342P EFFECT OF CULTURE MEDIUM ON ADRENERGIC RESPONSES IN RAT HEPATOCYTES

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Catecholamines increase glycogenolysis and in turn glucose output in the liver *via* two main pathways:- a cAMP-dependent β_2-adrenoceptor and a Ca^{2+}/PtdIns-dependent α_1-adrenoceptor pathway (Exton,1982). The extent to which each pathway is involved depends on age, sex and pathophysiological factors (Ishac *et al.*, 1992). The β-response is almost absent in the liver and freshly isolated hepatocytes of adult male rats. This study shows that the magnitude of the β-response may depend on the medium used to suspend the cells.

Hepatocytes were isolated from adult male rats (250-300g) by the two-step perfusion method of Seglen (1976). The effect of a 4-min exposure to various concentrations of phenylephrine (PHE) and isoprenaline (ISO) on glycogen phosphorylase *a* was determined as described by Ishac *et al.* (1992).

Results are shown in Table 1. EC_{50} values were estimated by iterative logistic curve-fitting and compared by the Student's *t* test. We observed that the β-effect was always significantly present in Williams' E medium compared to responses in Krebs'. The addition of L-amino acids (AA) or vitamins (VIT) (present in Williams' E but not in Krebs') to Krebs' solution shows that the amino acids component of Williams' E may be responsible for the 'unmasking' of the β-response. We have tested the effect of some amino acids and found proline (30mg/l) to be effective in this respect. The mechanism involved in this process is not clear, though some amino acids are known to induce glycogenolysis in the liver by causing cell swelling (Häussinger *et al.*, (1994).

Table 1. The Effect of culture medium on α- and β-adrenergic activation of glycogen phosphorylase *a*

Medium	Basal activity (units)†	EC_{50} (x 10^{-7} M) ISO (β)	PHE (α)
Krebs'	28.3±2.5	56.2±10.0**	5.3±1.5
Williams' E	31.4±4.1	6.3±1.5	7.1±2.0
Krebs' + VIT	25.2±2.3	n.d.	3.6±0.9
Krebs' + AA	36.2±3.2	6.0±1.3$^{n.s.}$	4.6±0.5
Krebs' + Pro	35.3±1.0	7.3±2.5$^{n.s.}$	4.4±1.0

† nmol/min/mg protein **P<0.01 & n.s.- not significant at P=0.05 compared to values in Williams' E (n=6). n.d. = not determinable

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343P GLUTATHIONE-S-TRANSFERASE ACTIVITIES AND THE TOXICITY OF 1-CHLORO-2,4-DINITROBENZENE AND ETHACRYNIC ACID IN IMMORTALISED RAT HEPATOCYTES

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Immortalised rat hepatocyte cell lines may provide an in vitro system for drug metabolism and toxicity studies. We have generated cell lines by transfection of SV40 DNA using both $CaPO_4$ precipitation (P9 cells) and electroporation (LQC 6 cells), and the stability of drug metabolising enzymes in these lines is being assessed. Glutathione-S-transferase (GST) activities provide crucial detoxification for electrophilic species. GST activities towards 1-chloro-2,4- dinitrobenzene (CDNB) and ethacrynic acid (EA), and the toxicity of these two chemicals, were compared in freshly isolated rat hepatocytes, and in P9 and LQC 6 cells.

GST activities were measured using either 50μM CDNB or 0.2mM EA in the presence of 1mM reduced glutathione. Toxicity was compared in hepatocytes (4-22h in culture) and in immortalised cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay following 18h exposure to concentrations between 0.05 and 200μM of CDNB or EA. Toxicity results are expressed as the MTT_{50}, the concentration of toxin which decreases viability to 50% of controls. These values are based on 8-16 measurements of viability at each of 11 concentrations of the two toxins and calculated from computer generated polynomials for the equation of the best-fit curves using Cricket Graph.

Table 1 shows that the immortalised cells contain lower levels of GST activity, particularly towards CDNB, and as a result the ratio of CDNB/EA GST activities is markedly different compared with freshly isolated cells. The MTT_{50} values for EA were similar in all three cell types, but that for CDNB was 10-fold higher in primary hepatocytes than in immortalised cells. Greater toxic potency of CDNB towards immortalised cells may be a reflection of their low GST activity towards this substrate. This reduction in GST expression may limit the use of these lines in prediction of drug toxicity.

Table 1 GST activities (nmol/min/10^6 cells) and toxicity of CDNB and EA in primary cultured and immortalised hepatocytes.

Cell type	GST-CDNB	GST-EA	Ratio CDNB/EA	MTT_{50} EA	MTT_{50} CDNB
Hepatocytes	467.34 +/- 145.20	796.0 +/- 96.0	0.579 +/- 0.117	37.40 μM	109.34 μM
P9	0.91 +/- 0.31*	97.34 +/- 9.98*	0.005 +/- 0.002*	32.54 μM	13.72 μM
LQC 6	1.08 +/- 0.32*	202.73 +/- 27.61*	0.009 +/- 0.003*	26.84 μM	11.18 μM

GST results are mean +/- s.d., of 4 experiments. The MTT_{50} values are based on 8-16 toxicity measurements at each of 11 different concentrations of toxin. *P<0.05, compared with hepatocytes, by ANOVA followed by Dunnett's test.

344P THE EFFECT OF LOW ENDOTOXIN MEDIUM ON THE MAINTENANCE OF STEROID METABOLISM IN CULTURED RAT HEPATOCYTES

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Hepatocytes in suspension have proved to be a useful tool in xenobiotic metabolism. One of the short comings of this model however is the rapid loss of cytochrome P-450 content and activity. Various researchers have tried to combat this by modifying the medium (Skett, 1994). Low endotoxin minimises immune stimulation. It is known that endotoxin causes a fall in cytochrome P-450 levels (Pittner and Spitzer, 1993). This investigation describes work on the effect of Williams' E medium supplemented with low endotoxin bovine serum albumin (LEBSA) on the metabolism of [^{14}C]-androstenedione. Isolation of hepatocytes from male Wistar rats was by the method of Seglen (1976). Hepatocytes were cultured for 24 hours and 48 hours.

After 24 hours in culture in the low endotoxin group, the activity of all the enzymes was higher with the exception of 7α- hydroxylase which was significantly lower as compared to control. This was also the case at 48 hours. Thus it can be concluded that low endotoxin is more effective at maintaining the activity of these enzymes when compared to the activity in normal bovine serum albumin.

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Table 1 The effect of Low Endotoxin medium (LEBSA) on the maintenance of steroid metabolism in cultured rat hepatocytes.

Values are expressed as % of zero time control ± s.d. (n=5); * P < 0.01 compared to respective control (Student's t-test). The enzymic activity for 7α, 6β, 16α, 17OHSD and 5α reductase at zero time was : 3.3±0.3,17.4±0.4,23.9±1.7,77.8± 19.2,205.3±56.9 pmole/min/10^6 cells respectively.

Treatment	Time (h)	7 alpha	6 beta	16 alpha	17 OHSD	5 alpha
BSA	24	246.2±53.7	131.0±5.8	134.2±2.6	85.0±1.8	163.2±8.3
LEBSA	24	136.9±29.0*	202.5±13.8*	181.4±20.7*	136.6±7.2*	236.9±44.4*
BSA	48	129.3±13.3	48.0±5.0	63.6±2.0	40.3±3.2	74.1±11.8
LEBSA	48	93.1±19.1*	76.2±13.6*	82.0±10.1*	53.3±4.5*	96.3±14.2

345P THE PROTECTIVE ACTION OF CYCLOSPORIN A ON ENERGY METABOLISM ASSOCIATED WITH RAT HEPATIC MITOCHONDRIA

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Mitochondrial calcium (Ca^{2+}) homeostasis is integral in regulating cellular energy production. Ca^{2+} overload within the mitochondria resulting from ischaemic/reperfusion injury coupled with high matrix [P_i] can induce the opening of a non-specific pore located within the inner mitochondrial membrane (Crompton *et al.*, 1988). Pore opening leading to loss of low molecular weight solutes, Ca^{2+} efflux and uncoupling of energy transduction. Uniporter uptake of released Ca^{2+} results in recycling of the cation, energy dissipation and collapse of membrane potential (Takeyama *et al.*, 1993). Cyclosporin A (Cyc A), an immunosuppressive and nephrotoxic agent inhibits pore opening. Previous reports indicate that toxicity relates to disruption of energy metabolism in kidney and liver mitochondria (Jung *et al*, 1985; Salducci, *et al*, 1992). Data produced here indicates a protective effect of cyclosporin A on energy production in rat hepatic mitochondria.

Rat liver mitochondria were prepared from female Wistar rats. Mitochondria incubated in the presence of 5mM glutamate plus 5mM malate, displayed respiratory control index (RCI) of 10.25 ± 3.09 (n=4). Oxygen consumption was measured using a Clark type O_2 electrode (Rank Bros, Bottisham, Cambridge).

Concentrations of Ca^{2+} known to induce pore opening (i.e. 25 μM; Al-Nasser & Crompton, 1986) in the presence of [P_i] produced a decrease in the state 3 value and an increase in the state 4 value which resulted in a reduction in the RCI from a control value of 7.58 ± 0.95 to 1.70 ± 0.70 (n=4; P<0.05). Cyc A (3 μM) had no effect upon the respiration parameters alone but in the presence of Ca^{2+} (25 μM) prevented the increase in state 3 and decrease in state 4 respiration (Table 1) with the RCI value increasing to 4.60 ± 0.53 (n=4; P<0.05).

Rates	Control	Ca^{2+} 25μM	Ca^{2+} 25μM plus CycA 3μM
Endogenous	13.75 ± 1.01	10.92 ± 1.98	13.46 ± 1.11
State 4	8.76 ± 1.46	47.68 ± 14.46	10.72 ± 2.64*
State 3	86.00 ± 4.27	50.53 ± 21.66	77.54 ± 16.55*
Return 4	12.00 ± 1.91	29.42 ± 0.84	16.81 ± 2.21*

Table 1. Effect of Ca^{2+} plus Cyc A on liver mitochondrial respiration, measured in ng atoms O min^{-1} mg protein^{-1},compared to Ca^{2+} alone; mean ± s.e. mean; n=4; *P<0.05.

This data shows that in rat liver mitochondria incubation with Cyc A can prevent the uncoupling action produced by high concentrations of Ca^{2+} and [P_i], by prevention of pore opening. Inhibition of pore opening may be beneficial in conditions such as ischaemic/reperfusion injury as shown by Smith *et al.* (1994).

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346P *IN VITRO* BINDING OF [^{14}C]SULPHAMETHAZINE TO MURINE HEPATIC MICROSOMES

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Sulphamethazine (SMZ) is widely used to control bacterial infections in livestock and poultry. Residues of this drug enter the human food chain through meat products and this is of toxicological concern because of the ability of sulphonamides to elicit hypersensitivity reactions (Rieder et al 1991). Such adverse drug reactions might result from metabolic activation of parent drug to form adducts that may act as immunogens (Hinson & Roberts 1992). In the present study, we have investigated the ability of [^{14}C]-SMZ to covalently bind to murine hepatic microsomes.

[^{14}C]-SMZ (0.2 μCi) and SMZ (1.67 mM) were incubated at 37°C and pH 7.4 for 20 minutes with mouse liver microsomes (0.8 mg ml^{-1} protein) in the presence of an NADPH-generating system. Protein was precipitated with 3M trichloroacetic acid and trapped on Whatman GF/B filters. Filters were washed to remove unbound 14-carbon and residual radioactivity was quantified by liquid scintillation counting. Data are presented as mean ± s.e. mean and statistical significance was determined by non-paired Student's t-test.

Table 1 shows that removal of NADP$^+$, incubation in an anaerobic atmosphere, incubation at 4°C and heat denaturation of microsomes resulted in statistically significant decreases in binding. Furthermore, binding was diminished in the presence of carbon monoxide and SKF-525A (0.33 mM), and was reduced when either glutathione (0.42 mM) or cysteine (0.42 mM) was added to the reaction medium. Treatment of incubation mixture with either protease (subtilisin) or ribonuclease (RNAse T$_1$) decreased binding by 56 and 20%, respectively.

These results suggest [^{14}C]-SMZ is metabolised by hepatic microsomal mono-oxygenases and the resultant metabolite(s) interact covalently with microsomal protein and RNA. The metabolite(s) may be electrophilic as the nucleophiles glutathione and cysteine inhibit binding.

Supported by MAFF contract N2059.

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Table 1 Covalent binding of SMZ to murine hepatic microsomes

Group	pmol SMZ bound mg^{-1} protein min^{-1}	% Inhibition	Group	pmol SMZ bound mg^{-1} protein min^{-1}	% Inhibition
Complete	425 ± 45 (15)	0	SKF-525A	205 ± 13 (5)***	52
-NADP$^+$	245 ± 50 (5) *	42	Carbon monoxide	178 ± 17 (5)***	58
Nitrogen	175 ± 43 (5)**	59	Glutathione	148 ± 20 (5)***	65
4 °C	178 ± 12 (5)***	58	Cysteine	123 ± 23 (5)***	71
Boiled for 5 min	120 ± 18 (5)***	72			

* $p<0.05$, ** $p<0.01$, *** $p< 0.001$ relative to group with complete incubation mixture.

