (Hp+, 125.3±46.7 pg/mg, n=25) compared to gastritis (Hp-, 47.2 ±2.5 pg/ml, n=25) or controls (48.4 ±13.3 pg/mg, n=16). Here we show that COX-1 and COX-2 inhibitors reduce PGE_2 released by human inflamed gastric tissue (Fig 1). Only indomethacin, which selectively inhibits COX-1, increased GMCSF production.

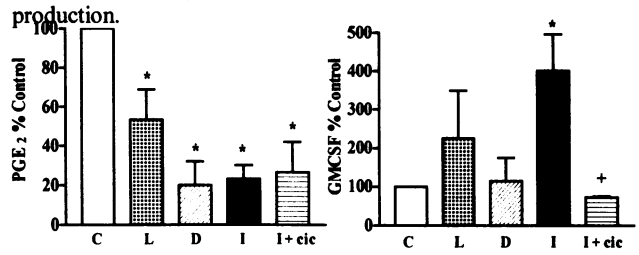

Figure 1: NSAID-induced changes in PGE_2 and GMCSF production by antral biopsies from patients with gastritis and Hp infection. Mean ± s.e.m of 6 patients. Mann-Whitney U test *p<0.05 v control (C), +p<0.05 indomethacin (I) v I +cicaprost (cic), DFP(D), L745,337 (L).

Since GMCSF increases neutrophil dependent damage, this data may provide a novel explanation for the mechanism of NSAIDs induced side effects in the gut. The validity here being strengthened as a prostacyclin mimetic, which reverses damage in animal models, completely blocked the effects of indomethacin on GMCSF release.

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20P STUDY ON THE PROTECTIVE ROLE OF *NIGELLA SATIVA* OIL ON STEROID-INDUCED OSTEOPOROSIS IN RATS

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Osteoporosis is a disease characterized by low bone mass and architectural deterioration of bone tissues. It was observed that some hypolipidaemic drugs were able to suppress steroid-induced damage to osteocytes (Zhou *et al.*, 1996). Due to the favourable effect of *Nigella sativa* oil (NSO) on lipid profile in rats (El-Dakhakhny *et al.*, 1997), it was thought interesting to evaluate its possible role in protection against prednisone induced osteoporosis in rats.

Four groups of female albino rats (8 animals each group, 180-200 g each) were used. The first group acted as a control group, the second group received prednisone (1 mg/Kg), the third group received NSO (0.88 g/Kg) & the fourth group received NSO with prednisone as groups 2 and 3 daily orally for 6 weeks. The animals were then exposed to light anaesthesia and blood samples were taken. Daily urine was also collected.

It was found that the intake of NSO had a protective effect against prednisone-induced osteoporosis as evidenced by the significant (P<0.05) elevation in serum alkaline phosphatase (24±3.79 to 41±3.9 mean±S.D. K&K), serum calcium (7.8 ±1.2 to 10±1.01 mg/dl) & serum osteocalcin (46.4±6.9 to 80.7±9.8 μg/l). There was also a significant decrease in serum cholesterol (75±3.6 to 68±2.7 mg/dl), triglycerides (130±8.5 to 107 mg/dl and urinary hydroxyproline (138±14 to 84±9.8 μmol/day) and urinary calcium (1.8±0.2 to 0.7±0.3 mmol/24h) in the NSO treated group when compared to the osteoporotic group.

The possible mechanism involved may be due to the hypolipidaemic effect of the NSO, the mild ethinyl-estradiol activity (Keshri *et al.*, 1995) and/or the elevated interleukin 3,1B (Hag *et al.*, 1995) This study shows that the intake of NSO may have a beneficial protective effect against incidence of osteoporosis and has to be confirmed in man.

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21P GLUCOCORTICOID HORMONES REDUCE INTEGRIN EXPRESSION ON MURINE EOSINOPHILS

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The involvement of eosinophils (EØ) in the pathophysiology of allergic diseases such as asthma is well-established and glucocorticoid hormones (GC) are used as first line treatment of these condition. Adhesion molecules play a major role in EØ adhesion and migration and in a recent study we showed that dexamethasone (DEX) can reduce cell surface $ß_2$ integrin levels on human EØ (1). The aim of this study was to investigate DEX effect on murine EØ integrin expression and to assess the functional relevance of this observation .

Blood EØ were isolated from mice over-expressing the Interleukin 5 (IL-5) gene using negative selection in a Macs column as previously described (2). Purified cells (10^6 per well) were cultured in complete medium supplemented with 1 ng/ml mouse IL-5, in the absence or presence of DEX (0.01-1 µM) or hydrocortisone, prednisolone, progesterone and testosterone (10 µM). In selected experiments, the effect of 10 µM RU486 co-incubation with 1 µM DEX was also determined. In all cases, cells were collected after 16 or 40 h incubation, and (i) viability assessed using the Trypan Blue exclusion test; (ii) CD11b and VLA_4 (CD49d) levels (cell surface and intracellular) measured using single cell labelling and flow cytometry (data being reported as units of median intensity fluorescence, MFI) in untreated or saponin-permeabilised cells, respectively; and (iii) EØ chemotaxis was assayed using chemoTXs™ plates from Neuroprobe with the migration index (MI) defined as the number of cells which migrated to the chemoattractant divided by the number migrating to the vehicle. Statistical analysis was performed using the paired student t – test and ANOVA followed by Dunnett's test, taking a P value <0.05 as significant.

Addition of IL-5 into the culture medium increased the 40 h viability of control cells from 19.7 ± 3.9% (n=11) to 92.1 ± 0.9% (n=19) and this was unaffected by 1 µM DEX (84.1 ± 2.3%, n=17). In cultured EØ, CD11b levels were 347 ± 74 MFI units, with VLA_4 values being 195 ± 14 MFI units (n=5). EØ incubation with DEX produced a reduction in CD11b expression which was only significant at the 1µM concentration and at the 48 h time point (43.8± 4.8% inhibition, n =15; P < 0.05). Co-incubation with RU486 blocked the effect of DEX on both CD11b levels (1 ± 3.8 % inhibition, n=6) and VLA_4 levels (2.2 ± 4.1 % inhibition, n=6). The DEX inhibitory action was mimicked by hydrocortisone (31 ± 9.6 % inhibition of CD11b expression, n=4) and prednisolone (32.6 ± 5.8 % inhibition of CD11b expression, n=6), but not testosterone or progesterone (not shown). Finally, DEX did not alter total EØ CD11b levels (as measured in permeabilised cells). In the chemotaxis assay, treatment of EØs with 1 µM DEX for 48 h inhibited the cell migratory response to both 10 ng/ml eotaxin (2.9 ± 0.3 *vs* 2.1 ± 0.3 MI, P< 0.05, n=9) and 0.1 µM platelet activating factor (1.9 ± 0.2 *vs* 1.4 ± 0.1, P< 0.05, n = 9).

In conclusion, inhibition of integrin expression on the cell surface of EØ contributes to the powerful inhibitory effect exerted by GC on EØ trafficking. This effect is mediated by the GC receptor and may be due a reduced externalisaton of the adhesion molecule on the plasma membrane.

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(2) Teixera MM, *et al.*, *Br J Pharmacol* 123: 538-44, 1998.

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22P CC CHEMOKINE EXPRESSION IN A MURINE MODEL OF CHRONIC GRANULOMA

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Several studies have investigated the expression of chemokines in acute models of inflammation, but less information is available in the literature with respect to chronic inflammatory models. Here we have investigated the profile of expression of two CC chemokines, macrophage inflammatory protein-1α (MIP-1 α) and monocyte chemoattractant protein-1 (MCP-1) in a murine model of chronic granuloma [1].

Female Swiss Albino mice (28-32 g) were treated s.c. with 3 ml of air 1 day prior to the local administration of 0.5 ml Complete Freund's Adjuvant (5 mg/ml *Myc. Tuberculosis*) supplemented with 0.1% croton oil [1]. At several days post-injection, animals were sacrificed and air-pouches collected: skin and granuloma samples were divided into two parts: a portion was snap frozen in iso-pentane and stored for histochemistry. Another portion was stored in liquid nitrogen before homogenisation and i) determination of chemokine contents by ELISA, and ii) assessment of enzymatic activities. Myeloperoxidase (MPO) reaction: 20 µl sample with 2 mM 3,3',5,5'-tetramethylbenzidine in acetate buffer, pH 6. Readings at 620 nm wavelength were interpolated on a standard curve constructed with human neutrophil MPO (Calbiochem). N-acetyl-ß-D-glucosaminide (NAG) reaction: 25 µl samples with 2.5 mM *p*-nitrophenyl-N-acetyl-ß-D-glucosaminide in citrate buffer, pH 4.5. Readings at 405 nm wavelength were interpolated on a standard curve constructed with different concentrations of *p*-nitrophenol. Specific ELISA for mouse MIP-1α and MCP-1 were purchased from R&D Systems (Abingdon, UK) and BioSource International. Data are mean ± s.e.mean of n mice per group, and were statistically analysed using ANOVA plus Bonferroni test taking a P value <0.05 as significant.

Only 10 ± 4 pg MIP-1 α per mg of protein were measured in skin samples in the absence of inflammation. MIP-1 α protein expression increased sharply after induction of inflammation, with >200 pg/mg values being measured in the granuloma between day 1 to 5 post-injection (peak at day 3: 284 ± 57 pg/mg, n=12). These amounts decreased steadily up to 4 weeks post-induction of inflammation (e.g. 91 ± 15 and 49 ± 6 pg/mg at day 7 and 14, respectively, n=17). MPO activity mirrored MIP-1 α expression, with the highest values being measured within the first week of inflammation: 120 ± 16 and 155 ± 19 mU/mg at day 3 and 5, respectively (n=12). Immunohistochemical analysis showed MIP-1 α to be associated predominantly with the monocyte/macrophage population infiltrating the granuloma. MCP-1 immunoreactivity in non inflamed skins was 4 ± 1 ng/mg (n=6). In contrast to MIP-1 α, MCP-1 levles increased steadily reaching maximal values at 14 day post-injection (27.4 ± 5.7 pg/mg, n=10-17). Similarly, NAG activity increased steadily reaching significantly higher values (P<0.05) vs. non-inflamed skins at days 21 and 28: 30 ± 1 (n=3), 40 ± 3 (n=12) and 50 ± 4 (n=11) nmol/mg at day 0, 21 and 28, respectively.

In conclusion, MIP-1 α and MCP-1 expression followed a different pattern of expression. Higher MIP-1 α values were measured during the first week of inflammation (inflammatory phase) and appeared to be associated with the influx of polymorphonuclear leukocytes. In contrast, MCP-1 values were significantly higher during the late phase of the response (repair phase), and were associated with an influx or maturation of monocytes/macrophages. These two chemokines, therefore, may play a distinct role in this model of chronic inflammation.

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23P SALICYLATE ANTAGONIZES THE EFFECTS OF SELECTIVE CYCLOOXYGENASE-2 INHIBITORS ON GASTRIC MUCOSAL INTEGRITY IN RATS

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The affinity of salicylate for cyclooxygenase (COX)-1 and COX-2, the key enzymes in prostaglandin biosynthesis, is controversial (Frölich, 1997, Botting & Vane, 1997). We investigated the interaction of salicylate with selective COX-2 inhibitors using 2 established models of gastric damage.

Male Wistar rats (180-200 g, n=6 each group) were used. 1) Gastric ischaemia-reperfusion: Rats were anaesthetized with pentobarbital (50 mg • kg^{-1}, i.p.). The pylorus was ligated and 1 ml of 0.1 N HCl instilled p.o. The celiac artery was occluded for 15 min followed by 30 min reperfusion. Then gastric mucosal damage was assessed by calculation of a lesion index (LI). Groups of rats were pretreated with the selective COX-2 inhibitors DFU (5.5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl) phenyl-(5H)-furanone (2 mg • kg^{-1}, s.c., 30 min) or NS-398 (N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide) (4 mg • kg^{-1}, s.c., 30 min) or the NO synthase inhibitor L-NAME (N^G-nitro-L-arginine methylester) (10 mg • kg^{-1}, i.v., 10 min). Further rats received sodium salicylate (0.01-0.05 mg • kg^{-1}, s.c.) 30 min before DFU and NS-398 or 50 min before L-NAME. 2) Mild irritant-induced gastroprotection: Conscious rats received 1 ml of 20% ethanol p.o. followed by 1 ml of 70% ethanol 30 min later. After further 5 min, mucosal damage was assessed. Groups of rats received DFU (0.2 mg • kg^{-1}, p.o., 30 min), NS-398 (1 mg • kg^{-1}, p.o., 30 min) or L-NAME (10 mg • kg^{-1}, i.v., 10 min) before instillation of 20% ethanol. Further groups of rats receiced salicylate (0.01-0.05 mg • kg^{-1}, p.o.) 30 min before DFU, NS-398 or L-NAME. Values are expressed as mean ± s.e.m. Statistical significance was assessed using Wilcoxon rank test.

Mucosal damage was minor after ischaemia-reperfusion alone (LI 4±1) but was significantly ($p<0.001$) aggravated by pretreatment with DFU (LI 34±2), NS-398 (LI 39±2) or L-NAME (LI 42±2). Salicylate (0.05 mg • kg^{-1}) reversed ($p<0.001$) the effects of DFU (LI 10±1) and NS-398 (LI 10±1) but not L-NAME (LI 38±3). The salicylate effect was dose-dependent (ID_{50} against NS-398: 0.02 mg • kg^{-1}).

Instillation of 20% ethanol prevented mucosal damage caused by 70% ethanol (LI 4±1 vs. 32±1, $p<0.001$). The protection was inhibited ($p<0.001$) by DFU (LI 33±1), NS (LI 29±2) or L-NAME (LI 43±2). Salicylate (0.05 mg • kg^{-1}) given alone did not protect against damage caused by 70% ethanol (LI 29±3) but abolished ($p<0.001$) the inhibition of mild irritant-induced protection evoked by DFU (LI 10±3) and NS-398 (LI 4±1) but not L-NAME (LI 41±3). The salicylate effect was dose-dependent (ID_{50} against NS-398: 0.02 mg • kg^{-1}).

In conclusion, salicylate at very low doses inhibits the effects of selective COX-2 inhibitors on rat gastric mucosal integrity. It remains to be investigated whether this drug interaction occurs at the level of COX-2 - similar to the interaction of salicylate and aspirin on platelet COX-1 (Dejana et al., 1981) - or is a prostanoid-independent phenomenon.

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24P PHARMACOLOGICAL EXAMINATION OF NEUROGENIC RESPONSES OF THE SHEEP ISOLATED INTERNAL ANAL SPHINCTER

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Recent evidence implicates nitric oxide as the principal inhibitory neurotransmitter controlling the internal anal sphincter (IAS) of man (Kelly et al., (1993), the smooth muscle involved in the control of defaecation. To date, the most widely studied animal model of this tissue is that from the opossum, for which pharmacological and biochemical evidence also supports a role for nitric oxide in the inhibitory neurogenic response (Chakder and Rattan, 1993). Since the opossum is not indigenous to the British Isles, we have examined the properties of the sheep IAS with the view to assessing its suitability as a pharmacological model for man.

Anal tissue of male and female sheep was obtained from a local abattoir within one hour of slaughter. The anal canal was opened and the mucosa removed to expose the underlying circular smooth muscle fibres of the IAS. Strips of the distal IAS (10mm x 2mm x 2 mm) were mounted between two platinum electrodes, within a perspex holder, and placed in a 20 ml organ bath containing Krebs-Henseleit saline (gassed with 95% O_2/5% CO_2 and maintained at 37°C). Following the application of 2 g wt. tension the tissues were allowed to equilibrate for 40 min and stimulated transmurally (1-20 Hz, 0.5 ms, 200 mA every 180 s) in the absence or presence of 0.1 µM atropine, 1 µM phentolamine or 100 µM L-NAME. Some segments were also exposed to cumulative concentrations of sodium nitroprusside. Responses have been expressed as the g wt., or as a percentage of the resting tone, and differences (mean ± sem) considered significant if $p < 0.05$ (Student t-test).

Within 30-40 min of the application of resting tension the segments developed stable myogenic tone (3.8±0.2 g wt., n=17). Electrical stimulation caused rapid, frequency-dependent relaxation; the amplitude of the maximum response (10 Hz) was approximately 60% of the myogenic tone (2.3±0.2 g wt., n=17). Tetrodotoxin (0.3 µM) abolished the inhibitory responses to electrical stimulation (n=4). Relaxations to 10 Hz stimulation were not signiicantly affected either by 0.1 µM atropine (2.13±0.39 g wt., n=4) or 1 µM phentolamine (2.60±0.69 g wt., n=4). The nitric oxide synthase inhibitor L-NAME (100 µM) abolished the inhibitory response to 10 Hz stimulation and uncovered a neurogenic contraction (1.20±0.28 g wt, n=7). Subsequent addition of 1 mM D-arginine failed to affect the neurogenic contractions (1.11±0.31 g wt., n=4) but 1 mM L-arginine reversed the neurogenic contractions to relaxations (0.69±0.19 g wt., n= 9). Sodium nitroprusside caused concentration-dependent inhibition of myogenic tone (equivalent to 100%) with a pIC_{50} of 6.45±0.13 (n=3).

In conclusion we have demonstrated that the sheep IAS develops myogenic tone to stretch and responds to electrical stimulation by relaxing. These neurogenic, inhibitory responses are mediated by nitric oxide, with no evidence for the involvement of either noradrenaline and acetylcholine and, as such, are pharmacologically similar to those observed in the opossum and human IAS. The sheep IAS represents a good model for the human IAS.

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25P ACETYLCHOLINESTERASE ACTIVITY IS ELEVATED IN DIVERTICULAR DISEASE

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Acetylcholinesterase (AChE) activity was investigated biochemically and pharmacologically in the muscularis externa of the sigmoid colon in diverticular disease, in order to determine if changes in this enzyme could be responsible for some of the dysfunction of the colon that occurs during this condition.

Samples of sigmoid colon were obtained from 20 patients with diverticular disease (eight males and 12 females, mean age 71 yr). Samples were also obtained from a control group seven males and seven females, mean age 65 yr) undergoing resection for rectal carcinoma. The criteria for exclusion included the presence of obstruction or gross inflammation. The presence of diverticular disease was confirmed by barium enema or colonoscopy, and gross and microscopic examination of the specimen. At the time of obtaining the samples of tissue ethical permission was not required, and all material used was surgically redundant.

In the muscularis externa, which included taenia coli, AChE activity was greater in the diverticular group than in controls, being 295.2 ± 27.1 (n = 10) $\mu moles.h^{-1}.(mm^2\ tissue)^{-1}$ and 211.8 ± 22.2 (n = 7) $\mu moles.h^{-1}.(mm^2\ tissue)^{-1}$, respectively ($P < 0.05$, Student's *t*-test). In isolated preparations of longitudinal muscle (i.e. taenia coli, LM) and circular muscle (CM), acteylcholine (ACh) evoked concentration-dependent contractions. In the absence of eserine there was no indication that a maximum level of contraction had been reached at the highest concentration tested (1 mM). In the presence of eserine (1 μM), the concentration-response curves reached a clear maximum level, and in their construction the responses to acetylcholine were expressed relative to the maximum response obtained in the presence of eserine. Because a clear maximum level had been reached it was possible to calculate $p[A]_{50}$ values for ACh (Table 1), where $p[A]_{50}$ is the negative logarithm of the concentration of ACh that produced a half maximal (50%) response. These results are summarised in Table 1. The $p[A]_{50}$ values for ACh were significantly lower in LM and CM from diverticular disease than in the controls (Student's *t*-tests). Eserine produced greater increases in the $p[A]_{50}$ values of tissues from diverticular disease patients that from control patients.

Thus it is evident that AChE activity is elevated in colonic smooth muscle in patients with diverticular disease. This may be responsible for some of the dysfunction of the colon that occurs during this condition.

Table 1. $p[A]_{50}$ values for acetylcholine in longitudinal (LM) and circular muscle (CM) in the absence and presence of eserine.

			+ eserine (1 μM)			+ eserine (1 μM)
Control	LM	4.3 ± 0.82 (6)*	6.4 ± 0.77 (6)	CM	4.3 ± 0.33 (6)*	6.9 ± 0.75 (6)
Diverticular disease	LM	3.3 ± 0.13 (9)	6.5 ± 0.74 (9)	CM	2.8 ± 0.36 (9)	6.0 ± 0.89 (9)

* $P < 0.05$ versus corresponding diverticular disease group. In each tissue eserine significantly raised the $p[A]_{50}$ ($P < 0.001$).

26P THE α_{1L}-ADRENOCEPTOR MEDIATES CONTRACTION IN HUMAN VAS DEFERENS

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Three α_1-adrenoceptor subtypes have been cloned (α_{1A}, α_{1B} and α_{1D}) and an additional α_{1L}-adrenoceptor has been identified in functional studies (Michel et al. 1995). In the human prostate, radioligand binding studies with [^{3}H]tamsulosin have been used to demonstrate the presence of the α_{1L}-adrenoceptor (Mason *et al.*, 1997). We have previously reported that the adrenoceptor mediating contraction of the human vas has the pharmacological properties of the α_{1A}-receptor (Furukawa *et al.*, 1995). However the possibility that the α_{1L}-subtype mediates contraction was not considered and a selective antagonist at the α_{1D}-receptor was not available. The aim of this study was to identify the role of the α_{1L}- and α_{1D}-adrenoceptors in mediating contraction of the human vas deferens using prazosin and RS17053, antagonists which distinguish between the α_{1A}- and α_{1L}-adrenoceptor, and BMY7378 an α_{1D}-selective antagonist (Saussey et al., 1994).

Human epididymal vas deferens from patients undergoing vasectomy were suspended under 1g tension in gassed Krebs at 37°C. Responses to phenylephrine were obtained in the absence and presence of antagonist (60min equilibration period). Only one non-cumulative concentration-response curve was obtained on each tissue. All experiments were conducted in the presence of corticosterone (10μM), cocaine (10μM) and propranolol (1μM).

Saturation experiments with Scatchard analysis were performed on membranes from human vas deferens using [^{3}H]tamsulosin. Competition experiments were performed using 0.5nM [^{3}H]tamsulosin and analysed using Graphpad PRISM software.

Phenylephrine produced concentration-dependent contractions of isolated human vas deferens. BMY7378 (300nM) failed to antagonise responses to phenylephrine (n=6), but higher concentrations (1 & 3μM) shifted the phenylephrine curve to the right yielding a mean (±sem) apparent pK_B value of 6.5±0.1 (n=12). Prazosin (10-100nM) antagonised responses with low affinity (pK_B = 8.6±0.1, n=12). Maximum responses to phenylephrine were not altered by this antagonist and the Schild plot had a slope of unity (1.1±0.3). RS17053 (100 & 300nM) also antagonised responses yielding low affinity estimates (7.1 for 100nM, n=14; 7.2 for 300nM, n=8). Increasing the concentration of RS17053 to 1μM did not shift the phenylephrine curve any further but maximum response was reduced by 54% although this was not significant (One way ANOVA).

[^{3}H]tamsulosin bound to membranes from human vas deferens with high affinity (pK_D of 10.0 ± 0.24) and with a density of binding sites of 27.8 ± 11.9 fmoles mg^{-1} protein. Prazosin (n=4) and RS17053 (n=3) displaced [^{3}H]tamsulosin from a single site which had a low affinity for both antagonists (pK_i values of 8.5±0.1 and 7.2±0.1 for prazosin and RS17053 respectively). Hill slopes for both antagonists were similar to unity (prazosin, 1.1±0.1; RS17053, 0.9±0.1).

The functional studies suggest that the main mediator of contraction in the human vas deferens is the α_{1L}-adrenoceptor subtype. The radioligand binding data supports this conclusion and furthermore suggests that the α_{1L}-adrenoceptor is the only subtype present in this tissue at the protein level.