347P A COMPARISON OF DRUG BINDING SITES ON HUMAN AND CHICKEN ALBUMINS

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Human and other mammalian albumins have two structurally selective ligand binding sites. Site I binds drugs such as warfarin and phenylbutazone, whereas benzodiazepines and some dansyl amino acids interact with site II (Sudlow *et al* 1976; Panjeshahin *et al* 1992). However, it is not known if similar binding loci exist in non-mammalian albumins. In this study, drug binding sites on chicken albumin have been investigated using site selective fluorescent probes and 4-nitrophenyl acetate (NPA); the hydrolysis of which is inhibited by site II ligands (Panjeshahin *et al* 1992).

The binding of warfarin (site I probe, 2μM) and dansylsarcosine (site II probe, 2μM) to defatted human and chicken albumins (20μM) was monitored by fluorescence spectroscopy at 31°C and pH 7.4. Displacement studies were done with phenylbutazone (site I selective, 20μM) and diazepam (site II selective, 20μM). The effect of increasing pH from 6.0 to 9.0 on the fluorescence of albumin-bound warfarin and dansylsarcosine was also examined; and in addition, the influence of site I and site II selective ligands on the hydrolysis of NPA by human and chicken albumins was investigated at 24°C and pH 8.0 (Means & Bender, 1975). Data are given as mean ± s.e. mean (n=3-5) and statistical significance was assessed by non-paired Student's t-test.

With human albumin, the fluorescence of bound warfarin was decreased ($p<0.001$) by 59.6 ± 0.3% of the control value when phenylbutazone was present. In the presence of diazepam, the fluorescence of dansylsarcosine was reduced ($p<0.001$) by 71.5 ± 1.2 % of the control value. With chicken albumin, however, the extent to which phenylbutazone and diazepam reduced the fluorescence of warfarin (14.8 ± 0.4%) and dansylsarcosine (6.1 ± 0.2%) was significantly less ($p<0.01$) than the decreases noted with human albumin. Increasing the buffer pH, from 6.0 to 9.0, enhanced fluorescence intensity of warfarin bound to human albumin by 61.9 ± 3.7 %; but by contrast, fluorescence with chicken albumin was decreased by 18.9 ± 1.4 %. There were no marked pH-induced changes in the fluorescence of dansylsarcosine when bound to either human or chicken albumin. The rate constant for hydrolysis of NPA was about five-fold greater for human than chicken albumin (0.065±0.003 vs. 0.013±0.001 sec^{-1}). The rate of NPA hydrolysis by human albumin was reduced by 62.8±5.1% in the presence of diazepam (50μM); but by only 10.1±7.7% with phenylbutazone (50μM). However, for chicken albumin, hydrolysis of NPA with either diazepam or phenylbutazone present was decreased by 22.3±3.5% and 17.2±4.7%, respectively.

The results suggest marked differences exist between human and chicken albumins with respect to the characteristics of the warfarin and dansylsarcosine binding sites and the ability of these albumins to hydrolyse NPA. These dissimilarities might be accounted for by differences in ligand affinity and this is currently under investigation.

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348P CHANGES IN HIPPOCAMPAL GENE EXPRESSION ASSOCIATED WITH THE INDUCTION OF LONG-TERM POTENTIATION

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Induction of long-term potentiation (LTP) in the hippocampus has been shown to be accompanied by changes in levels of particular mRNA species, both *in vivo* and *in vitro* (Cole *et al.*, 1989; Wisden *et al.*, 1990; Mackler *et al.*, 1992). While the increased expression of two genes, αCalcium/Calmodulin dependent protein kinase II (αCAMKII) and zif/268, is thought to be most closely correlated to the induction of LTP (Mackler *et al.*, 1992), it is not clear to what extent alterations in the expression of these, or other genes contribute to LTP in the Schaffer collateral/CA1 neurone synapses. In view of this, we have studied the expression of 4 genes following the induction of LTP in rat hippocampal slices.

Hippocampal slices (450μm) were prepared from male Wistar rats (150-250g), and placed in the recording chamber (30°C), superfused with artificial cerebrospinal fluid, composition (mM) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, Glucose 10, gassed with 95% O_2/5% CO_2. Population spikes (PS) were recorded in area CA1 following stimulation of Schaffer collaterals. LTP slices received 5 repetitions of a 20ms train delivered at 200Hz, which produced a 50-100% increase PS size. Control slices received low frequency, non-potentiating stimulation. Slices were then incubated at 30°C for either 45min or 2h before sectioning for in-situ hybridisation with [^{35}S]-dATP-labelled 45-mer oligonucleotide probes (Morris, 1989). The sections were then exposed to photographic emulsion and the hybridisation signal in area CA1 quantified using computerised image analysis. Results were expressed as a mean percentage change (±s.e.mean) compared to control, and analysed for significance using a one sample Wilcoxon test.

Levels of c-fos mRNA and tubulin (T26) mRNA in area CA1 were unchanged 45min after induction of LTP, however, zif/268 mRNA levels showed a significant increase compared to non-potentiated controls in the same area (22.2±7.6%; $P<0.05$; $n=6$). The levels of T26 mRNA were also unchanged 2h after the induction of LTP, but levels of αCAMKII mRNA were significantly increased over control values at this time point (21.0±7.5%; $P<0.05$; $n=7$).

It is possible, therefore, to measure changes in gene expression following induction of LTP *in vitro*. These results strengthen the theory that zif/268 and αCAMKII are involved in some aspect of the induction or maintenance of hippocampal LTP.

This work was supported by Glaxo and the Wellcome Trust

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349P APOLIPOPROTEIN E IMMUNOREACTIVITY IS ALTERED FOLLOWING TRANSIENT CEREBRAL ISCHAEMIA

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The role of apolipoprotein E (ApoE) in the pathogenesis of AD has attracted considerable research interest, not least because of its potential for pharmacologic intervention. However, although a role for ApoE in the peripheral response to injury is well documented, there is a paucity of information regarding ApoE in the injured CNS. This study sought to examine possible alterations in ApoE in the rodent hippocampus following an ischaemic challenge in which the involvement of excitotoxins and impaired energy metabolism is well defined.

Transient cerebral ischaemia was induced for 15 minutes in anaesthetised male, adult Wistar rats (300-400g) by occlusion of both carotid arteries with hypotension followed by reperfusion for 4h (n=5), 24h (n=5) or 72h (n=5). In 9 sham operated controls the carotids were exposed but not occluded. In this model, ischaemia results in selective neuronal degeneration in the CA1 pyramidal cell layer after 72h reperfusion while there is no damage to the CA3 hippocampal region. The brains were perfusion fixed with 4% paraformaldehyde, cryoprotected and processed for immunohistochemistry. Sections were immunostained with antibodies to ApoE and glial fibrillary astrocytic protein (GFAP) and counterstained with haematoxylin. Adjacent sections were stained with cresyl violet for histological verification of ischaemic damage.

In sham animals, ApoE immunoreactivity was confined to astrocytes and their processes and there was no neuronal staining observed in any of the 9 sham animals. ApoE immunoreactivity was not altered following 4h reperfusion in any animal. In 5 out of 5 animals at 24h reperfusion, intense ApoE staining of the cytoplasm of astrocytes and neuropil within the CA1 region of the hippocampus was observed. At 72h reperfusion in all 5 animals, intense ApoE staining of pyramidal cell bodies and dendrites was consistently observed in the CA1 region of the hippocampus and this was coincident with reduced ApoE staining of astrocytic processes. GFAP staining indicated astrocytes were present within the CA1 region of the hippocampus at 72h after reperfusion in all 5 animals.

This study demonstrated the localisation of ApoE to degenerating neurons and their processes following an ischaemic insult. The results are consistent with the release of ApoE from astrocytes and the uptake of ApoE into neurons following injury. Acute cerebral ischaemia may provide a model to examine the role of ApoE in the pathogenesis of neurodegenerative disorders.

350P SELECTIVE ALTERATIONS IN AMYLOID PRECURSOR PROTEIN FOLLOWING TRANSIENT CEREBRAL ISCHAEMIA

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Amyloid precursor protein (APP) is a transmembrane protein which can be cleaved to generate amino (N)-terminal and carboxy (C)-terminal fragments that can have both neurotrophic and neurotoxic properties. Abnormalities in the metabolism of APP may be a key event in the pathogenesis of Alzheimer's disease (Selkoe, 1991). The mechanisms which regulate APP processing and the generation of APP fragments remain to be elucidated. The present study sought to determine alterations in APP following a transient ischaemic insult.

Transient cerebral ischaemia was induced in anaesthetised male, adult Wistar rats (300-400g) by occlusion of both carotid arteries with hypotension followed by reperfusion for 4h (n=5), 24h (n=5) or 72h (n=5) (Smith et al. 1984). In 9 sham operated controls the carotids were exposed but not occluded. In this model, ischaemia for 15 minutes results in selective neuronal degeneration in the hippocampal CA1 pyramidal cell layer after 72h reperfusion while there is no damage to the CA3 region. The brains were perfusion fixed with 4% paraformaldehyde, cryoprotected and processed for immunohistochemistry. Adjacent sections were immuno-stained with antibodies which recognise different APP domains (N-terminus 60-100, 22C11 Boehringer; N-terminus near membrane, 527-540 and C-terminus 676-695 gifts from Dr. Selkoe) and counterstained with haematoxylin. Corresponding tissue sections were stained with cresyl violet to define the ischaemic damage.

In sham operated animals, all APP antibodies stained mainly the periphery of neuronal perikarya and their processes. At 4h reperfusion in all 5 animals, the pattern of APP staining was similar to sham-operated animals. At 24h reperfusion in 5 out of 5 animals, N-terminal APP immunostaining resembled staining in stam animals in the CA1 region whereas increased staining of pyramidal cells in the CA2/CA3 region was observed. C-terminal APP staining was similar to sham-operated animals in 5 out of 5 animals at 24h reperfusion. At 72h reperfusion, N-terminal staining in the CA1 region was lost but striking astrocytic staining in the stratum laconosum moleculare of the hippocampus and corpus callosum was apparent in all 5 animals. In contrast, C-terminal APP immunoreactivity intensely stained the perikarya of degenerating neurons and dendrites in the CA1 region at 72h reperfusion in all 5 animals.

These results demonstrate that ischaemia and reperfusion is associated with selective alterations in the cellular distribution of N-terminal and C-terminal APP immunoreactivity. Furthermore, the differences in cellular alterations and the direction of these alterations between the APP domains would suggest that transient ischaemia alters the regulation of APP.

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351P EFFECT OF CCK-4 ADMINISTRATION ON PANIC-LIKE REACTIONS PRODUCED BY STIMULATION OF THE DORSAL PERIACQUEDUCTAL GREY AREA IN THE RAT

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Intravenous administrations of CCK-4, a peptide present in the gut, triggers panic attacks in panic disorder patients (Bradwejn et al., 1991). This peptide is also found in the CNS suggesting that it could act as a modulator in the neuroprocessing of panic. The dorsal periacqueductal grey area (DPAG) in the brainstem is believed to be the final pathway for the integration of panic. Stimulation of the DPAG in humans induces tachycardia and outburst of fear similar to a full-blown panic attack, while in animals it triggers tachycardia and aversive behaviours such as flight (Graeff, 1990). The present studies investigate whether the tachycardia and the flight response induced by the injection of DL-homocysteic acid (DLH) into the DPAG are potentiated by CCK-4.

Male Lister Hooded rats (180-200g) were implanted with a guide cannula placed 2 mm above the DPAG under chloral hydrate anesthesia. Rats were then allowed to recover from the surgery for at least one week before behavioural experiments. A dose of 5 nmol of DLH was injected in the DPAG (with an injector protruding 2 mm below the guide cannula) inducing a flight response that was monitored with a video camera and subsequently analysed by computer to calculate the average speed and total distance travelled during 20 s. The tachycardia induced by 10 nmol of DLH injected into the DPAG was subsequently monitored under urethane anesthesia using a heart rate meter fed from an electrocardiogram signal.

In a first study, CCK-4 (2 µg in 1 µl) or saline were injected directly into the DPAG 5 min before triggering the defense response with DLH. This administration of CCK-4 did not change the average speed of the flight response (SAL: 0.15 ± 0.05 m/s; CCK: 0.16 ± 0.05 m/s; N=10) nor the total distance traveled (SAL: 3.05 ± 0.99 m; CCK: 3.29 ± 1.04 m; N=10). The tachycardia produced by DLH also was not changed by CCK-4 directly injected into the DPAG (Table 1).

In a second study, CCK-4 (40 µg in 20 µl) or saline were injected intracerebroventricularly (I.C.V.) 5 min prior to the DPAG stimulation. Here again the CCK-4 did not changed the average speed of the flight response (SAL: 0.35 ± 0.11 m/s; CCK: 0.43 ± 0.13 m/s; N=10) nor the total distance traveled (SAL: 6.95 ± 2.14 m; CCK: 8.53 ± 2.68 m; N=10). However, the duration (but not the amplitude) of the DLH-induced tachycardia was significantly increased by I.C.V. CCK-4 (Table 1).