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27P DETERMINATION OF CYTOCHROME P450 3A4 INDUCTION IN PRIMARY HUMAN HEPATOCYTES USING QUANTITATIVE REAL-TIME PCR

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Drug-induced induction of hepatic cytochrome P450 (CYP) is a key cause of drug-drug interactions. Such enzyme induction can modify drug clearance and thus alter the efficacy of drugs unrelated to the inducer. Pre-clinical prediction of CYP induction by novel compounds in primary hepatocytes is now routinely used to determine whether novel compounds are likely to induce CYP expression in the clinical phase. Commonly, protein expression and substrate metabolism are used as assay endpoints, but given the homology between the numerous CYP isoforms, these methods may not offer absolute specificity. Since it is thought that most cases of drug-induced CYP induction are through increased gene transcription (Gonzalez, 1988), quantification of CYP mRNA offers an alternative highly isoform specific *in vitro* test of CYP induction. Here, we describe the application of 96-well based quantitative real-time PCR (QRT-PCR) to the study of CYP3A4 induction in human hepatocytes.

All tissues were obtained through medically qualified intermediates, with the full consent of the donor, and with the approval of the local ethics committee. Hepatocytes were isolated from liver wedges by two-step collagenase digestion (Strom *et al.*, 1982), resuspended in William's E medium and cultured in collagen-coated 6-well plates. After 3 days of culture, hepatocytes were treated either with the CYP1A inducer 3-methylcholanthrene (3-MC; 1μM) or the CYP3A4 inducer rifampicin (50μM) for a further 2 days. Control and solvent (DMSO) treated cultures were also prepared. Total RNA was isolated and CYP3A4 mRNA expression quantified using QRT-PCR (Page *et al.*, 1998). Data are presented as the logarithm of the number of mRNA molecules in 100ng of total RNA (Log Cn).

Induction of CYP3A4 was studied in preparations of human hepatocytes isolated from three donors. Basal expression of CYP3A4 mRNA in the three donors was 3.13, 4.08 and 4.83. Rifampicin significantly ($P<0.05$ by paired t-test, n=3 donors) induced CYP3A4 mRNA expression (Figure), the mean fold increase in expression over control being 17.4 (4.6, 20, 28). Neither 3-MC nor DMSO had any significant effect on CYP3A4 expression. These data are consistent with the established effects of these treatments on CYP3A4 protein/activity (Li *et al.*, 1997).

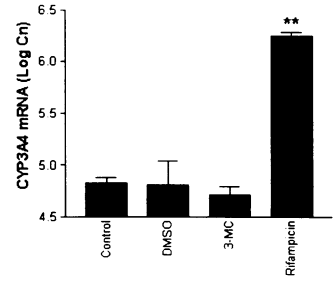

Figure. Effect of 3-MC and rifampicin on CYP3A4 mRNA expression in a representative experiment. Data are mean ± s.e.mean (n=3). (** $P<0.01$; ANOVA and Dunnett test).

These data demonstrate the applicability of 96-well-based QRT-PCR to the study of gene dynamics in human hepatocytes. This method represents a highly specific alternative to quantification of CYP induction by immunodetection and substrate metabolism.

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28P MUTATION OF THE POTENTIAL PALMITOYLATION SITE OF THE RAT NEUROPEPTIDE Y Y_1 RECEPTOR SUPPRESSES ITS FUNCTIONAL DESENSITISATION

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Palmitoylation of G protein-coupled receptors may be a dynamic modification altering signalling efficacy or internalisation (Bouvier *et al.*, 1995). We have previously used clones from human colonic epithelial cell lines to investigate the properties of stably transfected rat Y_1 receptors using [^{125}I]peptide YY (PYY) binding assays and functional measurement of short-circuit current responses (I_{SC}; Holliday & Cox, 1996). Here, we examine the possible effects of Y_1 receptor palmitoylation by comparing HCA-7 Colony 1 clones expressing the native rat Y_1 receptor (Y1-6, Y1-15) or a mutant which replaces the potential acylation site (Cys^{337}) with Ser (Y1C-3, Y1C-5).

Competition assays were carried out on membrane preparations using 20 pM [^{125}I]PYY and 10 pM - 1 μM unlabelled ligand; in saturation experiments, non-specific binding was assessed with BIBP 3226 (1 μM). After 120 min, incubations at 20°C were terminated by rapid filtration over GF/B filters soaked in 0.3 % polyethylenimine. In functional studies, epithelial layers (area 0.2 cm^2) were voltage-clamped at 0 mV (Holliday & Cox, 1996) and peak changes in the resulting I_{SC} were measured in response to basolateral peptide additions. PYY concentration-response curves were constructed non-cumulatively.

Specific [^{125}I]PYY binding was displaced in Y1 and Y1C clones by PYY (IC_{50}: 1.8 - 6.5 nM; n = 4 - 12) or BIBP 3226 (IC_{50}: 2.1 - 4.3 nM; n = 4), and reduced by GTPγS (10 μM; by 28 - 42 %) or NaCl (100 mM; by 39 - 52 %) added to the binding buffer (n = 3 - 4). Scatchard analysis of saturation isotherms (Table 1) also yielded similar PYY binding affinities (pK_i) for each clone and B_{max} values ranging from 80 - 533 fmol mg^{-1} membrane protein. In Y1 and Y1C epithelial layers, PYY inhibited VIP-stimulated I_{SC} with similar potency but varying maximal response size (Table 1). Responses in native Y1 clones became more transient with increasing agonist concentration, while those in Y1C-3 and Y1C-5 cells were sustained even after 1 μM PYY. Reductions after 100 nM PYY were significantly greater ($P < 0.001$, Student's t test) 10 min after agonist addition in Y1C-3 (-82.2 ± 5.9 % of peak response at 3 - 4 min, n = 7) and Y1C-5 epithelia (-91.4 ± 4.3 %, n = 8) than in either Y1-6 (-24.1 ± 3.2 %, n = 8) or Y1-15 cells (-20.7 ± 5.3 %, n = 6). Subsequent 1 μM UK 14,304 responses (via α_{2A}-adrenoceptors) exhibited similar time-profiles in all four clones. Thus Y_1(C337S) receptors bind agonist with high affinity and are capable of coupling to G_i proteins, but appear more resistant to functional desensitisation than the native form.

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Table 1. Summary of binding and functional data in Y1 and Y1C clones.

	Saturation binding		*I_{SC} measurement*			
Clone	*pK_i*	*B_{max} (fmol mg^{-1})*	*30 nM VIP (μA cm^{-2})*	*100 nM PYY (μA cm^{-2})*	*100 nM PYY (% total I_{SC})*	*PYY EC_{50} (nM)*
Y1-6	9.02 ± 0.14 (3)	145 ± 9 (3)	+51.6 ± 1.6 (215)	-35.1 ± 4.8 (8)	-38.4 ± 3.3 (8)	29.9 (11.1 - 80.7)
Y1-15	8.70 ± 0.10 (3)	80 ± 24 (3)	+37.0 ± 2.1 (54)	-12.5 ± 3.0 (6)	-18.4 ± 2.5 (6)	25.6 (13.6 - 48.6)
Y1C-3	8.69 ± 0.09 (4)	232 ± 47 (4)	+10.5 ± 0.7 (38)	-4.6 ± 0.5 (7)	-24.1 ± 3.5 (7)	26.7 (16.1 - 44.4)
Y1C-5	8.58 ± 0.04 (4)	533 ± 102 (4)	+49.4 ± 2.8 (66)	-5.6 ± 0.8 (8)	-5.8 ± 0.9 (8)	53.1 (13.7 - 207)

29P P2Y RECEPTOR-MEDIATED INHIBITION OF STRESS ACTIVATED PROTEIN KINASES IN THE HUMAN ENDOTHELIAL CELL HYBRID EAH926

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The mitogen-activated protein (MAP) kinases consist of the p42/44 MAP kinases and the stress-activated protein (SAP) kinases, c-Jun N-terminal kinase (JNK) and p38 MAP kinase. Stimulation of P2Y receptors in endothelial and smooth muscle cells activates both p42/44 MAP kinase and JNK and may mediate the proliferative effects of UTP in these cell types (Graham *et al.*, 1995, Huwiler *et al.*, 1997). However, in other cells p42/44 MAP kinase may function to inhibit SAP kinase activity through an undefined mechanism which may involve induction of a MAP kinase phosphatase (MKP) (Chen & Feldman, 1998). In this study we examined the ability of UTP to stimulate JNK and p38 MAP kinase and to regulate the responses to TNFα in the human endothelial hybrid cell line EAhy926.

JNK, p38 MAP kinase and MAP kinase-activated protein (MAPKAP) kinase-2 activities were assayed in quiescent EAhy926 cells by solid-phase/immunocomplex kinase assay. Activation of p42/44MAP kinase was determined by gel retardation assay and cellular MKP-1, JNK and p38 MAP kinase were visualised using confocal microscopy following staining with specific antibodies. Data is expressed as mean ± s.e.m. n = 4.

In EAhy926 cells, UTP (50 μM) did not stimulate the activity of either SAP kinase. However, preincubation with UTP for 30 min resulted in a substantial inhibition of 25 ng/ml^{-1} TNFα-stimulated JNK and p38 MAP kinase activity (JNK fold stim, TNFα = 4.99±0.90 TNFα + UTP = 1.12 ± 0.13). UTP also inhibited TNFα -stimulated MAPKAP kinase-2 activity (fold stim, TNFα = 5.1 ± 0.8, TNFα + UTP =1.1 ± 0.1). In 13121N cells expressing the recombinant human P2Y2 receptor, UTP stimulated JNK activity suggesting that the inhibitory effect of UTP in EAhy926 cells is mediated via a receptor other than P2Y2 or is cell type specific. In EAhy926 cells UTP also stimulated the induction of MKP-1, however, this phosphatase was found to be nuclear located whilst JNK and p38 MAP kinase were mainly cytosolic. Pretreatment of the cells with the MAP kinase kinase-1 inhibitor PD098059 (50 μM, abolished UTP-stimulated p42/44 MAP kinase activity but had no effect upon the inhibitory action of UTP. Preincubation (1-2 h) with the protein kinase C (PKC) activator phorbol myristoyl acetate (PMA) (100 nM) also inhibited TNFα-simulated JNK activity and, following chronic pretreatment (18 h), reversed the inhibitory effect of UTP.

These results show for the first time a novel inhibitory effect of P2Y receptor stimulation upon SAP kinase activity, which is distinct from phosphatase induction or p42/44 MAP kinase activation, but involves PMA -sensitive isoforms of PKC.

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30P α_2-ADRENOCEPTORS IN PORCINE CEREBRAL CORTEX SLICES DO NOT AFFECT [^{3}H]-CYCLIC AMP ACCUMULATION

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We have previously demonstrated that vasoconstrictor α_2-adrenoceptors are present on many blood vessels from the pig and cause inhibition of forskolin-stimulated [^{3}H]-cyclic AMP accumulation (Wright *et al.*, 1995ab). Trendelenburg *et al.*, (1996) have recently provided evidence for α_2-adrenoceptors mediated inhibition of [^{3}H]-noradrenaline release in the porcine cerebral cortex. We have examined (i) the pharmacological characteristics of α_2-adrenoceptor binding sites on the porcine cerebral cortex and (ii) whether the receptor is negatively coupled to adenylyl cyclase.

Brains from male and female pigs were collected from a local abattoir and transported to the laboratory on ice within 60 min. Pellets of the P_2 fraction of membranes were prepared from the grey matter of the cortex, using the method of Wright *et al.*, (1995a) and the effect of various adrenoceptor ligands against [^{3}H]-RX-821002 (0.5 nM) binding determined. [^{3}H]-cyclic AMP accumulation in 350x350 μm thick slices of the grey matter was determined by a modification of the method described by Wright *et al.*, (1995b). Following 60 min pre-labelling with [^{3}H]-adenine for 1 hour in gassed Krebs-Henseleit solution, gravity packed slices (25 μl) were added to incubation tubes containing gassed Krebs-Henseleit (final volume 300 μl), equilibrated at 37°C for 10 min before further additions. UK14304 (10 μM), a selective α_2-adrenoceptor agonist, was added and 10 min later either forskolin (30 μM) or 5'-*N*-ethylcarboxamidoadenosine (10 μM; NECA, an adenosine A_2 receptor agonist) added to the appropriate tubes. The reaction was stopped after a further 10 min by 200 μl of 1M HCl and 750 μl distilled water. [^{3}H]-cyclic AMP was determined following alumina column chromatography and liquid scintillation counting and has been expressed as % conversion of [^{3}H]-adenine. All experiments were performed in triplicate and the data expressed as the mean ± sem of either 3-5 (binding) or 4-7 (cyclic AMP) separate experiments.

Saturation analysis of membranes revealed that [^{3}H] RX821002 labelled a single site of α_2 adrenoceptors with a K_d of 0.54±0.13 nM and a density (B_{max}) of 115.2±24.5 fmolmg^{-1}. Rauwolscine (pK_i - 8.65±0.18), phentolamine (pK_i - 7.63±0.09), UK-14304 (pK_i - 7.57±0.13) and prazosin (pK_i - 5.63±0.03) caused complete displacement of [^{3}H]-RX-821002 binding with Hill Slopes not significantly different from unity. As shown in Table 1, 30 μM forskolin and 10 μM NECA caused a 5-8 fold increase in [3H]-cyclic AMP accumulation which was not significantly affected by 1μM UK-14304 ($p>0.05$, paired Student t-test). Furthermore, 1μM UK-14304 failed to affect basal accumulation of [3H]-cyclic AMP.

Table 1: [3H]-cyclic AMP accumulation (% conversion) for slices of the porcine cerebral cortex membrane (n= 5-6).

	Basal	Forskolin	NECA
Control	0.23±0.04	1.52±0.43	1.19±0.55
UK14304 (1μM)	0.23±0.04	1.30±0.37	1.24±0.58

We have confirmed the observations of Trendelenburg *et al.*, (1997) that the porcine cerebral cortex is endowed with α_{2A}-adrenoceptors (rauwolscine > phentolamine >> prazosin) but surprisingly, unlike the vascular counterpart, these do not appear to be negatively coupled to adenylyl cyclase.

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31P Ca^{2+} ENTRY ASSOCIATED WITH HISTAMINE INHIBITION OF DRUG-INDUCED CYCLIC AMP ACCUMULATION IN HUMAN U373 MG ASTROCYTOMA CELLS

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Thapsigargin, which induces capacitative Ca^{2+} entry into human U373 MG astrocytoma cells, inhibits drug-induced cyclic AMP accumulation (Wong *et al.*, 1998), probably via an action on a Ca^{2+}-inhibitable isoform of adenylyl cyclase (Cooper *et al.*, 1995). The inhibition requires Ca^{2+} entry and two separate pathways appear to be involved, which are distinguished by La^{3+} (Wong & Young, 1999). We have investigated whether the inhibition of the cyclase by histamine, which also releases Ca^{2+} from intracellular stores, shows the same properties.

U373 MG cells were cultured on multi-well plates as described previously (Wong *et al.*, 1998). Sub-confluent monolayers were incubated at 37°C for 3 h in DMEM F12 medium containing 3 μCi (0.12 nM) [^{3}H]-adenine and then equilibrated, with or without 1 μM La^{3+}, for 10 min in Ca^{2+}-free HEPES medium containing 0.5 mM isobutylmethylxanthine (IBMX). Stimulation with 10 μM forskolin or 1 μM isoprenaline in HEPES medium (Wong *et al.*, 1998) was terminated by addition of 5% trichloroacetic acid and [^{3}H]-cyclic AMP separated by the method of Salomon *et al.*, (1974). Corrections were made for variations in cell density between wells and for the recovery of ATP and cyclic AMP (Wong *et al.*, 1998).

The inhibition by 10 μM histamine of cyclic AMP accumulation stimulated by 1 μM isoprenaline measured over a 4 min incubation period (66 ± 1%) was abolished when Ca^{2+} was omitted from the medium and 0.2 mM EGTA added. A near maximal inhibition by histamine was achieved by 1 min when histamine and isoprenaline were added together. The time-course of the extent of the inhibition was similar with 10 μM forskolin as cyclase stimulant.

In the presence of 1 μM La^{3+} histamine caused an increase in $[Ca^{2+}]_i$ which returned over *circa* 100 s to near basal levels, although in each of 3 measurements there was a small secondary increase of approximately 10 nM. Where the Ca^{2+} signal in response to 10 μM histamine in normal medium had a 'plateau' phase, addition of 1 μM La^{3+} at the plateau reduced $[Ca^{2+}]_i$ rapidly to resting levels. In marked contrast, 1 μM La^{3+} only partly reversed the inhibition of isoprenaline-induced cyclic AMP accumulation by 10 μM histamine, measured over a 4 min incubation. However, the inhibition produced by 5 μM thapsigargin, measured concurrently, was effectively reversed by 1 μM La^{3+}, as reported previously (Wong & Young, 1999).

The inhibitory action of histamine apparently did not involve protein kinase C, since the broad-spectrum kinase inhibitor K-252A, 1 μM, did not produce any reversal. There was no indication that the inhibition produced by 10 μM histamine and 5 μM thapsigargin was additive, but in the presence of histamine + thapsigargin, 1 μM La^{3+} appeared to completely reverse the inhibition of the response to either 10 μM forskolin (116 ± 2% of control, n=6) or 1 μM isoprenaline (110 ± 4%, n=2, weighted mean).

These observations suggest that histamine activates a Ca^{2+} entry pathway distinct from or in addition to those opened by thapsigargin. However, the action of thapsigargin may be complex and it may also promote the inactivation of a Ca^{2+} entry pathway activated by histamine. This could be the thapsigargin/store-refilling-activated, La^{3+}-insensitive, channel, which appears to inactivate rapidly (Wong & Young, 1999).

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32P DIFFERENTIAL COUPLING TO ENDOGENOUS G PROTEIN SUBPOPULATIONS IN CHO AND BHK CELL LINES RECOMBINANTLY EXPRESSING mGlu1α RECEPTORS

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The mGlu1α receptor activates phospholipase C-β (PLC-β), however the G protein(s) responsible for receptor-effector coupling have been the subject of some debate. Recent studies in BHK cells recombinantly expressing rat mGlu1α receptors have provided evidence to suggest a dual regulation of PLC-β by $G\alpha_{i/o}$-, as well as $G\alpha_{q/11}$-type G proteins (Carruthers *et al.*, 1997). In the present study we have compared receptor-G protein coupling using a combined [^{35}S]-GTPγS binding and Gα subunit-specific immunoprecipitation protocol in BHK-mGlu1α cells and a CHO cell-line inducibly expressing human mGlu1α receptors (Hermans *et al.*, 1998).

mGlu1α receptor expression was induced in CHO cells with IPTG (100 μM, 20 h). BHK and CHO cell membranes were prepared, and [^{35}S]-GTPγS-binding/immunoprecipitations were performed as described by Akam *et al.*, (1998). Agonist-stimulated [^{3}H]-$InsP_x$ accumulations were assessed following [^{3}H]*myo*-inositol labelling (2.5 μCi ml^{-1}, 48 h) and incubation with glutamic-pyruvic transaminase (3 U ml^{-1}), pyruvate (3 mM) and LiCl (10 mM) for 30 min prior to challenge (Hermans *et al.*, 1998). Where indicated, cells have been treated with pertussis toxin (PTx; 100ng ml^{-1}) for 20 h prior to membrane preparation or assay.

Immunoprecipitation of [^{35}S]-GTPγS-Gα subunits revealed that in BHK-mGlu1α cells stimulated with quisqualate (10 μM), the activated receptor couples to $G\alpha_{q/11}$, $G\alpha_{i3/o}$, and $G\alpha_{i1/i2}$. Conversely in agonist-stimulated CHO cells mGluR1α only couples to $G\alpha_{q/11}$. Basal [^{35}S]-GTPγS binding to $G\alpha_{i3/o}$, and $G\alpha_{i1/i2}$, compared to $G\alpha_{q/11}$, was very high in both cell-lines, and treatment with PTx substantially reduced this high basal to 10-15% of that observed in untreated cells, indicative of a constitutive coupling to $G\alpha_{i/o}$ -type G Proteins. The PTx treatment also abolished any agonist-stimulated increases in [^{35}S]-GTPγS binding using the $G\alpha_{i/o}$ antibodies in the BHK cell-line.

To investigate whether PTx pre-treatment causes a reciprocal increase in $G\alpha_{q/11}$-[^{35}S]-GTPγS binding, agonist concentration-effect curves were generated in both cell-lines. Quisqualate (0.003-30 μM) caused concentration-dependent increases in [^{35}S]-GTPγS-bound to immuno-precipitated $G\alpha_{q/11}$ subunits in both BHK and CHO cell-lines, reaching a maximum of 518 ± 32% and 389 ± 26% over basal, respectively. PTx pre-treatment did not alter maximal agonist-stimulated binding, nor did it affect the pEC_{50} for quisqualate-stimulated $G\alpha_{q/11}$-activation in BHK or CHO cells (- PTx, 7.67 ± 0.14 and 6.99 ± 0.05; + PTx, 7.42 ± 0.10 and 7.22 ± 0.05, respectively).

To confirm the apparent absence of coupling of the mGlu1α receptor to $G\alpha_{i/o}$-type G proteins in CHO cells, [^{3}H]-$InsP_x$ assays were performed in control and PTx-treated cell monolayers. Unlike in the BHK-mGlu1α cells where PTx treatment increases both the potency and the maximal response to quisqualate (Carruthers *et al.*, 1997), in CHO cells no significant difference was seen in either of these parameters following PTx treatment (pEC_{50} 6.69 ± 0.03 and 6.91 ± 0.01 , maximum response 1653% ± 222 and 1763% ± 110 over basal in the absence and presence of PTx respectively).

Thus, we have presented evidence to support the conclusion that G protein coupling of the mGlu1α receptor can vary between cell types, perhaps depending on the available G protein complement of the cell-line for coupling. It has also been shown that upon removal of the $G\alpha_{i/o}$-mediated inhibitory tone on the PLC-β activity, agonist-stimulated $G\alpha_{q/11}$-activity does not appear to be altered confirming that the enhanced mGluR1α activation of PLC-β seen in BHK cells upon PTx treatment is due to the loss of the negative regulation exerted by $G\alpha_{i/o}$ rather than recruitment of further $G\alpha_{q/11}$ G proteins.

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33P S-SECOVERINE: A DEFINING LIGAND IN MUSCARINIC M_5 RECEPTOR CHARACTERIZATION

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The pharmacological properties of secoverine have been characterized previously (Zwagemakers & Claassen, 1980). According to this early report, secoverine exhibited antimuscarinic properties without antinicotinic or antihistaminic activity. The aim of the present study was to characterize the *in vitro* pharmacology of the S-enantiomer of secoverine at recombinant and native muscarinic receptor subtypes and compare it with that obtained with AQ-RA 741 (Doods *et al.*, 1991).

Methods.

Competition radioligand binding studies were performed at human recombinant muscarinic receptors stably expressed in CHO cells, using [^{3}H]*N*-methyl scopolamine as the radioligand and experimental conditions similar to that described by Hegde *et al.*, (1997). Affinity estimates (pK_i values) were calculated by the method of Cheng and Prusoff (1973).

Functional studies were conducted in guinea-pig atria (GPA, M_2 receptor assay) and in rat bladder (RB, M_3 receptor assay) using methods previously described (Watson *et al.*, 1995; Hegde *et al.*, 1997) and either (+)-cis-dioxolane or carbachol as the agonist. Tissues were incubated with antagonist for at least 60 minutes. Antagonist affinity estimates (pK_B's) were determined using the Gaddum equation (Gaddum, 1943).

Results

As shown in Table 1, S-secoverine, like AQ-RA 741, displayed a low affinity for M_5 receptors (> 19-fold lower when compared to other subtypes). Unlike AQ-RA 741, however, S-secoverine does not markedly discriminate between M_1, M_2, M_3 and M_4 receptors.

Table 1. Affinity estimates (pK_i) of secoverine and AQ-RA 741 at human recombinant muscarinic receptors

Antagonist	M_1	M_2	M_3	M_4	M_5
S-Secoverine	8.5	8.9	8.3	8.6	7.0
AQ-RA 741	7.6	8.9	7.5	7.9	6.0

Values shown are means (s.e.mean < 5%, n ≥ 3).
Hill slopes were not significantly different from unity.

Both S-secoverine and AQ-RA 741 behaved as silent antagonists in GPA and RB and produced surmountable rightward displacement of agonist concentration-effect curves in these tissues (see Table 2 for pK_B's). Unlike S-secoverine, which displayed similar affinities for M_2 (GPA) and M_3 (RB) muscarinic receptors, AQ-RA-741 exhibited 10-fold selectivity for M_2 receptors.