In a third study, the enzyme resistant BOC-CCK-4 (butyl-oxy-carbonyl-CCK-4; 40 µg in 2 ml) was administered intraperitoneally (i.p.) 10 min before injecting DLH into the DPAG. Both the average speed of the flight response (SAL: 0.13 ± 0.03 m/s; CCK: 0.25 ± 0.05 m/s; N=10) and the total distance traveled (SAL: 2.64 ± 0.72 m; CCK: 4.95 ± 0.91 m; N=10) were found nearly doubled following i.p. BOC-CCK-4, but these changes did not reach statistical significance (P=0.06) using the Student's t-test. In contrast, the DLH-induced tachycardia was not changed by a similar administration of BOC-CCK-4 (Table 1).

Table 1. Effect of CCK-4 on the DLH-induced tachycardia in the rat.

	Effect of DLH on the heart rate (pulses/min)		
Drug administration	prior to	60 s after	210 s after
saline into DPAG	325 ± 22	371 ± 15	347 ± 16
CCK-4 into DPAG	332 ± 21	374 ± 20	352 ± 20
saline i.c.v.	358 ± 14	401 ± 18	380 ± 10
CCK-4 i.c.v.	378 ± 14	407 ± 15	418 ± 20 *
saline i.p.	346 ± 10	378 ± 10	367 ± 19
BOC-CCK-4 i.p.	354 ± 12	387 ± 5	372 ± 14

(N=6 for each group, * $P < 0.01$ using ANOVA and Dunnett's post-hoc test.)

Overall these results suggest that CCK-4 potentiates the tachycardia component of panic induced by the DPAG stimulation by an action within the CNS, however this effect of CCK-4 cannot be accounted by a direct action in the DPAG. CCK-4 may also potentiate the behavioural component of panic by acting on some peripheral site rather than in the CNS. Further studies need to be undertaken to verify this possibility.

Acknowledgement, This work was supported by a fellowship from the Human Frontier Science Program (R.M.).

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352P LACK OF EFFECT OF YOHIMBINE ON EXPRESSION OF C-FOS mRNA IN RATS

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It has been proposed that the induction of the immediate early gene c-fos in response to environmental and pharmacological stimuli may be used as a metabolic marker in neuroanatomical mapping (Dragunow & Faull, 1989). Previous studies have shown increased expression of c-fos mRNA in response to the anxiogenic α_2 antagonist yohimbine, using Northern blot hybridisation (Gubits *et al.*, 1989). The aim of the present study was to map areas of brain involved in the response to yohimbine using *in situ* hybridisation to localise c-fos mRNA.

Male hooded Lister rats (235-300g) were handled once daily for 2 days. On the 3rd day rats received either yohimbine 5 mg kg^{-1} or water i.p. and were killed by cervical dislocation 30 min later. *In situ* hybridisation was performed on 20 μm coronal sections according to the method of Wisden *et al.* (1989) using a [^{35}S]-labelled oligonucleotide probe complementary to nucleotides spanning amino acids 1-15 of the Fos protein. Non-specific hybridisation, determined in the presence of a 50-fold excess of unlabelled probe, was negligible.

The hybridisation signal for c-fos mRNA was very low in control animals. In the treated animals, no area showed significantly different levels of c-fos mRNA from controls (Table 1). In particular, areas in which increased levels of Fos protein have been demonstrated in reponse to stress, such as the central amygdala and the paraventricular nucleus (PVN) of the hypothalamus (Honkaniemi, 1992), and to yohimbine, such as the cingulate cortex (Stone *et al.*, 1993) showed no increased c-fos expression.

The reason for this discrepancy is unclear. It may be that determination of changes in c-fos mRNA levels is insufficiently sensitive, compared with Fos immunocytochemistry, to be useful in mapping responses to the relatively mild stimulus of an anxiogenic drug.

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Table 1. Expression of c-fos mRNA after yohimbine 5 mg kg^{-1} i.p. Data are expressed as relative optical density on autoradiographs (minus background) ± s.e.mean., n=6 per group.

	control	yohimbine
Temporal cortex	0.257 ± 0.007	0.273 ± 0.009
Posterior PVN	0.231 ± 0.006	0.232 ± 0.005
Central amygdala	0.246 ± 0.006	0.263 ± 0.008
Anterior cingulate ctx	0.283 ± 0.007	0.300 ± 0.009

353P ARACHIDONYL ETHANOLIMIDE (ANANDAMIDE) MODULATION OF CYTOKINE-STIMULATED THYMIC LYMPHOCYTE PROLIFERATION

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Arachidonyl ethanolamide (AE), also known as Anandamide, has been identified as an endogenous ligand that binds effectively to both the cannabinoid receptor on rat synaptosomal membranes (Devane *et al.*, 1992) and to the cloned human cannabinoid receptor (Felder *et al.*, 1993). Following recent demonstration of cannabinoid receptor mRNA in several classes of immune cells including thymocytes (Bouaboula *et al.*, 1993), the goal of this study was to investigate the effect of arachidonyl ethanolamide (AE) upon cytokine-stimulated thymic lymphocyte proliferation.

Thymic lymphocytes (non-adherent) were obtained from male 5-6 week old NIH strain mice and resuspended in RPMI 1640 tissue culture media supplemented with 100 IU/ml Penicillin, 100 µg/ml Streptomycin, 5% (v/v) foetal calf serum, and 1 µg/ml phytohaemagglutinin (PHA). The thymic lymphocyte suspension was then transferred to 96-well tissue culture plates. IL-1α (100 ng/ml) was added, immediately followed by the addition of varying concentrations of AE. Control cultures were prepared as above with a percentage of ethanol (AE solvent) identical to that present in the cultures containing the test agent. Thymocytes were incubated at 37 °C, 5% CO_2, 100% humidity for 72 hours, and cells were pulsed with [^{3}H] thymidine for the final 24 hours of the incubation period. Assays were terminated by addition of trichloroacetic acid. The acid-insoluble radioactivity was measured by scintillation counting.

A concentration-dependent increase above IL-1α-stimulated control levels of [^{3}H] thymidine incorporation was observed for concentrations of AE between 10^{-8} M and 10^{-6} M. For example, at 10^{-7}M AE radioactivity increased to 9800 ± 1138 cpm from 6096 ± 861 cpm for controls. A maximal response occurred at 10^{-6} M AE where levels exceeded controls by a factor of 1.78 ± 0.12 ($P< 0.001$). AE was also observed to increase levels of [^{3}H] thymidine incorporation in incubations carried out in the absence of IL-1α. For example, at 10^{-7} M AE radioactivity increased to 2593 ± 270 cpm from 2104 ± 170 cpm for incubations without AE. A maximal response occurred at 10^{-6}M AE where levels exceeded IL-1α-negative controls by a factor of 1.45 ± 0.25 ($P< 0.01$). All values represent the mean of n = 4 ± s.d. and results are representative of at least three separate experiments. Results were confirmed by cell counting.

The results of this study indicate that arachidonyl ethanolamide enhances thymocyte proliferation in response to PHA alone, and that arachidonyl ethanolamide also enhances IL-1α-stimulated increases in thymocyte proliferation. These results suggest a possible modulatory role for arachidonyl ethanolamide, an arachidonic acid derivative, in immune cell responses.

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354P PROSTAGLANDIN E_2 AND FATTY ACID SUPPRESSION OF CYTOKINE-STIMULATED THYMIC LYMPHOCYTE PROLIFERATION: THE ROLE OF CYCLIC AMP

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An important axis of immune responsiveness is the amplification of lymphocyte proliferation by cytokines, particularly the stimulation of thymic lymphocytes by interleukin-1 (IL-1). IL-1 can also induce the biosynthesis of prostaglandins (PG) from immune cells especially PGE_2 which appears to suppress the IL-1-stimulated proliferation of T-cells. The immediate fatty acid precursors of PGs have also been shown to suppress IL-1-stimulated responses, however, this does not involve conversion to PGs (Rotondo *et al.*, 1994). PGE_2 can lead to activation of adenylate cyclase and consequently raise intracellular levels of cyclic AMP in many cell types. The aim of the present study was to investigate whether the suppression of IL-1-stimulated thymic lymphocyte proliferation by either PGE_2 or fatty acids involves cyclic AMP.

Thymic lymphocytes (non-adherent) were obtained from male 5-6 week old NIH strain mice and resuspended in RPMI 1640 culture medium containing 1 μg/ml phytohaemagglutinin, 5% (v/v) foetal calf serum. The cells (6×10^6) were then transferred to 96-well cell culture plates immediately followed by the addition of IL-1α or IL-1β (both 25 ng/ml), PGE_2 (100 nM), 100 μM of the fatty acids dihomo-γ-linolenic (DGLA) or arachidonic (AA), and of the adenylate cyclase activator forskolin (FS, 1 μM) and the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX, 1 μM). Control cultures were prepared as above with a percentage of ethanol or DMSO (PGE_2/fatty acid and FS/ IBMX solvents) identical to that present in the cultures containing the test agent and not exceeding 0.01 % (v/v). Cells were incubated at 37 °C, 5% CO_2, 100% humidity for 72 hours, and 0.5 μCi [^{3}H] thymidine (TdR) was added for the final 24 hours after which the radioactivity in the acid-insoluble fraction was measured. Cyclic AMP was measured by radioimmunoassay.

IL-1 enhanced the incorporation of [^{3}H]-TdR into thymocytes to 7945 ± 289 cpm from 2013 ± 156 in controls. PGE_2 inhibited the IL-1-stimulated response to 40.3 % ± 7.1 ($P < 0.01$). AA and DGLA inhibited the response to 10.1 % ± 6.1 and 46.5 % ± 8.5 ($P < 0.01$) respectively of the level with IL-1 alone. FS and IBMX also inhibited the response to 28.2 % ± 4.3 and 43.1 % ± 12.6 ($P < 0.01$). Inhibition of the IL-1 response by DGLA was not further enhanced by IBMX. However, PGE_2 inhibition of the IL-1-stimulated level was enhanced by IBMX from a level of 54.5% ± 5.6 to 21.2 % ± 6.3 ($P < 0.05$). PGE_2 also increased the intracellular level of cyclic AMP under similar conditions by 7.1-fold ± 1.2 over control cells (all values n= 4).

The data obtained show that the incorporation of [^{3}H]-TdR in response to IL-1 can be inhibited under conditions which increase cellular levels of cyclic AMP, suggesting that PGE_2 suppresses thymocyte proliferation by raising levels of cyclic AMP. However, this does not appear to be the case for the mechanism by which fatty acids suppress lymphocyte proliferation.

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355P IP-PROSTANOID RECEPTOR-MEDIATED INHIBITION OF CALCIUM CHANNEL CURRENTS IN NG108-15 NEUROBLASTOMA CELLS IS MEDIATED THROUGH G_s ACTIVATION

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The effects of iloprost, a selective IP-prostanoid receptor agonist (Williams & Kelly, 1994), on voltage-gated calcium channel currents (I_{Ca}) were investigated in NG108-15 neuroblastoma x glioma hybrid cells using the whole-cell variant of the patch-clamp technique. Cells were differentiated by treatment with IBMX (50μM) and prostaglandin E_1 (10μM) for three days and then superfused (5-7 ml.min^{-1}) at room temperature with a solution containing (mM): TEACl 132.5; CsCl 5.4; $BaCl_2$ 10; $MgCl_2$ 1; HEPES 10; sucrose 50 and glucose 10. Whole cell patch clamp recordings were made with electrodes containing (mM): CsCl 168; MgATP 5; BAPTA 10; GTP 5; HEPES 10. All solutions were adjusted to pH 7.3 with CsOH. Cells were held at -90mV and I_{Ca} was activated by 200 ms depolarising pulses.

T-type calcium channel currents were isolated by first pretreating cells for 15 minutes with ω-conotoxin GVIA (1μM) and then exposure to nimodipine (3μM) throughout the period of recording. L-type currents were isolated by first pretreating cells for 15 minutes with ω-conotoxin GVIA (1μM) and introducing a depolarising prepulse to 0mV for 100ms, then briefly restoring to -90mV for 10ms prior to applying the test pulse. N-type currents could not be completely isolated so N-type currents contaminated with T-type currents were studied after inhibiting L-type currents by exposure to nimodipine (3μM) throughout the period of recording. IP-prostanoid receptor activation by exposure to iloprost (1μM) for 4 minutes had no effect on T-type currents but produced inhibition of L-type currents (31 ± 6% at +10mV; n=8). When N- and T-type currents were evoked together, iloprost produced an inhibition of 23 ± 5% at +10mV (n=4).

The inhibition of L- and N-type I_{Ca} was abolished by prolonged cholera toxin treatment (3hrs; 50ng.ml^{-1}) but was insensitive to pertussis toxin (17hrs; 50ng.ml^{-1}). Pretreatment with cholera toxin abolishes the effect of iloprost on cyclic AMP accumulation by down-regulation of G_s (Williams & Kelly, 1994). We repeated these experiments using cyclic AMP accumulation assays to demonstrate the effectiveness of our cholera toxin. Similarly pertussis toxin pretreatment abolished the inhibitory effect of the δ-opioid selective agonist, [D-Pen^2, D-Pen^5]-enkephalin on cyclic AMP accumulation.