Table 2. Affinity estimates (pK_B) for S-secoverine and AQ-RA-741 in guinea-pig atria (GPA) and rat bladder (RB)

Antagonist	GPA	RB
S- Secoverine	8.9 ± 0.1	8.3 ± 0.1
AQ-RA 741	8.3 ± 0.1	7.0 ± 0.1

Values shown are means ± s.e.mean, n ≥ 3.

In conclusion, S-secoverine represents a useful and distinct pharmacological tool for defining the M_5 muscarinic receptor.

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34P ANTAGONISM OF MUSCARINIC CHOLINOCEPTORS BY ESERINE IN THE ILEUM OF THE NMRI MOUSE AND MYELENCEPHALIC BLEBS MOUSE MUTANT

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The mouse is increasingly being used in genetic experimentation, but compared to other laboratory animals its enteric neuromuscular transmission has been little studied. Also, there are many strains of laboratory mouse, and there may be important differences in the characteristics of enteric neuromuscular transmission amongst the many strains.

The aim of the current study was to investigate cholinergic neuromuscular transmission in the ileum of the myelencephalic blebs (*my*) mouse mutant. There is an abnormal interaction between the mesoderm and ectoderm during development, and phenotypically the adult mouse may suffer from cryptophthalmia, syndactyly and renal malformation. It has been suggested that this mutant is a model of Fraser's syndrome (Darling & Gossler, 1994). The genetic background mouse for *my* is the NMRI mouse.

NMRI and *my* mice were killed by asphyxiation with carbon dioxide. Segments of mid ileum (2 cm) were removed, flushed with cold Krebs' solution and mounted in standard organ-baths (5 or 10 ml) for isometric recording of contractile activity of the longitudinal muscle. Electrical field stimulation (EFS, supramaximal V, 0.3 ms biphasic pulses, 0.5 - 32 Hz for 30 s) was facilitated by Pt ring electrodes (3 mm diameter, 1 cm apart) through which the preparations were threaded. Frequency-response relationships and concentration-response relationships for acetylcholine (ACh) were established in the absence and presence of the cholinesterase inhibitor, eserine (physostigmine, 1μM).

ACh (10 nM - 1 mM) caused concentration-dependent contractions with a maximum response evoked by 10 -100 μM. The $p[A]_{50}$ (i.e. -log EC_{50}) values in the absence of eserine were 6.4 ±0.3 and 6.5 ± 0.2 for NMRI and *my* ilea, respectively (n = 6). In the presence of eserine the $p[A]_{50}$ value did not change significantly, being 5.9 ±0.7 and 6.1 ± 0.2, respectively ($P > 0.05$, paired *t*-tests). In the presence of eserine the level of maximum response decreased significantly to 44.1 ± 9.1 % and 41.9 ± 10.4 % relative to the control conditions, for NMRI and *my* preparation, respectively ($P < 0.002$, paired *t*-tests). A small proportion of this inhibition was due to time, which alone caused reductions of 15.7 ± 3.5 and 14.1 ±3.3 % (n = 6) for NMRI and *my* ilea, respectively ($P < 0.001$, paired *t*-tests). Time alone had no significant effect on the $p[A]_{50}$ values.

EFS caused frequency-dependent contractions, with a maximum response at 16 or 32 Hz. Addition of eserine did not significantly affect the frequency-response relationships for either NMRI or *my* ilea (two-way ANOVA with repeated measures), nor on a point-by-point analysis did eserine have a significant effect at any single stimulation frequency.

These results indicate that either there is no acetylcholinesterase activity in the NMRI and *my* mouse small intestine or that this enzyme is resistant to eserine in these two strains. Furthermore, in both these types of mice eserine appeared to be a non-competitive antagonist of the muscarinic receptors since it reduced the level of maximum responses to ACh without altering the $p[A]_{50}$ values.

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35P STIMULATED ADENOSINE A_1-RECEPTOR EXPRESSION IN RESPONSE TO HYPOXIA

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Fully functional human adenosine A_1-receptors have been expressed in CHO-K1 cells (Townsend-Nicholson & Shine, 1992). The coding region of the human A_1-receptor has a putative hypoxia-response element, which may bind the hypoxia-inducible transcription factor HIF-1. The minimum binding sequence for HIF-1 is 5'-CGTG-3'. Disruption of this site in other genes results in loss of response to hypoxia (Semenza, 1996). We have therefore studied the effect of hypoxia (3% O_2) on A_1-receptor expression.

Human A_1-receptor cDNA was inserted into the pGL3-promoter plasmid (Promega) at *HindIII/XbaI*. Disruption of the HIF-1 binding site in the A_1R/pGL3 construct was from 5'-AAC GTG CTG-3' to 5'-AAT GTA TTG-3' (mutA_1R/pGL3). This change did not alter the amino acid sequence of the A_1-receptor ($Asn^{27}Val^{28}Leu^{29}$). CHO-K1 cells were transfected with A_1R/pGL3 (CHO-A_1) or mutA_1R/pGL3 (CHO-mutA_1) directly into 24well plates as previously described (Dickenson & Hill, 1998). Twenty-four hours post-transfection cells were transferred to 3% O_2 for 24hrs. Control cells were maintained at 21% O_2. A_1-receptor expression was measured by 8-cyclopentyl-[^{3}H]-1,3-dipropylxanthine ([^{3}H]DPCPX) binding, as previously described (Dickenson & Hill, 1998). Assays were performed at 21% atmospheric O_2. Non-specific binding was measured with 10μM xanthine amine congener (XAC) and was <10% of total binding. Results are mean±s.e.mean.

Saturation analysis of [^{3}H]DPCPX binding to CHO-A_1 cells demonstrated a significant increase in receptor expressions at 3% O_2 compared to 21% O_2 controls ($p<0.0001$, ANOVA, n=5). Kd values were 2.88±0.35nM at 21% O_2 and 2.82± 0.55nM at 3% O_2. Hypoxia increased Bmax from 2.53±0.04 to 5.43±1.34 pmol/mg protein. There was no significant difference in the protein content of 21% and 3% O_2 treated cells (0.14±0.01 and 0.15±0.01 mg/well respectively). Displacement analysis with 3nM [^{3}H]DPCPX and unlabelled DPCPX gave -logIC_{50} values of 8.37±0.15 (21% O_2) and 8.44±0.23 (3% O_2). Equivalent values for XAC were 7.54±0.0.19 and 7.12±0.11 respectively. Mutation of the HIF-1 binding site within the A_1-receptor construct significantly reduced the effect of hypoxia by 27% ($p<0.05$, paired t-test, n=8). The percentage increase in A_1-receptor expression for CHO-A_1 cells was 76.9±12.1% and for CHO-mutA_1 cells was 53.5±8.7%. The effect of hypoxia, therefore, was not completely abrogated suggesting other hypoxia-regulatory sites within the A_1R/pGL3 construct.

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36P USE OF RECOMBINANT AEQUORIN TO INVESTIGATE THE AUGMENTATION BY ADENOSINE A_1-RECEPTORS OF CALCIUM RESPONSES TO ATP IN CHO-K1 CELLS

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Adenosine A_1-receptor agonists can augment ATP-stimulated (P_{2y2}-receptor) inositol phospholipid hydrolysis in CHO-K1 cells transfected with the human adenosine A_1-receptor (Megson et al.,1995). We have previously shown that expression of Gβγ scavenging proteins (such as the C terminus of βARK1) can partially attenuate this effect of A_1-agonists on inositol phosphate accumulation in these cells (Dickenson & Hill, 1998). Here we show that the calcium-sensitive bioluminescent protein aequorin can be used to investigate the role of Gβγ subunits in these synergistic interactions at the level of intracellular calcium.

CHO-K1 cells stably transfected with the human adenosine A_1 receptor (CHO-A1 cells) were grown in DMEM/F12 medium supplemented with 10% FCS and 2mM L-glutamine in humidified air:CO_2 (95:5). Cells were transiently transfected with pcDNA3 containing the cDNA for apo-aequorin (Brini et al., 1995; pcDNA3-Aeq) and/or the cDNA for the βARK minigene (pcDNA3-βARK; Dickenson & Hill, 1998). Cells, in 96-well plates were then incubated with coelenterezine (1μM) for 1 h prior to addition of agonists. Luminescence was measured using a Dynex MLX luminometer for 5 sec following agonist addition.

Addition of ATP (1μM) to CHO-A1 cells produced a rapid and marked rise in aequorin luminescence. Simultaneous addition of the selective A_1-agonist cyclopentyl-adenosine (CPA;10μM) with ATP (1μM) produced a significant augmentation of the ATP-induced aequorin signal in CHO-A1 cells (an increase in response from 38.4 ± 3.0 to 67.9 ± 7.9 %; control response to 10μM ATP=100%; $p < 0.001$, n=14; 2-way ANOVA). CPA (10μM) produced only a small direct response alone (3.0 ± 1.3 % of response to 10μM ATP; n=13). CPA (10 μM) shifted the EC_{50} for ATP to lower agonist concentrations (log EC_{50} = -6.02 ± 0.09 [+CPA], -5.68 ± 0.06 [-CPA]; $p<0.05$, n=8, paired t-test). This effect was abolished following treatment of cells with pertussis toxin (3h; 200ng/ml). Co-transfection of cells with apo-aequorin and pcDNA3-βARK also attenuated the augmentation by CPA (10μM) of the response to ATP (1μM; Table 1).

	PcDNA3 (control)	PcDNA3-βARK
CPA (10μM)	1.9 ± 0.1	-0.3 ± 0.6
ATP (1μM)	17.8 ± 1.2	17.6 ± 2.5
ATP+CPA	31.5 ± 2.8	19.9 ± 1.2*

Table 1. Aequorin luminescence (% of response to 20μM ionomycin; n=3 experiments, each in triplicate) * $p < 0.001$ versus control, 2-way ANOVA, post hoc Neuman Keuls.

These data suggest that Gβγ-subunits are involved in the augmentation by CPA of calcium responses to ATP.

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37P KINETICS OF NO LIGATION WITH NITRIC OXIDE SYNTHASE BY FLASH PHOTOLYSIS AND STOPPED-FLOW SPECTROPHOTOMETRY

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Nitric oxide synthase (NOS) catalyzes conversion of L-arginine to nitric oxide, which subsequently stimulates a host of physiological processes (Knowles,1989). Prior work suggests that NOS is inhibited By NO, providing opportunities for autoregulation (Klatt,1992). The question how NOS can still function mechanistically with this autoinhibition and how autoregulation works was addressed based on the determination of equilibrium constants for NO-binding. Since NOS is involved in the regulatorycycle it is appropriate it is regulated by NO but not poisonrd by very high affinity for NO, Is affinity for NO reasonably low? Does affinity differ between ferric and ferrous oxidation states? Is low affinity achieved by slow association or by fast dissociation and if the kinetics and the equilibria are affected by cofactors or substrates.

Rat nNOS-Holoenzyme and the amino-terminal haeme-binding domain (residues 1-714) of rat nNOS and were expressed in E. Coli purified and reconstituted as described previously (McMillan and Masters,1995). Flash photolysis used an improved version of an instrument described previously (Jongeward,1988). Photolysis laser pulses were 4 ns in duration at 545 nm with up to 4 mJ of energy over an area of 0.1cm^2. The probe had an 8-nm bandwidth selected from a stable tungsten lamp, except that measurements of geminate recombination required a pulsed xenon flash. The photomultiplier was wired with both a large standing current and large interdynode capacitors to optimize linearity. The digitizer had been upgraded to a Lecroy Model 9361 digitizing oscilloscope.

Kinetics of NO dissociation from ferric nNOS-NO were determined using a Durrum stopped-flow instrument NO dissociation was measured by monitoring absorption increase at 412 nm during reacttion with oxymyoglobin, which is converted to metmyoglobin by reaction with NO, a method we used recently for another protein (Kharitonov,1997). Dissociation from ferrous nNOS-NO is so slow that it could be measured in an ordinary spectrophotometer, utilizing sodium dithionite (SDT) as a scavenger, in the presence of CO, and monitoring absorbance change at 420 nm (Moore and Gibson,1976).

This contribution reports that NO reacts rapidly (ka= $2x10^{-7}$ M^{-1} s^{-1}) with neuronal NOS in both its ferric and ferrous oxidation states. Association kinetics are almost unaffected by L-arginine or the cofactor tetrahydrobiopterin. Some slight heterogeneity can be detected in association kinetics of CO. Small amounts of geminate recombination of NO trapped in a protein pocket can be observed over nanoseconds, and a much larger is inferred to take place at picosecond time scales. Dissociation rates are also very fast from the ferric form, in the neighbourhood of 50 s^{-1}, when measured by extrapolating association rates to the zero NO concentration limit. Scavenging experiments give dissociation rate constants more than an order of magnitude slower-still quite fast. For the ferrous species, extrapolation is not distinguishable from zero, while scavenging experiments give a dissociation rate constant near $10^{-4}s^{-1}$.

We have demonstrated that insights generated by studies on the fine details of ligation in Hb and Mb, such as the prominent role of protein caging and geminate recombination, are applicable to the haeme enzymes. We have shown that fast ligand association in nNOS combines with fast dissociation to keepaffinities reasonable in the ferric form, but an additional mechanism (cycling between oxidation states) plays an important role in avoiding inactivation by NO binding to the ferrous state of nNOS.

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38P OPERATIONAL PROPERTIES OF MOUSE P2X$_4$ RECEPTORS: A SPECIES COMPARISON

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P2X receptors comprise a family of ligand-gated, non-selective cation channels. Seven genes encoding P2X receptor subunits (P2X$_1$-P2X$_7$) have been identified, each capable of forming functional homomeric receptors of unknown stoichiometry, when expressed in heterologous systems. The P2X$_4$ receptor is widely distributed in the brain, and is thought to be involved in regulation of synaptic transmission. The aim of this study was to functionally characterise the recently cloned mouse P2X$_4$ receptor (Simon *et al.*, 1999) and to compare its pharmacological properties with the human and rat orthologues.

Whole cell recordings were made from rafts (Miller *et al.*, 1998) of HEK-293 cells stably expressing recombinant mouse, rat or human P2X$_4$ receptors, using Cs-aspartate containing electrodes (3-8MΩ), in a HEPES-buffered extracellular medium. Data are mean with 95% confidence intervals, or ± SEM. In a total of 81 experiments, at a holding potential of -90mV, 10μM ATP induced inward currents in all cells expressing murine P2X$_4$ receptors, with a amplitude of 3.6±0.37nA and a rise time of 132±9.9ms (n=9).

The agonist potency of ATP at the mouse, human and rat P2X$_4$ receptors was similar, with mean EC$_{50}$ values of 2.4μM [1.3-4.3] (n=16), 1.4μM [0.8-2.6] (n=6) and 5.5μM [2.6-11.6] (n=7) respectively. By comparison, both AP4 and αβmeATP acted as partial agonists at the mouse P2X$_4$ receptor, reaching maximum responses of 69% and 29%, of that achieved with 100μM ATP, respectively, with EC$_{50}$ values of 2.6 [1.9-3.7] and 7.1 [2.3-21.6] μM (n = 6 and 9, AP4 and αβmeATP, respectively).

Similar results were found at the human P2X$_4$ receptor, with AP4 having an EC$_{50}$ value of 3.0μM [1.5-6.0] (n=6) and reaching a maximum of 72% of the maximum ATP reponse, while αβmeATP elicited a maximum response of 24% (EC$_{50}$=19.2μM [11.432.4], n=6). At the rat P2X$_4$ receptor, AP4 acted as a partial agonist with respect to ATP (EC$_{50}$= 20.0μM [4.6-87.4], n=6) and at 100μM, elicited 49% of the maximum current. In contrast to the other species orthologues, however, αβmeATP failed to elicit a significant agonist response at rat P2X$_4$ receptors, even at concentrations of 100μM.

Although the P2X$_4$ receptor was initially distinguished from the other P2X subunits on the basis of its relative insensitivity to purinergic antagonists, mouse P2X$_4$ receptors were found to be sensitive to the antagonist PPADS (IC$_{50}$=10.5μM [7.5-14.8], n=6), as were human P2X$_4$ (IC$_{50}$=9.6μM [4.8-19.1], n=6). The rat receptor however, showed a low sensitivity to PPADS (IC$_{50}$ > 100 μM). All three species orthologues were insensitive to suramin (IC$_{50}$ > 100 μM). Uniquely, at the rat P2X$_4$ receptor, αβmeATP acted as an antagonist, with an IC$_{50}$ of 4.6μM [2.4-8.6], n=9. KN62, a potent antagonist at the P2X$_7$ receptor, was found to be inactive at all 3 species orthologues of the P2X$_4$ receptor (IC$_{50}$> 3μM).

In summary, we have pharmacologically characterised three orthologues of P2X$_4$ receptors using identical experimental conditions. We conclude that the responses to ATP and AP4 at the three receptor types are similar, but that differences exist in the antagonist profiles. The mouse and human P2X$_4$, but not the rat receptor, were sensitive to the antagonist, PPADS. Surprisingly, the human, like the rat and mouse P2X$_4$ receptors, was insensitive to suramin. In addition, αβmeATP behaved as a partial agonist but was devoid of intrinsic activity at the rat P2X$_4$ receptor, where it behaved as an antagonist.

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39P UCL 1848: A NOVEL BIS-QUINOLINIUM CYCLOPHANE WHICH BLOCKS APAMIN-SENSITIVE K^+ CHANNELS WITH NANOMOLAR AFFINITY

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Small conductance Ca^{2+}-activated K^+ channels (SK) are widely distributed throughout the body. Certain subtypes of SK channel, e.g. in rat superior cervical ganglion (SCG) neurones and guinea-pig hepatocytes, are sensitive to block by apamin while others, e.g. those believed to underlie the slow afterhyperpolarization (AHP) in rat hippocampal pyramidal cells, are insensitive. Recently three clones, SK1, SK2 and SK3, have been described which when expressed in *Xenopus* oocytes form functional SK channels. SK1 is insensitive to apamin whereas SK2 and SK3 are blocked by apamin with IC_{50}'s of 60 pM and 2 nM respectively (Kohler *et al.* 1996). A closely related gene (hIK, hSK4) is believed to code for the apamin-insensitive intermediate conductance Ca^{2+}-activated K^+ channel found in erythrocytes (Ishii *et al.*, 1997, Joiner *et al.*, 1997).

We have recently shown that a novel bisquinolinium cyclophane (UCL 1684) blocks apamin-sensitive SK channels (Campos Rosa *et al.*, 1998). Here we describe a more potent compound, UCL 1848 (1,1'-(1,5-pentan-diyl)-N,N'-(1,5-pentan-diyl)-bis-(4-aminoquinolinium) di-trifluoroacetate), in which the quinolines are linked by short alkyl chains rather than by xylyl groups as in UCL 1684.

UCL 1848 was tested for its ability to block the slow AHP in rat SCG neurones using methods described by Dunn (1994). The IC_{50} was 2.7 ± 0.2 nM (s.e. mean, n = 6). It was even more potent as an inhibitor of the binding of ^{125}I-apamin to intact guinea-pig hepatocytes (see Castle *et al.*, 1993) with a K_I = 140 ± 10 pM (n = 4). Having shown that UCL 1848 is a highly active blocker of apamin-sensitive SK channels, we examined its effect on the IK-mediated Ca^{2+}-activated K^+ permeability present in rabbit blood cells, and on the slow AHP in hippocampal pyramidal neurones. These actions are thought to involve IK and SK1 channels respectively. Neither was affected by UCL 1848 at concentrations up to 100 nM.

In conclusion UCL 1848 is the most potent non-peptidic blocker of SK channels yet described and appears to be highly selective for apamin-sensitive sub-types. This and other related compounds should be powerful tools for the study of both native and cloned apamin-sensitive SK channels.

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40P A SIMPLE ASSAY FOR FATTY ACID AMIDE HYDROLYASE ACTIVITY

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Fatty acid amide hydrolase (FAAH) is a membrane-bound enzyme that hydrolyses a number of primary and secondary fatty acid amides to their parent fatty acids (Cravatt *et al.*, 1996). Its substrates include the endogenous cannabinoids and the sleep-inducing lipid *cis*-9,10-octadecenoamide (oleamide). Current assays for FAAH activity often involve the use of radiolabelled substrates and chromatographic techniques which are both expensive and laborious. Here we describe a simple, novel spectrophotometric assay for FAAH activity based on oleamide hydrolysis.

Oleamide is the primary amide of oleic acid and, therefore, its hydrolysis by FAAH yields oleic acid and ammonia. To measure the rate of ammonia release in this reaction, a dual-enzyme assay containing FAAH and L-glutamate dehydrogenase (GDH) was developed. GDH catalyses the condensation of ammonia and 2-oxoglutarate to glutamate, using NADH as a coenzyme. Since NADH has a large molar extinction at 340 nm, the rate of ammonia production and, hence, oleamide hydrolysis is directly proportional to the decrease in absorbance at 340 nm as NADH is converted to NAD^+. The assay was performed at room temperature using bovine GDH (Calbiochem) and FAAH activity from the crude particulate fraction of rat (Wistar, either sex, 180-250 g) liver solubilised with 1 % Triton X-100. Unless stated otherwise, data are represented as mean ± SEM of 3 separate experiments.

These assay conditions gave an apparent K_m of 110.7 ± 9.9 μM for oleamide hydrolysis with a V_{max} of 5.9 ± 0.5 nmol/min/mg protein. The serine protease inhibitor phenylmethylsulphonyl fluoride (PMSF) inhibited oleamide hydrolysis with an IC_{50} value of 23.0 ± 4.9 μM, while methyl arachidonyl fluorophosphonate (MAFP), a potent and irreversible inhibitor of FAAH (Deutsch *et al.*, 1997), inhibited oleamide hydrolysis with an IC_{50} value of 200.3 ± 20.4 nM (mean ± range, n=2). Anandamide (arachidonoyl ethanolamide), itself a substrate of FAAH, was shown to inhibit oleamide hydrolysis by 10.0 ± 2.1 % and 47.4 ± 3.0 % at 10 μM and 100 μM, respectively (mean ± range, n=2). To determine whether the inhibitors used had a direct effect on GDH, assays were performed with GDH alone, using ammonium acetate (100 μM) as the substrate. The rate of the GDH reaction was not affected by either PMSF (100 μM, 99.1 ± 0.6 % of control) or MAFP (1 μM, 97.0 ± 1.1 %).

We have therefore developed a novel, simple and inexpensive spectrophotometric assay for the activity of fatty acid amide hydrolase. As well as removing the need for radiochemicals and chromatography, this assay has the potential for adaptation for the high-throughput screening of compound libraries for FAAH inhibitors.

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41P REGULATION OF CYCLO-OXYGENASE ACTIVITY BY ACTIVATORS OF SOLUBLE GUANYLYL CYCLASE IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Nitric oxide synthase (NOS) and cyclo-oxygenase (COX) are enzymes that play an important role in the maintenance of a healthy vasculature, regulating tone and inhibiting platelet aggregation via the production of NO and prostaglandins (PGs) respectively. The actions of NO and PGs, such as PGE_2, are mediated via activation of guanylyl and adenylyl cyclase respectively. Inflammatory agents such as interleukin (IL)-1β or bacterial endotoxin induce the expression of 'inflammatory' forms of NOS and COX (iNOS and COX-2) in vascular smooth muscle. Studies using murine macrophages have shown that the co-expression of iNOS and COX-2 results in cross-talk between the two pathways (Salvemini et al., 1993, Swierkosz et al., 1995). Here we have examined the potential for endogenous NOS, exogenous NO (in the form of sodium nitroprusside; SNP) or a non-NO related activator of soluble guanylyl cyclase (sGC), YC-1, to modulate COX-2 activity in human vascular smooth muscle (HVSm) cells.

HVSm cells were cultured from saphenous vein obtained from patients undergoing coronary artery bypass surgery according to Bishop-Bailey et al., 1998. After 6-10 weeks the primary cultures were fully confluent, and cells passaged into 96 well plates for treatment. Culture medium was replaced with medium containing IL-1β (10 ng/ml) to induce COX-2 (Bishop Bailey et al., 1998). After 24 hours the medium was removed and COX-2 activity measured by the release of PGE_2 by radioimmunoassay.