The inhibitory effect of iloprost on the total I_{Ca} (T, N and L) was attenuated from 20 ± 4% at 0mV (n=11) to 8 ± 4% (n=10) by intracellular dialysis for 10min with the Rp-isomer of adenosine-3',5'-cyclic monophosphothioate (100μM), a PKA inhibitor (Rothermel *et al.*, 1984). Superfusion of the cells with H89 (10μM), a membrane permeable PKA inhibitor, also significantly attentuated the inhibition produced by iloprost to 6 ± 2% (n=14)(Geilen 1992). However H89 itself inhibited I_{Ca} by 17± 4% (n=14).

These results indicate that IP-prostanoid receptor activation can produce inhibitory effects on L- and N-type calcium channel currents via G_s and possibly PKA.

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356P EFFECT OF A SELECTIVE PROSTAGLANDIN H SYNTHASE-2-INHIBITOR ON PROSTAGLANDIN PRODUCTION BY THE GUINEA-PIG UTERUS

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Increased prostaglandin production by the uterus (particularly the endometrium) towards the end of the cycle is involved in luteolysis in non-primates and menstruation in women. Dysmenorrhoea and menorrhagia are due to an overproduction or imbalance in endometrial PG production, respectively. Relief from these disorders is obtained by treatment with non-selective non-steroidal anti-inflammatory drugs (NSAIDs; Poyser, 1995). This study has investigated whether PG synthesis by the uterus is inhibited by a selective PGH synthase-2 (PGHS-2) inhibitor, namely NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methanesulphonamide; Futaki *et al.*, 1994). The effects of NS-398 (16 & 32 μM) and, for comparison, indomethacin (14 & 28 μM) on PG synthesis by homogenates of day 7 and 15 guinea-pig endometrium incubated for 1 h were studied. Also, the effects of NS-398 (16 μM) and indomethacin (14 μM) on PG output from cultured day 7 guinea-pig endometrium and myometrium, and from endometrial cells (i.e. epithelial and stromal) were investigated. The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced were measured by radioimmunoassays. Indomethacin (14 μM) significantly ($P < 0.05$, $n = 4$; Students t test) reduced the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesized by homogenates of endometrium by 75.4, 58.1 and 45.0%, respectively, on day 7 and by 49.5, 67.5 and 59.3%, respectively, on day 15. NS-398 (16 μM) significantly ($P < 0.05$, $n = 4$) reduced the amounts of the 3 PGs synthesized by endometrial homogenates by 42.6, 49.3 and 42.5%, respectively, on day 7 and by 58.8, 60.4 and 51.9%, respectively, on day 15. Indomethacin (28 μM) significantly ($P < 0.05$, $n = 4$) reduced the amounts of the 3 PGs synthesized by endometrial homogenates by 81.9, 68.9 and 55.0%, respectively, on day 7, and by 59.3, 71.4 and 66.7%, respectively, on day 15. NS-398 (32 μM) significantly ($P < 0.05$, $n = 4$) reduced the amounts of the 3 PGs synthesized by endometrial homogenates by 62.3, 58.1 and 50.0, respectively, on day 7, and by 61.5, 67.8 and 63.0%, respectively, on day 15. Indomethacin (14 μM) significantly ($P < 0.05$, $n = 4$) reduced the outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ by 98.5, 70.6 and 88.0%, respectively, from cultured endometrium and by 69.7, 60.0 and 86.1%, respectively, from cultured myometrium. NS-398 (16 μM) significantly ($P < 0.05$, $n = 4$) reduced the outputs of the 3 PGs by 98.1, 70.6 and 68.0%, respectively, from cultured endometrium, and by 60.6, 34.5 and 55.6%, respectively, from cultured myometrium. The outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from cultured epithelial cells were significantly ($P > 0.05$, $n = 4$) reduced by indomethacin (14 μM) by 94.1, 28.6 and 57.1%, respectively, and by NS-398 (16 μM) by 94.7, 61.9 and 38.9%, respectively. $PGF_{2\alpha}$ output from cultured stromal cells was not significantly inhibited by indomethacin or NS-398. However, PGE_2 and 6-keto-$PGF_{1\alpha}$ outputs from cultured stromal cells were significantly ($P < 0.05$, $n = 4$) reduced by indomethacin (14 μM) by 15 and 47.5%, respectively, and by NS-398 (16 μM) by 16.4 and 51.5%, respectively. In conclusion, NS-398 significantly inhibited PG production by both the endometrium and myometrium obtained from the guinea-pig uterus, and was found to be as effective as indomethacin. The results indicate that PGHS-2 is the predominant PG-forming enzyme in the guinea-pig endometrium, which is in agreement with a previous study using Western blotting to detect the enzyme (Naderali & Poyser, 1994). If PGHS-2 is the predominant form of PGHS in human endometrium, then selective inhibitors of PGHS-2 may prove as beneficial as the non-selective NSAIDs in the treatment of menstrual disorders with but fewer side-effects.

E.K. Naderali is supported by the MRC. NS-398 was kindly supplied by Taisho Pharmaceutical Co., Ltd, Tokyo, Japan.

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357P MODULATION OF INTERLEUKIN-1β PRODUCTION IN HUMAN BLOOD BY PROSTAGLANDIN E_2 AND ARACHIDONIC ACID

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Arachidonic acid (AA) can act as an intracellular regulator (Naor, 1991) in addition to its conversion to a range of oxygenated metabolites such as eicosanoids, particularly prostaglandins (PG). Although PGE_2 has been reported to suppress the release of pro-inflammatory cytokine mediators from immunostimulated isolated cell preparations in culture it is unclear what role the precursor fatty acid plays in modulating cytokine release and hence the overall inflammatory response. The aim of the present study therefore was to examine whether AA could modulate production of the cytokine interleukin-1β (IL-1β) by human blood.

Experiments were carried out using whole blood incubations in order to more closely mimic the *in vivo* environment. IL-1β release was stimulated using lipopolysaccharide (LPS). All incubations were carried out in sterile Eppendorf tubes and contained 0.1 IU/ml aprotinin. Blood was incubated with LPS and either PGE_2, the cyclooxygenase inhibitor ketoprofen (KP), AA, or vehicle at 37°C, 5 % CO_2 in air. At the end of the incubation period tubes were centrifuged (5,000g x 2 min), plasma transferred to new tubes and stored at -20 °C until analysed. IL-1β was estimated using a commercially available ELISA system. All values are expressed as means ± s.d., $n \geq 3$.

Initial experiments showed that maximum levels of IL-1 were detected after 20 h incubation with LPS, therefore all subsequent incubations were carried out for 20 h. There was no detectable IL-1 in samples incubated without LPS. LPS in the range 1 μg/ml - 1 mg/ml induced a concentration dependant increase in IL-1 release. Although there was a wide variation in the absolute amount of IL-1 detected in samples from different donors in response to any one concentration of LPS, EC_{50} values were remarkably consistent (0.98 ± 0.30 μg/ml, n = 6). PGE_2 (100 nM) reduced levels of IL-1 from 2,432 ± 9 pg/ml to 1115 ± 5 pg/ml in response to 1 μg/ml LPS ($P < 0.05$). No further suppression was observed with higher concentrations. KP (50 μM) increased LPS-induced IL-1 levels 1.2-fold from 9,700 ± 400 pg/ml in the absence of KP to 11,700 ± 300 pg/ml in the presence of KP ($P < 0.05$) with 1 μg/ml LPS. AA also enhanced LPS-induced IL-1-release when compared to control incubations without AA. For example, IL-1 levels increased from 450 ± 98 pg/ml in the presence of 1 μg/ml LPS alone to 2,173 ± 100 pg/ml, 2,113 ± 60 pg/ml and 1,899 ± 30 pg/ml in the presence of 1 μg/ml LPS plus either 1, 10 or 100 μM AA respectively.

The data obtained shows that the whole-blood model is useful for studying the modulation of cytokine production and that endogenous PGE_2 downregulates the production of IL-1. Also, AA can modulate cytokine release in a PG independent -manner.

This work was supported by Ministry of Agriculture Fisheries and Food.

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358P CHARACTERISATION OF PROSTAGLANDIN E_2 RECEPTORS IN RAT KIDNEY

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The presence of prostagladin E receptors in rat kidney homogenates was first reported by Oien *et al.* (1979). The EP_1 and EP_2 selective compounds SC19220 and SC4286, did not displace PGE_2 binding in kidney homogenates (Eriksen *et al.*; 1987; Eriksson *et al.*; 1990), and these authors concluded that EP_1 and EP_2 receptors were not present in this tissue. They did not use a selective EP_3 receptor compound in their studies and consequently, the aim of this study was to establish if the EP_3 receptor agonist, enprostil (Reeves *et al.*, 1988) displaced PGE_2 binding in rat kidney sections.

Renal tissue was obtained from CFY rats killed by decapitation. Ten micron sagittal sections were cut, thaw-mounted onto gelatinised slides and air dried. One hundred µl of 10 mM Hepes buffer (pH 7.4, bovine serum albumin 0.5 %), containing 1nM [^{3}H] PGE_2 and test substances, was placed on each section for a period of 60 min at 20°C, followed by 3 washes of 5 min, each in ice cold 0.9 % NaCl. Individual kidney sections were wiped from the slide, and bound radioactivity counted by liquid scintillation spectroscopy. Non-specific binding was defined as the binding observed in the presence of 1 µM unlabelled PGE_2. Enprostil was dissolved in 100% ethanol, while the EP1 antagonist, AH6809 (Coleman *et al.*, 1985) and the selective EP2 agonist, AH13205 (Nials *et al.*, 1991), were made up fresh in 1% $NaHCO_3$ daily. Binding parameters were estimated using the EBDA/LIGAND computer programme (Biosoft, Cambridge).

The binding of [^{3}H] PGE_2 to sections of rat kidney was of high affinity (Kd=31.4±6.5nM, n=5) and saturable. Bmax was (240± 165 fmoles/section, n=3). AH13205 did not displace [^{3}H] PGE_2 from its binding site at concentrations between 0.1nM and 3mM, n=6. AH6809 (10nM-0.3mM), displaced [^{3}H] PGE_2 binding only at high concentrations, 10µM-300µM yielded 14±3 % to 21±8 % displacement, n=4. [^{3}H] PGE_2 binding was completely displaced (n=3) with high affinity by enprostil, (concentration range used, 10fM-3µM) (Ki=0.84±0.19nM).

The results suggest that PGE_2 binding sites in rat kidney are predominantly of the EP_3 subtype. While AH6809 caused some displacment indicating that that EP_1 sites may also be present, inhibition occurred only at high concentrations of AH6809 (>10µM). Moreover, the low Ki value for enprostil provides clear evidence that the EP binding sites in rat kidney show characteristics typical of EP_3 receptors.

Acknowledgements: Enprostil was donated by Syntex Pharmaceuticals Ltd., Edinburgh. AH6809 and AH13205 were donated by Glaxo Group Research, Herts. This work was supported by the BBSRC.

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359P EFFECTS OF AH23848B ON PROSTANOID-INDUCED RELAXATION OF HUMAN MYOMETRIUM *IN VITRO*

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Human myometrium contains a variety of prostanoid receptors, both inhibitory and excitatory (Senior *et al.*, 1991, 1992). To date, no functional evidence has been provided to support the presence of EP_4 receptors in this tissue, although EP_4 receptor mRNA is expressed (Senchyna & Crankshaw, 1995). We have examined the effects of the EP_4 receptor antagonist AH23848B (Coleman *et al.*, 1994) on the relaxation of human myometrium induced by a variety of prostanoids that act predominantly at EP receptors.

Strips of human myometrium from non-pregnant, pre-menopausal donors were obtained and set-up for isometric recording as described by Fernandes & Crankshaw (1995). Strips were stimulated by cloprostenol (2 µM) in the presence of L670596 (50 nM) to prevent action at TP receptors. Concentration-effect curves to inhibitory prostanoids were obtained by cumulative addition to matched strips in the absence and in the presence of AH23848B (29 µM). The results are shown in Table 1.

The lack of effect of AH23848B on any parameter of the concentration-effect curves argues against a significant contribution by EP_4 receptors to the relaxant effects of any of the agonists tested. The order of agonist potency supports action at an EP_2 receptor (Coleman *et al.*, 1988) which is consistent with previous observations (Senior *et al.*, 1991).

Supported by the Medical Research Council of Canada.

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Table 1. Prostanoid-induced inhibition of human myometrial activity *in vitro* in the absence (CONTROL) and presence (TREATED) of 29 µM AH23848B. The relationship between the molar concentration of the agonist (C) and the effect (E) was fitted to the equation: $E = E_{max}/1 + e^{-k(\log C + pEC_{50})}$. Values are means ± SD from 4 paired experiments in all cases.

	CONTROL			TREATED		
Compound	pEC_{50}	k	E_{max}	pEC_{50}	k	E_{max}
Prostaglandin (PG) E_2	7.6 ± 0.2	3.6 ± 0.9	120 ± 50	7.5 ± 0.2	4 ± 1	94 ± 8
Misoprostol	6.6 ± 0.06	4 ± 1	100 ± 10	6.8 ± 0.1	4 ± 1	98 ± 5
16,16-dimethyl PGE_2	6.3 ± 0.7	5 ± 2	100 ± 10	6.1 ± 0.3	5 ± 2	95 ± 7
11-deoxy PGE_1	5.5 ± 0.2	4 ± 3	100 ± 20	5.8 ± 0.2	4 ± 2	130 ± 60
AH13205	5.5 ± 0.2	3 ± 1	97 ± 5	5.2 ± 0.3	3 ± 1	110 ± 20

No significant differences in any parameter between CONTROL and TREATED tissues, Student's paired t-test.

360P ANTI-INFLAMMATORY ACTIVITY IN EXTRACTS FROM THE SEEDS OF *PICRALIMA NITIDA* (FAM. APOCYNACEAE)

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The seeds of *Picralima nitida* are used in Ghanaian folk medicine for the relief of pain. Analgesic activity comparable to that of morphine has been reported for akuamine, the major alkaloid from the plant (Ansa-Asamoah & Ampofo, 1986). We have tested extracts from picralima seeds for anti-inflammatory activity. Suspensions of the dried extracts in 2% Cremophor EL (Sigma) were administered p.o. 1h before inducing carrageenan paw oedema in Wistar rats (Winter et al, 1962). Oedema was monitored at 1h intervals as percent increase in paw thickness during 6h and maximal and total (areas under time-course curves) responses over the 6h were determined.

The extract obtained by Soxhlet extraction caused a dose-dependent suppression of the oedema produced during 6h (Table 1). When 200 mg kg^{-1}, p.o. was administered 1h after induction of oedema, the maximal oedema response during 5h was significantly (n=5, P<0.05) reduced to 78.5±7.8% of the mean vehicle-treated control response. Daily administration of the extract (200 mg kg^{-1}, p.o.) to adjuvant arthritic rats (Pearson, 1956) reduced the mean knee joint swelling attained on the 23rd day to 76.2±7.1% of the mean control response (n=5, P<0.05). The methanol extract obtained after defatting the seeds with n-heptane reduced the peak carrageenan-induced paw swelling to 44.3±3.0% of the mean control response (n=5, P<0.05).

Table 1. Dose-effect relationship for the aqueous ethanol extract of picralima seeds on carrageenan-induced rat paw oedema.

	Response as % of Mean Control Value	
Treatment (mg kg^{-1})	Maximal Oedema	Total Oedema
Control	100±8.2	100±7.8
Extract (100)	87.2±2.0*	83.3±3.1*
Extract (200)	78.9±9.0*	76.9±8.1*
Extract (400)	57.5±5.2*	64.0±4.7*
Indomethacin (2.5)	57.1±8.8*	51.6±8.4*

Results given as Mean±s.e.m., n=5.
* Difference from control at P<0.05 (One way ANOVA plus Newman Keuls range test)

Picralima nitida extracts thus contain polar anti-inflammatory substances which are potentially anti-arthritic.

This work is funded by the International Foundation for Science, Stockholm, Sweden

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361P THE INHIBITORY EFFECTS OF ORG 20241 ON CLONED HUMAN MONOCYTE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE 4A

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Org 20241 is a novel compound with potential utility in the treatment of asthma (Nicholson *et al.*, 1995). It produces relaxation of airway smooth muscle and inhibition of inflammatory cell activity through a mechanism involving cyclic nucleotide phosphodiesterase (PDE) inhibition. Indeed biochemical experiments have shown Org 20241 to selectively inhibit PDE4 isoenzyme prepared both from animal and human airway smooth muscle (Nicholson *et al.*, 1995). The effects of Org 20241 on human inflammatory cell PDE4 has, however, not been examined. Consequently, the aim of the present study was to examine the effects of this compound on cloned human monocyte PDE4.

The entire PDE4A gene (2.1 Kb) was amplified from human monocyte (U937 cell line) cDNA using polymerase chain reaction methodology and checked by DNA sequence analysis. The PDE4 gene was subcloned into the expression vector pcDNA3 and transiently transfected into COS-7 cells using lipofectin. The cells were harvested 48h post-transfection and lysed in hypotonic buffer (20 mM Bis tris, 1 mM dithiothreitol, 2 mM benzamidine, 2 mM EDTA, 50 mM NaCl, pH 6.5). The extract was centrifuged and the cytosolic fraction used as source of human PDE4. Cyclic AMP PDE activity was assayed as described previously (Shahid *et al.*, 1991).

Transient transfection of COS-7 cells with the PDE4A gene produced a 5-10 fold increase in soluble cyclic AMP PDE activity. The cloned PDE isoenzyme had a high affinity for cyclic AMP (K_m: 3.1 μM) and was not affected by either cyclic GMP (1 μM) or calmodulin (1.5 μg/ml). Both Org 20241 and rolipram produced concentration-dependent inhibition of the cloned PDE4A with mean pIC_{50} values of 5.54±0.07 (n=3) and 6.30±0.11 μM (n=8), respectively. In contrast the PDE3 selective compound Org 9935 (Shahid *et al.*, 1991) was less active and produced only partial (<90%) inhibition (IC_{50} ~30 μM).

In conclusion, this study confirms that Org 20241 produces inhibition of human inflammatory cell PDE4A isoenzyme and that the level of activity is similar to that observed against human airway smooth muscle PDE4 (Nicholson *et al.*, 1995). Thus, based on these results Org 20241 may be expected to elicit both anti-inflammatory and bronchial relaxant effects in asthmatics.

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362P COMPARISON OF THE EFFECTS OF ORG 20241 AND ROLIPRAM ON CYCLIC NUCLEOTIDE PHOSPHODIESTERASE 4A EXPRESSION IN A HUMAN MONOCYTE (U937) CELL LINE

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Salbutamol and rolipram, through a cyclic AMP-dependent mechanism, have been shown to upregulate cyclic nucleotide phosphodiesterase (PDE) activity in human (U937) monocytes (Torphy *et al.*, 1992). Using an indirect method it was shown that the increase in PDE activity was largely due to enhanced expression of PDE4 isoenzyme. In the present study we have examined this aspect more directly by using the technique of quantitative polymerase chain reaction (PCR). The aim of the study was to compare the effects of PDE4 isoenzyme selective inhibitors, Org 20241 and rolipram on PDE4 expression in U937 monocytes.

U937 cells were grown in suspension culture using standard methodology and added to 1 ml of Krebs-Henseleit solution in 24 well plates (10^7 cells/well) before drug treatment. Monocytes were treated with salbutamol (10^{-8}-10^{-4} M), Org 20241 (10^{-7}-10^{-4} M) or rolipram (10^{-7}-10^{-4} M) and incubated for 2 hrs. At the end of this period cells were centrifuged (1000 g, 5 min) and washed twice with phosphate buffered saline. Total RNA was extracted (Ultraspec RNA kit, Biotecx) from the washed cell pellets and cDNA prepared (cDNA synthesis kit, Pharmacia). The cDNA was subjected to multiplex PCR with fluorescently tagged primers specific for PDE4A and the internal control gene, G3PDH. The PCR products were quantified using Fragment Manager Software on a Automated Laser Fluorescent DNA Sequencer (Pharmacia).

Salbutamol, rolipram and Org 20241 elicited time- and concentration-dependent increases in PDE4A expression. The peak response (2.8 fold increase over basal value) to salbutamol (10 μM) was observed after 2-3h treatment. Pre-incubation with propranolol (1 μM) blocked the increase in PDE4A message produced by salbutamol, reducing the maximal response to 23.4%±14.7% (n=3) at the highest concentration tested. Salbutamol was more potent than rolipram or Org 20241, the EC_{50} values being 24 nM, 3.1 μM and 42 μM, respectively. Whilst rolipram (92.4%±19.9% of salbutamol maximum, n=4) had a similar response to salbutamol, Org 20241 at the highest concentration only produced 55.1%±12.7% (n=4) of the salbutamol maximum response.

The results confirm that prolonged exposure to salbutamol or PDE4 isoenzyme selective inhibitors can enhance PDE4A expression in human monocytes. This may have implications for possible tolerance to the functional effects of these compounds in inflammatory cells. However, as indicated by the difference in the maximal responses to rolipram and Org 20241, PDE4 inhibitors are not all equieffective.

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363P THE EFFECT OF LIPOPOLYSACCHARIDE ON THE INTRACELLULAR DISTRIBUTION OF [^{3}H]-INOSITOL IN HUMAN MONOCYTIC CELLS

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Bacterial endotoxins (lipopolysaccharide, LPS) activate blood monocytes (Mø), which are important in the coordination of immune responses. The U937 cell line, when induced to differentiate by retinoic acid (RA), is a useful model for blood monocytes. The role of phospholipids in cell signalling has been extensively investigated, especially the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2). Recently a tri-phosphorylated inositol lipid (PIP_3) has been identified in activated neutrophils (Stephens *et al.*,1991). However, there is little information on the mechanisms by which LPS activates Mø and whether inositol lipids are involved. In this study, we investigated the incorporation of inositol into lipids in RA-treated U937 cells and a comparison with blood Mø.

U937 cells were grown in RPMI 1640, and incubated with 100 nM RA for 4 days prior to the experiment. Peripheral blood Mø were obtained by Histopaque density centrifugation and plastic adherence. Cells were then incubated with 10 μCi/ml [^{3}H] myo-inositol for 20 h at 37 °C, 5% CO_2 and 100% humidity. Cell aliquots ($2x10^6$/ml) were then incubated with 1 μg/ml LPS for a further 2 hours. Incubations were stopped by adding chloroform/methanol (2:1 v/v) to yield aqueous and organic phases. The inositol phosphates from the aqueous phase were separated by anion-exchange chromatography (Maslanski & Busa, 1990). Inositol lipids were separated by thin-layer chromatography (Mitchell *et al.*, 1986). Radioactivity was measured by scintillation counting.

In all cell preparations between 69 and 75 % of the total radioactivity was incorporated into the aqueous fraction. In the presence of LPS the levels of inositol phosphates in RA-treated U937 cells were increased from 9017 ± 369 cpm to 10212 ± 207 cpm and in Mø from 6343 ± 138 cpm to 7334 ± 66 cpm (all values represent the means of n = 3 ± s.d., $P < 0.05$). In parallel, the incorporation of [^{3}H]-inositol into organic compounds, especially into phosphoinositides decreased in RA-treated U937 cells from 1661 ± 37 cpm to 1427 ± 136 cpm and in Mø from 2595 ± 63 cpm to 2454 ± 54 cpm ($P < 0.05$). However, no changes were observed in the level of radioactivity incorporated into PIP_2 in either RA-treated U937 cells or Mø. In RA-treated U937 cells only, a decrease from 726 ± 57 cpm to 329 ± 153 cpm ($P < 0.05$) was observed for another inositol lipid which migrated behind the PIP_2 band, presumably PIP_3.

These data suggest that LPS-stimulation induces intracellular changes in the distribution of inositol in both Mø and RA-treated U937 cells. In addition, inositol may also be incorporated into another phosphorylated inositol lipid, possibly PIP_3, in response to LPS in human monocytic cells.

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364P A ROLE FOR PHOSPHATIDYLINOSITOL 3-KINASE-DEPENDENT p70 RIBOSOMAL S6 KINASE ACTIVATION IN PDGF-STIMULATED DNA SYNTHESIS IN BOVINE TRACHEAL SMOOTH MUSCLE CELLS

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In bovine tracheal smooth muscle cells a number of intracellular signalling pathways may regulate cell division in response to activation of both growth factor and G protein linked receptors including the activation of intracellular protein kinases such as mitogen-activated protein kinase (MAP kinase) (Malarkey *et al.*, 1995a). Both endothelin-1 (Et-1) and platelet-derived growth factor (PDGF) stimulate sustained activation of MAP kinase (Malarkey, *et al.*, 1995b), however, PDGF is 25 times more efficacious than Et-1 at stimulating DNA synthesis suggesting involvement of another signalling pathway. Growth factor activation of p70 ribosomal S6 kinase ($p70^{s6k}$) has been shown to be involved in the regulation of both protein and DNA synthesis (Lane *et al.*, 1993). $p70^{s6k}$ is believed to lie downstream of phosphatidylinositol 3-kinase (PtdIns 3-kinase) in this cascade (Chung *et al.*, 1994). We have investigated the role of this pathway in the regulation of DNA synthesis in bovine tracheal smooth muscle cells.

Bovine tracheal smooth muscle cells, passages 3-10, were rendered quiescent prior to stimulation by serum deprivation for 48 h. Agonist-stimulated $p70^{s6k}$ activity was assayed following immunoprecipitation by *in vitro* [γ-^{32}P]ATP phosphorylation of the S40 substrate peptide (Clavo *et al.*, 1994). Activity of PtdIns 3-kinase was determined in phosphotyrosine immunoprecipitates by assaying *in vitro* [γ-^{32}P]ATP phosphorylation of PtdIns to PtdIns-3-[^{32}P]phosphate. DNA synthesis was assessed by incorporation of [^{3}H]thymidine during the final 4 h of 24 h exposure to agonist. All values are expressed as mean± s.e.m.