SNP inhibited release of PGE_2 from HVSm cells in a concentration-dependent manner (IC_{50} 77μM) with a maximum reduction seen at 1mM (Figure 1A). The NO-independent sGC stimulator YC-1 (10 or 100μM), also inhibited the release of PGE_2 from IL-1β treated (Figure 1A). By contrast, the constitutive and inducible NOS inhibitor, N_ω-nitro-L-arginine methyl ester (L-NAME; 1mM) or the selective inhibitor of iNOS, 1400W (10μM) had no effect on PGE_2 release by these cells (Figure 1B). The COX-2 selective inhibitor, DFU (10μM) greatly reduced PGE_2 production by IL-1β stimulated HVSm cells (Figure 1B)

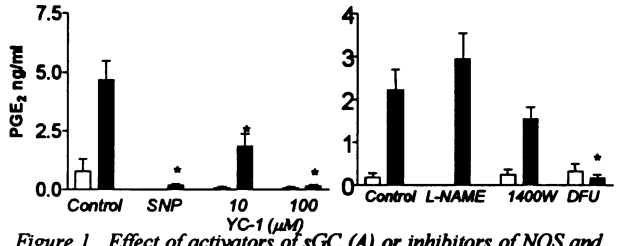

*Figure 1. Effect of activators of sGC (A) or inhibitors of NOS and COX-2 activity (B) on PGE_2 production. Open bars represent data from control cells, whilst filled bars data from cells treated with IL-1β. The data is the mean ±S.E.M for n=6-12 experiments. * represents a significant (one-way ANOVA followed by Dunns test for multiple comparisons) difference between responses of cells under control versus treated conditions.*

Here we have demonstrated that SNP, which activates sGC via the release of NO, inhibits COX-2 activity in HVSm cells. In addition, the NO-independent activator of sGC, YC-1, also inhibited COX-2 in these cells. The findings that L-NAME and 1400W had no effect on COX-2, suggests that insufficient levels of NO were formed endogenously during the conditions described in this study. These observations suggest that NO inhibits COX-2 activity via activation of sGC and may have important implications for our understanding of vascular dysfunction or defense during conditions of inflammation.

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42P ENDOTHELIUM-DEPENDENT RELAXATION TO K^+ IN THE RAT ISOLATED MESENTERIC ARTERIAL BED

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Edwards *et al.* (1998) have recently proposed that endothelium-derived hyperpolarizing factor (EDHF) may be K^+. By contrast, Chaytor *et al.* (1998) contend that gap junctions mediate EDHF responses. In this study, the effects of the gap junction inhibitor, 18α-glycyrrhetinic acid (18α-GA; Taylor *et al.*, 1998) and ouabain, a Na^+/K^+ATPase inhibitor which indirectly blocks gap junctions (Dhein, 1998), have been assessed against vasorelaxation to carbachol (acting via EDHF) and KCl.

Male Wistar rats (250-350g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1}, i.p.) and exsanguinated. The mesenteric arterial bed was isolated (Randall *et al.*, 1996) and perfused with oxygenated Krebs-Henseleit solution (K^+ free for KCl experiments). Following 20min equilibration, methoxamine was added to increase perfusion pressure (100-150mmHg). The vasorelaxant effects of carbachol (in the presence of 10μM indomethacin and 300μM L-NAME acting via EDHF) or KCl were then assessed in the absence and presence of 18α-GA (100μM) or ouabain (1mM). Each inhibitor was added 30min prior to construction of dose-response curves. The endothelium was removed by 3min perfusion with distilled water. All data were compared with ANOVA with Bonferroni's *post hoc* test.

Carbachol (5.48pmol-1.64μmol) induced dose-related relaxations (ED_{50}=4.4±1.6nmol, mean ±s.e.mean; maximum relaxation (R_{max})=79.5±3.5%, n=6). 18α-GA (100μM, n=6) caused a significant (P<0.01) inhibition of EDHF mediated vasorelaxation with an R_{max} of 44.3±10.4%. Ouabain (1mM) also inhibited this EDHF response (R_{max}=11.0±2.5%, P<0.001; n=6). The EDHF inhibitor clotrimazole (10μM) also abolished responses to carbachol (R_{max}=7.9±0.7%, P<0.001; n=4). In endothelium-denuded preparations, responses to carbachol were abolished (R_{max}=7.5±0.6%, P<0.001; n=5). KCl (1ml boluses of 1mM-25mM) induced dose-dependent relaxation (R_{max}=73.7±2.4%, n=7). These relaxations were sensitive to indomethacin (R_{max}=56.4±6.2%, P<0.05; n=5) but not to L-NAME (R_{max}=65.8±2.8%; n=5). Addition of 18α-GA significantly inhibited K^+ relaxation (Rmax=48.3±8.0%, P<0.01; n=6) whilst ouabain essentially abolished the responses (R_{max}=9.1±1.7%, P<0.001; n=5). Clotrimazole had no effect on KCl responses (R_{max}=70.5±2.0%, n=5). In endothelium-denuded preparations, relaxation to KCl was almost abolished (R_{max}=11.9±2.6%, P<0.001; n=6).

The results of the present study show that K^+ causes relaxations which are endothelium-dependent, therefore suggesting that K^+ is not an EDHF in the rat mesentery. K^+ probably induces relaxation by release of endothelial autacoids. The inhibitory effects of 18α-GA on carbachol responses implicate the involvement of gap junctions in EDHF-mediated relaxations.

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43P ENHANCED NO-MEDIATED VASODILATOR RESPONSES IN PRESSURIZED MESENTERIC RESISTANCE ARTERIES ISOLATED FROM HYPERTENSIVE, TRANSGENIC (mRen-2)27) RATS

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Hypertensive, transgenic (m(Ren-2)27) (abbreviated to TG) rats (Mullins *et al.*, 1990), represent a novel model of hypertension, produced by transfection of the mouse renin gene into the Hannover Sprague Dawley (SD) rat genome. Hypertension in TG rats is characterized by decreased renal, mesenteric and hindquarters vascular conductances (Gardiner *et al.*, 1995). In the present study, we have assessed vasodilator responses in mesenteric resistance arteries (MRA), isolated from SD and TG rats.

Male TG rats and SD rats (350-550 g) were anaesthetized with sodium methohexitone (40-60 mg kg^{-1} i.p.) and killed by exsanguination. The mesentery was removed and placed in ice-cold physiological salt solution containing 23 μmol l^{-1} disodium EDTA. Subsequently, a second order branch (200-400 μm) of the superior mesenteric artery was isolated and cleared of connective tissue. Vessels were then mounted on a pressure myograph (Halpern *et al.*, 1984). Intraluminal pressure of arterial segments was set to about 50% of the mean arterial pressure (i.e. 60 mmHg and 80 mmHg in SD rats and TG rats, respectively) and vessels were allowed to equilibrate for 60 min. Thereafter, vessel diameter was reduced by approximately 50% with noradrenaline. Subsequently, concentration-dependent vasodilator responses to acetylcholine (ACh), sodium nitroprusside (SNP) or papaverine were obtained. In some experiments, responses to ACh were obtained in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 0.3 mmol l^{-1}).

Responses to the endothelium-dependent vasodilator, ACh, and the nitric oxide (NO) donor, SNP, were enhanced in MRA isolated from TG rats compared to those isolated from SD rats. This enhanced sensitivity was not due to a non-selective increase in the ability of the MRA of TG rats to vasodilate, since the sensitivity of the responses to the phosphodiesterase inhibitor, papaverine, was similar in MRA from TG and SD rats. Co-incubation with L-NAME resulted in a 30-fold rightward displacement of the concentration response curve to ACh in MRA isolated from TG rats, but in only a 3-fold shift in the response to ACh in MRA isolated from SD rats. In the presence of L-NAME, responses to ACh in MRA from TG and SD rats were not different (Table 1).

Table 1. Responses to vasodilators (expressed as log IC_{50}: the concentration (mol l^{-1})which produced 50% of the maximal vasorelaxation) in MRA isolated from SD rats and TG rats. Values are mean ± s.e. mean. *P < 0.05 vs SD rats and #P < 0.05 vs ACh control (Mann-Whitney U test).

	n	SD rats	TG rats
ACh control	10 - 11	-7.32 ± 0.03	-8.23 ± 0.04*
ACh + L-NAME	6	-6.91 ± 0.02#	-6.74 ± 0.03#
SNP	6	-4.96 ± 0.13	-7.03 ± 0.18*
Papaverine	4 - 5	-5.35 ± 0.02	-5.28 ± 0.07

Taken together, these results indicate that the increased sensitivity to ACh in MRA from TG rats may be explained by a greater sensitivity of guanylyl cyclase to NO.

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44P DIFFERENCES IN ENDOTHELIAL FUNCTION IN PREGNANCY AND PRE-ECLAMPSIA

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A number of endothelium-derived factors modulate vascular tone. These include vasoactive prostanoids, nitric oxide (NO) and endothelium-derived hyperpolarising factor (EDHF). In the present study, we have assessed the relative contributions of NO and EDHF in mediating responses to bradykinin in isolated myometrial resistance arteries, and how responses are altered during normal and abnormal pregnancy.

Myometrial biopsies were obtained at Caesarean section from normal pregnant women (n=12) and women whose pregnancies were complicated by pre-eclampsia (n=6) and at the time of hysterectomy from non-pregnant controls. Myometrial resistance arteries (<400μm in diameter) were dissected and mounted in physiological salt solution on a pressure myograph (Wallis *et al.*, 1996). Following equilibration, vessels were contracted to approximately 50% of their original diameter with the thromboxane mimetic U46619 [10^{-10}M-10^{-8}M]. Vessels were then exposed to increasing concentrations of the endothelium-dependent vasorelaxant, bradykinin [10^{-11}M-10^{-6}M] in three-fold increments. Following washing, concentration-response curves to U46619 and bradykinin were repeated in the presence of the nitric oxide synthase (NOS) inhibitor, L-NAME [10^{-4}M]. In a separate set of experiments, in the presence of L-NAME to inhibit NO production, concentration response curves to bradykinin were obtained in the presence of raised extracellular KCl to inhibit EDHF activity. Indomethacin (10^{-5}M) was present throughout all experiments to inhibit prostanoid production.

Bradykinin produced concentration-dependent responses, which did not differ between groups with regard to sensitivity or maximum response:

Non-pregnant	$-\log EC_{50}$ 7.4 ±0.2,	Emax 82.4 ±6.0%
Pregnant	$-\log EC_{50}$ 7.3 ±0.2,	Emax 83.0 ±5.6%
Pre-eclamptic	$-\log EC_{50}$ 7.1 ±0.2,	Emax 78.8 ±2.1%

L-NAME [10^{-4}M] significantly attenuated the maximal relaxation to bradykinin in myometrial resistance arteries from non-pregnant women (Emax 38.7 ± 10.0% $p<0.0001$ ANOVA). In contrast L-NAME had no effect in vessels isolated from normal pregnant women (Emax 73.3 ± 18.7% $p>0.05$ ANOVA). In vessels from this group, the additional presence of 25mM KCl almost totally abolished the relaxation to bradykinin (Emax 17.6 ± 6.6% $p<0.0001$ ANOVA). In contrast, the relaxation to bradykinin was almost completely abolished in the presence of L-NAME [10^{-4}M] in vessels isolated from women with pre-eclampsia (Emax 19.6 ± 10.4% $p<0.0001$ ANOVA).

This study suggests that during normal human pregnancy, there is a conversion of the mechanism of endothelium-dependent relaxation from one predominantly involving NO, to one largely via EDHF. Similar pregnancy induced changes have been observed in rat abdominal aorta (Bobadilla *et al.*, 1997) and in rat mesenteric arteries (Gerber *et al.*, 1998). In contrast to normal pregnancy, endothelium-dependent relaxations in vessels from pre-eclamptic women were mediated almost exclusively by NO. Thus, pre-eclampsia may be associated with a failure of the endothelium to adapt appropriately to produce EDHF rather than NO during pregnancy.