PDGF, but not Et-1, stimulated a 20-fold increase in PtdIns 3-kinase activity in these cells. PDGF also stimulated a 15-fold increase in $p70^{s6k}$ activity which was time dependent, reaching a peak of activity at about 60 min (control = 0.015 ± 0.001; PDGF = 0.222 ± 0.024; pmoles Pi $min^{-1}mg^{-1}$ protein, n=3). This activation was abolished by the $p70^{s6k}$ inhibitor, rapamycin and the PtdIns 3-kinase inhibitor, wortmannin. In contrast, Et-1 stimulated a wortmannin-insensitive 3-fold increase in $p70^{s6k}$ activity which peaked at 10min (control = 0.015 ±0.001; Et-1 = 0.042 ± 0.002; pmoles Pi $min^{-1}mg^{-1}$ protein, n=3). PDGF stimulated a 25-fold increase in DNA synthesis (control = 2042±170; PDGF = 50627±3538; DPM/well, n=3) which was reduced >90% by both rapamycin (3977±130) and wortmannin (6600±745) whereas Et-1 only stimulated a 1.5-fold increase (Et-1 = 3416 ± 354) which was abolished by rapamycin (2028±131) but was unaffected by wortmannin (2976±258).

These results show that in bovine tracheal smooth muscle cells activation of the $p70^{s6k}$/PtdIns 3-kinase pathway appears to be an obligatory step for the initiation of DNA synthesis and may distinguish between agonists which stimulate comparable MAP kinase activities.

This work was funded by grants to Robin Plevin and Andrew Peacock from the Chest Heart and Stroke Association and National Asthma Campaign.

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365P TEMPERATURE-DEPENDENT EFFECTS OF ADENOSINE ON PRESYNAPTIC CALCIUM CURRENTS AND ACETYLCHOLINE RELEASE FROM MOUSE MOTOR NERVE TERMINALS

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Previous studies have shown that adenosine modulates presynaptic calcium currents and evoked release of acetylcholine from motor nerve terminals of rat and mouse (Hamilton and Smith, 1991; Rowan *et al.*, 1993), and that the effects of adenosine are mediated through presynaptic A_1 purinoceptors (Nagano *et al.*, 1992). However, these observations were demonstrated at non-physiological temperatures (below 30°C). Therefore, the relevance to a physiological modulatory action of adenosine on acetylcholine release at skeletal neuromuscular junctions remains uncertain. The aim of the present study was to determine the effects of adenosine on neuromuscular transmission at physiological temperature (37°C).

Conventional intracellular and extracellular recording techniques using mouse triangularis sterni nerve-muscle preparations (for details see Fatehi *et al.*, 1994) were employed to compare effects of adenosine on neuromuscular transmission and presynaptic calcium current at 20°C and at physiological temperature (37°C).

Adenosine (300 μM) decreased the average amplitude of endplate potentials by 31 ± 3% of control within 5 min exposure at 20°C (n = 4). Adenosine (300 μM) did not alter the evoked release of acetylcholine from mouse motor nerve terminals at 37°C (n = 4). However, in the presence of 10 μM erythro - 9 - (2 - hydroxy - 3 - nonyl) adenine hydrochloride (an adenosine deaminase inhibitor from Research Biochemicals Incorporated), adenosine (300 μM) reduced quantal release of acetylcholine by 25 ± 3% of control at 37°C (n = 3). Adenosine (300 μM) decreased the duration of the slow calcium current recorded extracellularly from mouse motor nerve endings by 26 ± 3% of control at 20°C (n = 4). No significant change in presynaptic calcium current was observed when 300 μM adenosine was applied exogenously at 37°C. All figures represent mean ± S.E. mean. The effects of adenosine on presynaptic calcium current and evoked quantal release of acetylcholine from mouse motor nerve terminals at 20°C but not at 37°C were significant at $P < 0.05$, using the Mann-Whitney U-test.

In conclusion, the present results indicate that exogenously applied adenosine (upto 300 μM) is not able to affect neuromuscular transmission at a mouse preparation at physiological temperature *in vitro*, probably due to enzymatic degradation (presumably by adenosine deaminases). Therefore, further experiments are required to elucidate the role of endogenous adenosine in modulating neuromuscular transmission under physiological temperatures.

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366P SELECTIVE DELIVERY OF DRUGS TO THE NERVE TERMINAL CYTOPLASM USING LIPOSOMES

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Liposomes have been investigated as a means of drug delivery for some time, but non selective uptake has been a drawback and led to various strategies for selective targeting of liposomes (Lasic, 1992). The theoretical advantage of being able to deliver substances directly to the cytoplasm is often outweighed by uncertainty over the actual destination. By virtue of its size, the mammalian nerve terminal presents unique problems of access which are not amenable to solution by microelectrode techniques. Although some use has been made of liposomes in delivering otherwise impermeant substances to the motor nerve terminal (Rahamimoff *et al.*, 1978; Silinsky *et al.* 1987), no direct visual confirmation of the selectivity of the process has been provided. We have investigated the conditions under which selective delivery of impermeant substances to the motor nerve terminal by liposome can be accomplished.

Liposomes, 60-85 nm in mean diameter, were prepared from a 7:2:1 w/w mixture of phosphatidylcholine, cholesterol and phosphatidylserine. For visualization, the contained solute was the green fluorescent dye, pyranine(1-hydroxypyrene-3,6,8-trisulphonic acid (HPTS, 35 mM). Hemidiaphragm muscles from adult male mice (n=4) were pinned in 35 mm Petri dishes, and incubated with HPTS-liposome suspension in Bretag's solution. The phrenic nerve of one of a pair of hemidiaphragms was stimulated at 1 Hz for 20 min. After stimulation, a solution of 50 μg/ml rhodamine labelled cobra venom was added to the dishes, and the tissues incubated for a further 50 min. After washing, the tissues were wet mounted on a microscope slide and viewed normally, and under u.v.light at 350-460 nm.

End plates lying in the vicinity of the phrenic nerve bundle were easily identified by rhodamine fluorescence. In stimulated tissues, accumulation of liposomes occurred in the cytoplasm of the nerve terminal, sufficient to outline its morphology which was seen to be coincident with the end plate, and of the terminal portion of axon. In unstimulated tissues, no uptake of liposomes into structures was observed.

It is concluded that specific uptake of liposomes to the terminal can occur under conditions associated with synaptic vesicle endocytosis.

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367P THE EFFECTS OF POLYAMINE FTX-3.3 AND POLYAMINE AMIDE sFTX-3.3 ON ACETYLCHOLINE RELEASE

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Polyamines found in the venom of arthropods have been shown to block some subtypes of voltage-dependent calcium channels Recently, it has been reported that a polyamine FTX-3.3, from the venom of the American funnel-web spider, *Agelenopsis aperta,* and its synthetic polyamine amide analogue, sFTX-3.3, inhibited voltage-activated calcium channels (see Scott *et al.*, 1993 for a review). The main objective of the present investigation was to compare the effects of polyamine FTX-3.3 (Blagbrough and Moya, 1994) and polyamine amide sFTX-3.3 (Moya and Blagbrough, 1994) on mouse neuromuscular transmission. It was also of interest to determine whether the effects of these substances on the evoked release of acetylcholine could be attributed to their differential effects on nerve terminal calcium currents.

Intracellular (Fatt and Katz, 1951) and extracellular (Penner and Dreyer, 1986) electrophysiological recording techniques were employed to examine the effects of FTX-3.3 and sFTX-3.3 on release of acetylcholine and presynaptic calcium currents. Presynaptic calcium currents were recorded in the presence of 3,4-diaminopyridine (400 μM) and tetraethylammonium chloride (3 mM) using extracellular microelectrodes. Experiments were carried out on mouse triangularis sterni preparations (McArdle *et al.*, 1981) at room temperature (22-25°C).

FTX-3.3 at 1 and 5 μM had no significant effect on evoked release of acetylcholine. However, at 10 μM, FTX-3.3 reduced the average amplitude of endplate potentials by 64 ± 3% of control, within 5 min. sFTX-3.3 at 10 and 50 μM did not significantly affect evoked release of acetylcholine; however, at 100 μM, sFTX-3.3 decreased the average amplitude of endplate potentials by 36 ± 2% of control, within 5 min. Neither FTX 3.3 nor sFTX 3.3 affected postsynaptic sensitivity or resting membrane potential of muscle fibres. FTX-3.3 (10 μM) reduced the average amplitude of the fast calcium current recorded extracellularly from motor nerve terminals by 55 ± 5 % of control, 5 min after exposure, while the duration of the slow calcium current was not significantly affected. sFTX-3.3 (100 μM) reduced the duration of the slow calcium current recorded extracellularly from motor nerve terminals by 50 ± 6% of control 5 min after exposure. It had little effect on the fast calcium current. The effects of these toxins on evoked release of acetylcholine and presynaptic calcium currents were significant at $P < 0.05$, using Mann-Whitney U-test. The figures represent the mean ± s.e.mean of 3 experiments.

FTX-3.3 is known as a P-type calcium channel blocker (Brown *et al.*, 1994) and sFTX-3.3 has been reported to be more selective towards low voltage-activated T-type calcium currents (Scott *et al.*, 1992). The present results show that FTX-3.3 is more potent than sFTX-3.3 at inhibiting neuromuscular transmission. This may imply that evoked release of acetylcholine is predominantly regulated by calcium currents flowing through P-type calcium channels.

The financial support of BBSRC is acknowledged.

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368P REGULATION OF ADENYLYL CYCLASE ACTIVITY BY CHRONIC ETHANOL

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We have previously reported chronic ethanol to alter both G-protein expression and cyclic AMP accumulation in intact NG108-15 cells (Williams *et al.*, 1993). The present study aimed to further investigate changes in adenylyl cyclase signal transduction using NG108-15 cell homogenates following chronic exposure of cells to ethanol.

NG108-15 cells (passage 20-40) were cultured for 7 days in DMEM containing 6% fetal bovine serum, in the presence/absence of 200mM ethanol. Cells were then harvested, washed and frozen at -70 °C. Adenylyl cyclase activity was assessed in cell homogenates using a binding protein assay (Williams *et al.*,1993).

As compared to controls, chronic ethanol treatment had no effect on the stimulation of adenylyl cyclase activity by submaximal concentrations of NECA (3μM), iloprost (30nM), sodium fluoride (10mM) or forskolin (10μM). The effects of chronic ethanol on receptor-mediated inhibition of adenylyl cyclase were next investigated via inhibition of iloprost (30nM)-stimulated adenylyl cyclase. Inhibition by the muscarinic receptor agonist carbachol (10μM) was 39.3 ± 1.2% and 31.1 ± 1.7%, for the α2-adrenoreceptor agonist UK14304 (1μM) 29.3 ± 1.4% and 20.0 ± 2.6% , and for the δ-opioid agonist deltorphin (100nM) 60.0 ± 4.5% and 47.7 ± 1.5% in control and ethanol treated cell homogenates respectively with all drug concentrations submaximal (n=3 for each, $P<0.05$ by Student's t test). Analysis of carbachol concentration effect curves revealed a decrease in maximum inhibition from 37.5 ± 4.1% to 20.9 ± 2.7% (n=6, $P<0.05$ by Student's t test) with a non-significant shift in the IC_{50} from 1.81 ± 0.5μM to 16.56 ± 8.5μM in control and ethanol treated cell homogenates respectively.

We have recently shown chronic ethanol to promote differentiation of NG108-15 cells (Kelly *et al.*, 1995). Differentiation with 0.5 mM sodium butyrate for 7 days caused no change in adenylyl cyclase activation in cell homogenates, but like ethanol reduced the ability of carbachol to inhibit iloprost-activated adenylyl cyclase activity (maximum inhibition in controls 25.9 ± 1.1% and in butyrate treated 17.8 ± 1.7%, n=6, $P<0.05$, Student's t test). In membrane competition binding assays using increasing concentrations of carbachol in the presence of 0.2nM ^{3}H-N-methylscopolamine, the displacement of the tritiated ligand was the same from membranes of control and ethanol treated cells.

These results indicate that chronic ethanol selectively inhibits receptor mediated inhibition of adenylyl cyclase activity in NG108-15 cell homogenates. This is not due to a reduction in inhibitory receptor number, but may reflect a reduction in Gi activity or expression. Furthermore this change may be the result of neuronal differentiation, since sodium butyrate produced identical changes.

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369P KININS IN BOVINE MASTITIS

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The presence of bradykinin (BK) and kininogen proteins has been reported in bovine milk (Wilson et al., 1989). Kallikrein has been also detected in bovine mammary gland (Peeters et al., 1979). Bovine mastitis is accompanied by marked oedema and tenderness in the affected quarters of the udder. Kinins are amongst the most potent known mediators of pain and oedema. In the present study we have measured kinin levels in milk from healthy and mastitic cows.

Quarter milk samples were obtained from healthy cows in which the somatic cell count (SCC) had been consistently less than 0.1×10^6 /ml milk for at least the past 3 months and there was no record of previous mastitis. Mastitic milk samples were obtained from udder quarters which had been diagnosed as clinically mastitic (milk clotting, udder hardness, and tenderness) and in which the SCC was higher than 1×10^6/ml milk and had been for the past 3 months.

The milk samples were acidified at pH 4.5 (glacial acetic acid) ultracentrifuged (75000g, 4° C, 15 min) and the bradykinin extracted with Sep-pak-vac cartridges (C18). Aliquots were radioimmunoassayed against synthetic bradykinin (Moshi et al, 1992). The mean recovery of radiolabelled Bk was 92.3 ± 1.3%. The Mann Whitney test was used for statistical comparisons. Table 1 shows that bradykinin levels in mastitic milk samples are significantly ($P=0.0202$) higher than in normal milk. Parallelism tests on the antibody binding curves showed the released kinin to be BK and not lys BK or des-Arg BK. Pathogens were only found in half of the mastitic samples. The previous 4 SCC's from the mastitic cows were all significantly raised ($p=0.001$). The presence of raised kinin levels in the mastitic milk may contribute to the symptomatology of the disease.

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Table 1. Difference in kinin levels in normal and mastitic milk samples [n=6, * represents Median (Q1, Q3)].

Milk samples	The last 4 Cell counts(x1000)	Sample Cell counts(x1000)	Pathogen isolated	Previous mastitis	Kinin levels (pg/ml)
Normals	43.6 (27.7, 59.5)	24 ± 13	(-)	(-)	444 (258,725) *
Mastitic	643 (223, 1668)	4760 ± 3550	*S.aureus*	3-cases	1111(807,1590) *

370P MANIPULATION OF DRUG METABOLISING ENZYME ACTIVITIES IN MCF 7 HUMAN BREAST CANCER CELLS

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The quinone anticancer drug, mitoxantrone (MTX), is metabolised to cytotoxic species by cytochrome P450 dependent mixed function oxidase (MFO), and may be detoxified by DT-diaphorase. It may be possible to manipulate the cytotoxicity of MTX by co-administration of a drug which inhibits the MFO system, and induces DT-diaphorase activity. To this end we investigated the effect of the flavonoids, myricetin (M), quercetin (Q) and epicatechin (E), which are widely distributed in our diet, and the antifungal agent, griseofulvin (G), on the activities of the enzymes involved in MTX metabolism in MCF 7 cells.

The drugs were added at 25, 50 and 100μM (in 0.1% dimethylsulfoxide (DMSO)) to MCF 7 cells cultured in Dulbecco's medium with 10%(v/v) foetal calf serum. They were added daily, for 3 days, from day 3 after seeding the cells, and their effect monitored in cell homogenates, prepared in 0.1M sodium phosphate buffer, pH 7.6, on day 6. None of the chemicals caused loss of cell viability (assessed by leakage of lactate dehydrogenase activity - results not shown), although 100μM Q and G caused inhibition of cell growth and/or detachment of cells (see Table 1). With the exception of E, all chemicals increased reduced glutathione (GSH) content. M increased MFO activity as measured by the O-dealkylation of ethoxyresorufin (EROD). The effect of Q is of particular interest in that it caused slight inhibition of EROD, while increasing the activities of DT-diaphorase, and glutathione and NADPH cytochrome c reductases. These properties of Q and M may prove useful in reducing the acute cytotoxicity associated with MTX.

Table 1 The effect of xenobiotic treatment on protein and GSH content, and drug metabolising enzyme activities of MCF 7 cells.

Treatment	Protein content	GSH content	EROD	DT-diaphorase	Glutathione reductase	NADPH cyt. c reductase
DMSO control	3.57 +/- 0.13	51.8 +/- 2.0	1.98 +/- 0.12	0.38 +/- 0.02	47.80 +/- 4.61	6.62 +/- 0.95
100μM M	3.97 +/- 0.12	77.3 +/- 2.6*	4.13 +/- 0.09*	0.37 +/- 0.02	33.58 +/- 6.91	6.67 +/- 0.36
100μM E	3.80 +/- 0.04	62.9 +/- 2.5	0.91 +/- 0.10	0.34 +/- 0.01	33.58 +/- 1.29	10.56 +/- 0.27*
100μM Q	1.45 +/- 0.20*	94.1 +/- 12.3*	0.86 +/- 0.22	0.54 +/- 0.02*	95.85 +/15.34*	17.45 +/- 1.10*
100μM G	2.50 +/- 0.16*	87.6 +/- 6.8*	1.96 +/- 0.64	0.65 +/- 0.08*	32.45 +/- 5.57	11.67 +/- 0.29*

Results are mean +/- s.e. mean, of 3 experiments. Protein content is mg/ml; GSH content nmol/mg protein; EROD pmol/h/ mg protein; DT-diaphorase μmol/min/mg protein and glutathione and NADPH cytochrome c reductases are nmol/min/mg protein. *$P < 0.05$, compared with DMSO control (ANOVA followed by Dunnett's test).

371P THE EFFECTS OF SELECTIVE TACHYKININ RECEPTOR AGONISTS ON HUMAN COLONIC CIRCULAR MUSCLE IN HEALTH AND INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory condition of unknown aetiology although the immune system is suspected of playing a major role. It has been proposed that neurones containing the tachykinin substance P and/or other neuropeptides are involved in regulating inflammatory and immune responses in peripheral tissues and these neurones may contribute to the abnormal inflammatory response seen in IBD (Mantyh *et al.*, 1989; Mazumdar & Das, 1992). The aim of this investigation was to determine if the responsiveness of human colonic circular muscle to tachykinins is altered in IBD.

Control specimens were obtained from the non-involved region of bowel from patients undergoing surgery for colonic cancer; n=7 (5 males and 3 females) age 66±5.4 yr. Diseased specimens, n=5 (3 males and 3 females) age 38±11 yr, were from patients with moderate to severe IBD; no differentiation has been made between Crohn's Disease and ulcerative colitis. Tissues were obtained from the operating theatre and placed immediately into ice-cold Krebs solution. The mucosa+ submucosa was removed and circular muscle strips (15x3 mm) prepared by cutting the full thickness *muscularis* in the direction of the circular muscle fibres. The muscle strips were set up in 3 ml organ baths containing oxygenated Krebs solution at 37 °C and contractions recorded isometrically.

In control and diseased tissues, neither the NK_1 receptor agonist Substance P methyl ester nor the NK_3 agonist Senktide had any contractile effects in concentrations up to 3 µM. The selective NK_2 agonist [β-Ala^8]-Neurokinin A(4-10) (β-Ala) however, produced well-maintained, concentration-dependent contractions of healthy colonic circular muscle with an EC_{50} of 13nM (range 7-16; n=7). These contractile responses were unaffected by hyoscine (300 nM), mepyramine (125 nM) and tetrodotoxin (500 nM) but were antagonised by the selective NK_2 antagonist SR 48968 (apparent $pK_B = 9$), demonstrating a direct action of β-Ala at tachykinin NK_2 receptors on the colonic smooth muscle. In contrast to other investigations (eg Guiliani *et al*, 1991), β-Ala caused rapid desensitisation in the human colon unless a 15 min interval was left between drug additions. In colonic circular muscle from patients with IBD, the EC_{50} for β-Ala was increased to 37nM (range 29-48; n=5; $p<0.002$ vs non-diseased colon). There was no change in the maximum contractile response elicited by β-Ala which was 8.0±3.0 g in healthy colon and 7.6±3.6 g in IBD. Moreover, the contractile activities of carbachol and histamine were the same in health and IBD.

In conclusion, the responsiveness of human colonic circular muscle to the tachykinin NK_2 receptor agonist [β-Ala^8]-Neurokinin A(4-10) is decreased in IBD.

This work is supported by GRID and ia.

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372P THE EFFECTS OF CAPSAICIN AND 6-HYDROXYDOPAMINE ON TNBS-INDUCED COLITIS IN RATS

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We have previously observed beneficial effects of lignocaine treatment in trinitrobenzene sulphonic acid (TNBS)-induced colitis in rats, suggesting a role for nerve input in the development of experimental colitis (McCafferty *et al*, 1994). In this study we have examined the role of capsaicin-sensitive nerves and sympathetic nerves in the development of experimental colitis in rats.

Rats were treated with 6-hydroxydopamine (6-OHDA) (300mg/kg, i.p.) as adults, with capsaicin (50mg/kg, s.c.) as neonates, or with both treatments. After treatment, colitis was induced in groups of at least 6 rats by intrarectal (i.r.) administration of 0.5 ml of TNBS (60mg/ml) in 50% ethanol. Rats were killed 5-7 days later and the colons excised and assessed for severity of colitis using previously described criteria (McCafferty *et al*, 1994). Samples of colonic tissue were also taken for determination of myeloperoxidase (MPO; an enzyme found in granulocytes) activity. In a separate experiment, colitis was induced in capsaicin-treated rats 30 minutes after lignocaine administration (10 mg/kg, i.r.). Lignocaine administration was continued daily for 7 days.

Myeloperoxidase levels in capsaicin-treated rats were not significantly (Student t-test) different from those of vehicle-treated controls with experimental colitis (55 ± 6 U/mg vs 70 ± 7 respectively). In contrast, 6-OHDA-treatment significantly raised MPO levels (132 ± 11), though combination of 6-OHDA- and capsaicin-treatment produced comparable levels to capsaicin alone (51 ± 8). Clinical damage assessment (using an arbitrary damage score) was not significantly different in capsaicin-treated rats compared to vehicle-treated controls (11.1 ± 0.9 vs 12.4 ± 0.6 respectively), however the mortality in all capsaicin-treated rats was higher than in controls (TNBS 29%; TNBS-lignocaine 40% compared to 0% in controls [non-ablated-TNBS] or 6-OHDA-TNBS). Capsaicin-treated rats also lost more weight over 7 days (-29 ± 3%) compared to vehicle controls (-7 ± 3%). 6-OHDA-treated rats had significantly less damage than vehicle controls (7.6 ± 0.2) or rats treated with 6-OHDA and capsaicin (11.8 ± 1.8). Histology showed that 6-OHDA-treated rats had a reasonably well preserved mucosal architecture compared to either capsaicin-treated rats or rats given both treatments. Lignocaine administration in capsaicin-treated rats did not significantly alter MPO levels (61 ± 7 U/mg) or damage score (12.7 ± 0.7) from levels in nonablated rats.

These data suggest that primary afferent and sympathetic nerves contribute to the pathogenesis of colitis. Primary afferent, capsaicin -sensitive nerves appear to play a protective role since some aspects of colitis are more severe when these are ablated. The beneficial effect of lignocaine in the TNBS-induced colitis model appears to be in part due to its action on primary afferent capsaicin-sensitive nerves. It also seems likely that sympathetic nerves contribute to inflammation and may interact with primary afferent nerves through as yet unknown mechanisms.

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373P BU 224, 2-BFI, CIRAZOLINE AND IDAZOXAN SHOW SPECIES DIFFERENCES FOR PUTATIVE IMIDAZOLINE SITES (I-SITES) IN KIDNEY CORTEX

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BU 224 (2-(4,5-dihydroimidaz-2-yl)-quinoline-HCl) and 2-BFI (2-(2-benzofuranyl)-2-imidazoline-HCl) are highly selective ligands for imidazoline$_2$-sites (I_2-sites) over α_2-adrenoceptors in rabbit brain (Hudson et al., 1994, 1995). These compounds also have high affinity for novel putative imidazoline sites (I-sites) in rabbit kidney cortex (Baines et al. 1995), labelled with [^{3}H]-clonidine, but distinct from I_1-sites in bovine brainstem (Ernsberger, 1992). This study compares the affinities of several ligands for these novel non-adrenergic I-sites in rat and rabbit kidney cortex.

Rat and rabbit kidney cortex membranes were prepared based on the methods of Bricca et al. (1993). [^{3}H]-Clonidine (6nM, in the presence of 10 uM noradrenaline) was used to label I-sites in kidney cortex membranes. Membranes (100 ug rabbit, 200 ug rat), [^{3}H]-ligand and inhibiting drugs were incubated in duplicate to equilibrium (45-60 min, room temperature). Non-specific binding was defined using 100 uM idazoxan (rabbit) or 100 uM clonidine (rat). Filtration was used to separate bound radioligand from free. Table 1 shows mean -logIC50 (pIC50) values ± s. e. mean.

Clonidine and rilmenidine had moderate affinity for I-sites labelled with [^{3}H]-clonidine in both rat and rabbit kidney cortex whilst adrenaline had very low affinity. BU 224, 2-BFI, idazoxan and cirazoline had high affinity for I-sites in rabbit kidney cortex but very low affinity for I-sites in rat kidney cortex. BU 224, idazoxan and cirazoline often showed biphasic inhibition curves suggesting the presence of a second site labelled with [^{3}H]-clonidine in rabbit kidney cortex.

Table 1. Mean pIC50 values ± s. e. mean (n = 3-5).

	Rabbit kidney cortex (pIC50)	Rat kidney cortex(pIC50)
BU 224	9.14 ± 0.09	5.90 ± 0.15
2-BFI	8.71 ± 0.27	6.11± 0.10
Idazoxan	8.67 ± 0.14	5.13 ± 0.04
Cirazoline	9.19 ± 0.20	5.17 ± 0.12
Clonidine	6.70 ± 0.14	6.92 ± 0.10
Rilmenidine	6.66 ± 0.10	7.39 ± 0.14
Adrenaline	< 4	< 4

These results show that BU 224, 2-BFI, cirazoline and idazoxan have over 400-fold higher affinity for novel I-sites labelled with [^{3}H]-clonidine in rabbit than in rat kidney cortex. Although the nature of these I-sites remains unclear, they are distinct from I_1-sites labelled with [^{3}H]-clonidine and its analogues as defined by Ernsberger (1992), and they differ in their pharmacological profile between species.