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45P *IN VITRO* CHARACTERISATION OF [^{18}F]-BQ3020, THE FIRST POSITRON EMITTING LIGAND SYNTHESISED FOR THE ENDOTHELIN ET_B RECEPTOR

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Alteration in ET function has been suggested to play a role in human vascular diseases. Positron emission tomography (PET) is a non-invasive technique for imaging and quantifying receptor-bound radioligands *in vivo* with high sensitivity. The aim of this work was to develop a positron emitting radioligand as a means to study the ET receptor *in vivo* in humans and animals using PET.

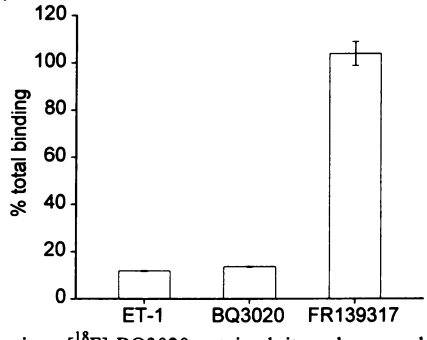
BQ3020, an ET_B selective agonist (Molenaar *et al.*, 1993) was labelled in the ε-amino group of lysine using *N*-succinimidyl 4-[^{18}F]fluorobenzoate a Bolton-Hunter type reagent developed for peptide labelling with the positron emitting radionuclide, ^{18}F (Vaidyanathan & Zalutsky, 1992). [^{18}F]-BQ3020 was characterised using *in vitro* binding assays. Sections of human tissue were incubated with increasing concentrations (10^{-11} - 10^{-9} M, saturation) or with 0.5 nM (inhibition) of [^{18}F]-BQ3020 in HEPES buffer for 1 hr. at 23°C. Non-specific binding (saturation) was defined using 1 μM unlabelled ET-1. Atherosclerotic coronary arteries were sectioned and stained for macrophages. Adjacent sections were incubated with [^{18}F]-BQ3020 using the same conditions as for the saturation experiment. Distribution of ET_B receptors were visualised using a phosphor imaging screen.

[^{18}F]-BQ3020 retained subnanomolar affinity with a Hill slope close to unity (Table 1) as well as the selectivity for the ET_B receptor (Figure 1) after introduction of the 4-[^{18}F]fluorobenzoyl group. [^{18}F]-BQ3020 bound as expected to left ventricle of heart with low levels of binding to smooth muscle of intramyocardial vessels. However high levels of [^{18}F]-BQ3020 binding to ET_B receptors were found to infiltrating macrophages within atherosclerotic lesions, as previously shown using [^{125}I]-BQ3020 (Bacon *et al.*, 1996).

Table 1. Saturation data for [^{18}F]-BQ3020 - human heart tissue

n	K_D (nM)	B_{max} (fmol/mg protein)	nH
3	0.48±0.46	14.3±.3.3	0.94±0.08

Figure 1. Inhibition of 0.5 nM [^{18}F]-BQ3020 binding to human kidney tissue (n=3) by 1 μM concentrations of unlabelled ET-1 (non-selective), BQ3020 (ET_B selective) and FR139317 (ET_A selective).



In conclusion, [^{18}F]-BQ3020 retained its subnanomolar affinity and selectivity for the ET_B receptor and might prove to be a potential radioligand for the study of atherosclerosis in human coronary arteries *in vivo* using PET.

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46P COMPARISON OF ENDOTHELIN-1 GENE EXPRESSION WITH EXPRESSION OF ISOFORMS OF ENDOTHELIN-CONVERTING ENZYME-1 IN BOVINE ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE CELLS

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The phosphoramidon-sensitive metallopeptidase, endothelin converting enzyme-1 (ECE-1), has been proposed as the physiologically relevant enzyme for hydrolysis of big endothelin-1 into mature endothelin-1 (ET-1). However, ECE-1 gene knockout experiments were inconclusive (Yanagisawa *et al.*, 1998). ECE-1 occurs as three isoforms (ECE-1a, 1b and 1c) encoded by three distinct mRNA transcripts which differ as a result of alternate splicing at their 5′ ends. The three ECE-1 isoforms are expressed in endothelial cells, but the regulation of their expression and their relationship with ET-1 synthesis and secretion remains to be elucidated (Schweizer *et al.*, 1997). In this study we have compared the expression of all three ECE-1 isoforms with ET-1 gene expression in bovine aortic endothelial cells (BAEC) and bovine pulmonary artery smooth muscle cells (BPASMC) under conditions which alter ET-1 synthesis.

Cells were cultured in Dulbecco's modified Eagle's medium containing 10% v/v foetal bovine serum. Experiments were carried out under serum free conditions on confluent cultures. ET-1 release was measured by immunoassay (Corder *et al.*, 1998). At the end of the treatment period, after removal of conditioned media for ET-1 measurement, total RNA was extracted from cell monolayers and gene expression was assessed using reverse transcriptase polymerase chain reaction (RT-PCR) (Corder & Barker, 1999). BAEC were treated for 6h with or without 10 μM cytochalasin B (an inhibitor of actin polymerisation) which causes a concentration-dependent inhibition of ET-1 synthesis. BPASMC were incubated for 24h in the presence or absence of tumour necrosis factor-α (TNFα, 100 ng.ml^{-1}). Significant differences were determined by Student's t-test.

Cytochalasin B (10 μM) reduced ET-1 secretion from BAEC by 78 ± 2% ($p=<0.001$, n=6; basal secretion 46 ± 4 fmol.cm^{-2}.6 h^{-1}). PreproET-1 gene expression showed a corresponding reduction to <5% of basal levels ($p<0.001$; n=5) when normalised to expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In contrast, no significant changes were observed in expression of ECE-1a, 1b or 1c in BAEC. In BPASMC, treatment with TNFα increased preproET-1 mRNA levels in parallel with ET-1 release. ECE-1a mRNA was not detectable under any conditions in BPASMC. Levels of ECE-1c mRNA were not significantly altered by treatment with TNFα, whereas ECE-1b mRNA was reduced by 68 ± 10% ($p<0.01$, n=4).

These data show a lack of co-ordination between ET-1 synthesis and the putative processing enzyme ECE-1. In addition, the effects of cytochalasin B show that ET-1 gene expression in endothelial cells is dependent on actin filaments a feature, which is not shared by ECE-1.

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47P INVOLVEMENT OF GAP JUNCTIONAL COMMUNICATION IN CARDIAC PRECONDITIONING IN RAT ISOLATED HEART

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Recent interest has focused on the ability of short periods of ischaemia to protect the heart against prolonged ischaemia and this protective effect has been termed cardiac preconditioning (Murry *et al.*, 1986). To date the mechanisms underlying preconditioning are unclear, although protein kinase C activation has been implicated. Cardiac function is dependent on normal conduction of electrical impulses, and it is now hypothesised that gap junctional communication may play a role in preconditioning. In order to test this hypothesis, hearts have been preconditioned in the absence and presence of a selective gap junction inhibitor, 18α-glycyrrhetinic acid (18GA; Davidson *et al.*, 1986).

Male Wistar rats (250-300g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1} i.p.) and exsanguinated. Following a thoracotomy, the heart was rapidly excised and perfused in the Langendorff mode at constant flow (20 ml min^{-1}) with oxygenated Krebs-Henseleit buffer (Randall *et al.*, 1997). A fluid-filled balloon catheter was inserted in to the left ventricle in order to measure left ventricular developed pressure (LVDP), from which heart rate (HR) was derived. After 45min equilibration preconditioning was induced by 3 cycles of 5min ischaemia with 5min reperfusion prior to the 30min ischaemic insult, which was followed by 60min reperfusion (15 ml min^{-1}). The non-preconditioned hearts were continuously perfused prior to the 30min global ischaemia. The hearts treated with the gap junction inhibitor were continuously perfused with 18GA (2μM; Davidson *et al.*, 1986) 15min after starting in Langendorff mode.

In the control hearts (n=9) the baseline LVDP=92.4±6.7mmHg (mean±s.e.mean) and HR=253±10beats min^{-1}, and these were no different from the corresponding values in hearts receiving 18GA (LVDP=116±13mmHg, HR=247±9beats min^{-1}, n=8). In the preconditioned controls the preservation of cardiac performance after 30min of ischaemia was appreciable with recovery of LVDP to 24.8±6.9mmHg and HR=208±45beats min^{-1} after 60min of reperfusion, compared to values of 4.1±2.0mmHg (LVDP) and 93.0±44.4beats min^{-1} (HR) in non-preconditioned hearts (n=7). In the group of hearts which were preconditioned and reperfused in the presence of 18GA, cardioprotection was abolished, such that after 60min of reperfusion LVDP=4.7±2.9mmHg ($P<0.05$, compared to preconditioned control hearts, ANOVA) and HR=109±82 beats min^{-1}.

The results of the present investigation clearly show that although inhibition of gap junctional communication does not affect the heart under baseline conditions, 18GA does abolish the cardioprotective effects of preconditioning. These findings may suggest that modulation of gap junctional communication may be central to the mechanisms underlying cardiac preconditioning.

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48P A_1 ADENOSINE RECEPTORS, BUT NOT P2 PURINE RECEPTORS, INHIBIT PREJUNCTIONALLY SYMPATHETIC NEUROTRANSMISSION IN THE HAMSTER ISOLATED MESENTERIC ARTERIAL BED

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Recent evidence has shown that P2 purine receptors are expressed by the peripheral terminals of sympathetic nerves and modulate neurotransmitter release upon activation by ATP and adenosine 5'-O-(3-thiotriphosphate (ATPγS) in a number of different tissues from rat and mouse (von Kügelgen *et al.*, 1996; Koch *et al.*, 1998). The present study characterizes inhibitory purine receptors modulating sympathetic neurotransmission in hamster mesenteric arteries.

Male Syrian hamsters (100-140g) were killed by exposure to CO_2 and decapitation. Mesenteric beds were isolated and perfused with oxygenated Krebs solution at 5 ml min^{-1} (Ralevic *et al.*, 1997). After 30 min equilibration, the effects of purines on contractions to electrical field stimulation (EFS) were investigated. The tone of a group of mesenteric beds was raised with methoxamine (ME; 10-80μM) in order to evaluate relaxation by purines. pIC_{20} is the negative logarithm of the molar concentration of agonist required to elicit inhibition of the response by 20%.

Repetitive pulses of EFS (64Hz, 90V, 1ms, 5s) at 2 min intervals elicited reproducible contractile responses (10-30 mmHg) of the mesenteric arterial beds (n=32). Responses were blocked by guanethidine (5μM) or prazosin (1μM) indicating mediation by noradrenaline released from sympathetic nerves. The selective P2 receptor agonists ATPγS (0.1-10μM; n=4) and adenosine 5'-O-(2-thiodiphosphate) (100μM; n=4) had no significant effect on contractions to EFS. In contrast, contractions were attenuated by the selective A_1 receptor agonist N^6-cyclopentyladenosine (CPA) (1-10μM; n=7) (Table 1). The A_2 selective agonist 5'-(N-ethylcarboxamido)-adenosine (NECA; 0.1-10μM; n=5) and adenosine (1-100μM; n=6) were less potent as inhibitors of contractions to EFS (Table 1). The concentration-inhibitory effect curve to CPA was shifted to the right by an adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX; 10nM; apparent pK_B value 9.6; n=6-7). Tone of a group of mesenteric arterial preparations was raised with ME (to 29.6±2.5mmHg; n=14). CPA (0.001-10μM) did not elicit vasorelaxation (Table 1).

Table 1. Inhibition by adenosine P1 receptor agonists of contractions to EFS and methoxamine of mesenteric arterial beds.

		CPA	NECA	adenosine
EFS	pIC_{20}	8.2±0.1 (7)	7.6±0.2 (5)	5.8±0.1 (6)
	Maximal inhibition	37.4±4.3%	>46%	>45%
MX	pIC_{20}	<5 (4)	<5 (5)	3-4 (5)
	Relaxation at 10μM	0	14.9±3.6%	1.7±1.1%

These data show that P1 receptors inhibit prejunctionally sympathetic neurotransmission in hamster mesenteric arteries and that the receptors are of the A_1 subtype. The evidence does not support a role for P2 receptors in inhibitory modulation of sympathetic neurotransmission in hamster mesenteric arteries. This is in contrast to findings reported in a variety of tissues from rat and mouse (von Kügelgen *et al.*, 1996; Koch *et al.*, 1998).

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