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374P BU 224 AND 2-BFI HAVE HIGH AFFINITY FOR TWO DIFFERENT IMIDAZOLINE SITES (I-SITES) IN THE RABBIT

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The presence of several non-adrenergic binding sites recognising various imidazolines is well established, although subclassification of these imidazoline sites (I-sites) remains unclear. BU 224 (2-(4,5-dihydroimidaz-2-yl)-quinoline-HCl) and 2-BFI (2-(2-benzofuranyl)-2-imidazoline-HCl) are highly selective ligands for I_2-sites over α_2-adrenoceptors (Hudson et al. 1994; 1995). The present study compared the affinities of BU 224 and 2-BFI for I_2-sites in rabbit cerebral cortex and for novel putative I-sites in rabbit kidney cortex.

Rabbit cerebral and kidney cortex membranes were prepared based on the methods of Bricca et al (1993). [^{3}H]-Clonidine (6 nM, in presence of 10 uM noradrenaline) and [^{3}H]-idazoxan (1 nM) were used to label I-sites in kidney and cerebral cortex membranes respectively. Membranes (100 ug), [^{3}H]-ligand and inhibiting drugs were incubated in duplicate to equilibrium (45-60 min, room temperature). Non-specific binding was defined using 100 uM idazoxan in both assays. Bound radioligand was separated from free by filtration. Mean -log IC50 (pIC50) values ± s. e. mean are shown in Table 1.

In the present study, [^{3}H]-idazoxan binding in rabbit cerebral cortex showed the pharmacology expected of an I_2-site as defined by Ernsberger (1992). [^{3}H]-Clonidine labelled different putative I-sites in rabbit kidney cortex that had higher affinity for clonidine than the I_2-sites in cerebral cortex. However, the putative I-sites described here differed from I_1-sites as defined by Ernsberger (1992) in bovine brainstem in having lower affinity for clonidine, and much lower affinity for oxymetazoline and efaroxan. BU 224, 2-BFI and idazoxan had high affinity in the present study for both I_2-sites and novel putative I-sites labelled with [^{3}H]-clonidine. In addition, BU 224 and idazoxan often showed biphasic inhibition curves suggesting the presence of a second site labelled by [^{3}H]-clonidine in rabbit kidney cortex.

Table 1. Mean pIC50 values ± s. e. mean (n = 3-5).

	[^{3}H]-clonidine Kidney cortex pIC50	[^{3}H]-idazoxan Cerebral cortex pIC50
BU 224	9.14 ± 0.09	8.71 ± 0.15
2-BFI	8.71 ± 0.27	9.07 ± 0.06
Idazoxan	8.67 ± 0.14	8.31 ± 0.17
Oxymetazoline	4.41 ± 0.11	4.67 ± 0.16
Clonidine	6.70 ± 0.14	4.67 ± 0.05
Efaroxan	4.25 ± 0.12	4.41 ± 0.05
Adrenaline	< 4	< 4

These results show that BU 224 and 2-BFI have high affinity for I_2-sites labelled with [^{3}H]-idazoxan and novel putative I-sites labelled with [^{3}H]-clonidine. We suggest that whilst BU 224 and 2-BFI show good selectivity for I_2-sites over α_2-adrenoceptors, they also show high affinity for novel putative I-sites in rabbit kidney cortex distinct from I_1- and I_2-sites as defined by Ernsberger (1992).

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375P THE EFFECT OF THE NOVEL ECTO-ATPase INHIBITOR ARL 67156 ON NEUROTRANSMISSION IN THE GUINEA-PIG ISOLATED VAS DEFERENS AND URINARY BLADDER

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ATP and acetylcholine (ACh) mediate contractions of the guinea-pig urinary bladder evoked by stimulation of the parasympathetic nerves. The larger, initial, phasic component is mainly purinergic, whilst the smaller, tonic portion is largely cholinergic. (Kasakov & Burnstock, 1983). The ecto-ATPase inhibitor ARL 67156 (6-N,N,diethyl-D-β,γ-dibromomethylene ATP, formerly FPL 67156, Crack *et al.*, 1995) enhances responses to sympathetic nerve stimulation and to exogenous ATP in the guinea-pig isolated vas deferens, but not those to the stable analogue, α,β-methyleneATP (α,β-meATP, Sneddon *et al.*, 1995). We have now compared the effect of ARL 67156 on the guinea-pig urinary bladder and vas deferens. Strips of guinea-pig urinary bladder or whole vas deferens were bathed in Krebs' solution at 37°C and bubbled with 95% O_2, 5% CO_2. Contractions were evoked by field stimulation (supramax voltage, 0.5ms pulse width) at 1-8 Hz for 20s, or by adding ATP, α,β-meATP, ACh or histamine to the bath. Data were analysed by Student's paired t-test and considered significant at $P<0.05$. In the vas deferens, ARL 67156 (100μM) significantly enhanced peak responses at 1, 2, 4 and 8 Hz from 0.33±0.07g to 0.69±0.14g; 0.55±0.11g to 0.88±0.12g; 1.32±0.20g to 2.08±0.23g, and from 2.89±0.22g to 4.00±0.45g respectively ($n=7$, $P<0.05$). In the bladder, ARL 67156 (100μM) produced a smaller, but still significant increase in peak response at 1, 2, 4 and 8 Hz from 1.03±0.14g to 1.33±0.16g; 1.33±0.18g to 1.68±0.17g; 1.91±0.41g to 2.46 ±0.53g and from 3.21±0.39g to 4.07±0.42g respectively ($n=8$, $P<0.05$). Atropine (1μM) abolished responses to ACh in the bladder and the cholinergic component of the neurogenic contraction. Under these conditions ARL 67156 (100μM) enhanced contractions at 4 Hz from 1.43±0.23g to 1.85±0.36g ($n=6$, $P<0.05$). ARL 67156 (100μM) also enhanced responses to exogenous ATP (100μM) from 0.98±0.16g to 1.63±0.18g ($n=16$) ($P<0.05$), but had no effect on those to α, β-meATP (5μM) (from 0.95±0.27g to 0.96±0.34g, $n=7$). Surprisingly, contractions to exogenous ACh (1μM) and histamine (3μM) were also potentiated by ARL 67156 (100μM) from 0.90±0.26g to 1.14±0.30g ($n=8$) and from 1.51±0.16g to 1.78±0.16g ($n=18$) respectively ($P<0.05$). The increase in response to histamine was not seen when the P_2-purinoceptor antagonist PPADS (100μM) was present (control 0.86±0.16g, 0.57±0.08g in ARL 67156) suggesting that an increase in endogenous extracellular ATP levels by ARL 67156 increases the excitability of the smooth muscle to non-purine agonists.

The results of this study show that ARL 67156 potentiates the purinergic component of neurogenic contractions in the guinea-pig isolated vas deferens and urinary bladder. However, the effect is greater in the vas deferens, suggesting that ecto-ATPases may have a greater role in modulating neurotransmission in this tissue.

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376P ENDOGENOUS $GABA_B$ RECEPTOR MODULATION OF $GABA_A$ RECEPTOR FUNCTION IN MOUSE ILEUM

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In previous studies we have shown that electrical field stimulation (EFS) of guinea-pig and mouse ileum was able to inhibit contractile responses to γ-aminobutyric acid (GABA) and the $GABA_A$ receptor agonist muscimol (Alyami *et al.*, 1990, 1991). This inhibitory action was selective and was not modified by a range of antagonists, enzymes and enzyme inhibitors. Since EFS provokes the neuronal release of GABA from ileum (Taniyama *et al.*, 1983), it is possible that endogenous GABA may mediate the observed inhibition. The present study explores the ability of the $GABA_B$ receptor antagonist CGP35348 (CGP; Bowery 1993) to protect $GABA_A$ receptor-mediated responses against the inhibitory effect of EFS in mouse ileum.

Segments of ileum taken from adult female BKW mice were suspended under 0.5g tension in Krebs' solution at 37°C and gassed with 95% O_2/5% CO_2. Increases of isometric tension were recorded in response to EFS (0.6Hz, 1ms duration, supramaximal voltage), GABA (0.3-1000μM) and muscimol (3-100μM), precautions being taken to avoid desensitisation to the agonists. Submaximal contractions to GABA (100μM) or muscimol (30μM) were elicited prior to (control) and immediately following (test) a period of EFS of 60s duration: test responses to GABA and muscimol were significantly reduced, to 40±6 and 47±2% of control respectively (mean ±s.e. mean, n ≥ 4 for each agonist throughout, $p<0.05$). CGP (10-30μM), in contact with the tissue for 5 min, was able to protect the test responses to GABA and muscimol in a concentration dependent manner: in the presence of CGP (30μM), EFS was no longer found to inhibit the test responses to GABA or muscimol (99±9, 93±11% of control respectively).

In order to establish that the protective action of CGP was mediated through antagonism at $GABA_B$ receptors, the selective $GABA_B$ receptor agonist 3-aminopropylphosphinic acid (3-APPA; Bowery, 1993) was employed. 3-APPA (0.1-10μM) caused concentration-related inhibition of responses to EFS, inhibition being maximal (59±11% of control) with 3.2μM 3-APPA. At this concentration, 3-APPA reduced submaximal responses to GABA (100μM) and muscimol (30μM) to 56±2 and 60±9% of control respectively. Incubation of the tissue with CGP resulted in concentration-dependent antagonism of all of the effects of 3-APPA: CGP (30μM) significantly reversed the inhibitory effects of 3-APPA to 98±5 and 97±8% of control for responses to GABA and muscimol respectively, and at 80μM, CGP antagonised the 3-APPA-induced inhibition of responses to EFS (to 83±8% of control).

These results support the hypothesis that GABA, released during EFS, activates $GABA_B$ receptors which in turn diminishes responses mediated via $GABA_A$ receptors.

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377P ANTAGONISTIC EFFECTS OF GLIBENCLAMIDE AND GLIPIZIDE AT β-ADRENOCEPTORS IN MOUSE ILEUM

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Glibenclamide (GBC) is widely recognised as a blocker of the ATP-sensitive potassium channels found in smooth muscle cells. We have previously reported the antagonism by GBC of the relaxant action of isoprenaline (ISO) in the mouse intestine (Yeung *et al.*, 1994). Narishige *et al.* (1994) have shown that GBC prevents coronary vasodilatation induced by β_1-adrenoceptor stimulation in dogs. The present study aims to investigate further the blocking effect of GBC and the structurally related compound, glipizide (GPZ) on the β-adrenoceptors in mouse ileum.

Mucosa-free preparations derived from the distal ileum of male BKW mice were placed in Krebs' solution under 0.5g tension (37°C, 95%, O_2/5% CO_2). Following equilibration, isometric contractions were elicited by electrical field stimulation (EFS, 0.5Hz, 30V). When consistent twitches were generated, cumulative concentration-response curves to ISO (1nM-100μM), dobutamine (DOB, 30nM-100μM), salbutamol (SAL, 30nM-300μM) or vehicle were performed. This procedure was repeated following 20 min incubation with a single concentration of GBC (0.3-1μM) or GPZ (10-60μM) or a β_1 selective blocker, atenolol (ATEN, 0.1-1μM) or their vehicles. The potencies of relaxants were expressed as mean ± s.e.mean of their pD_2 values (-log molar [EC_{50}]). The effects of blockers against ISO were expressed as dose ratios (the EC_{50} of ISO in the presence of blocker vs EC_{50} of ISO alone).

ISO was a more potent relaxant than DOB and SAL; pD_2 values 7.21±0.03 (n=57), 5.64±0.05 (n=9) and 4.81±0.04 (n=4) respectively. The relaxant effect of ISO was antagonised by GBC, GPZ and ATEN (Table 1). The relaxant response to DOB was also antagonised by GBC (μM): Dose ratios: 3.3±0.7 (0.3, n=4) and 2.7±0.3 (1.0, n=5). Neither cumulative administration of GBC (0.01-10μM) nor GPZ (1-30μM) had any effect on the response to a single concentration of SAL (20μM, giving approx. 50% maximum relaxation). There was no change in tissue sensitivity with time and none of the vehicles had any effect (n>4).

The results show that GBC and GPZ had the ability to antagonise the actions of the non-selective β-adrenoceptor agonist ISO and the β_1 adrenoceptor agonist DOB, but had no significant effect on the β_2-adrenoceptor agonist SAL. The possible involvement of β_3-adrenoceptors cannot be eliminated at this stage. The concentration ranges in which GBC and GPZ antagonise β-adrenoceptors are similar to those in which they modulate potassium channels (Yeung *et al.*, 1995).

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Table 1. Mean dose ratios for isoprenaline in the presence of glibenclamide, glipizide or atenolol (*P<0.05, **P<0.01, EC_{50} of ISO in the presence of blockers vs EC_{50} of ISO alone, Student's paired t-test)

GBC (μM)	Dose Ratio	n	GPZ (μM)	Dose ratio	n	ATEN (μM)	Dose ratio	n
0.3	2.1 ± 0.2*	4	10	3.3 ± 0.4*	4	0.1	3.8 ± 0.7*	4
0.6	5.3 ± 2.3	4	30	5.4 ± 1.1*	4	0.3	6.6 ± 0.9*	4
1.0	6.8 ± 1.8**	7	60	2.9 ± 0.9	4	1.0	4.1 ± 0.8**	6

378P SELECTIVE ANTAGONISM BY CHLORPROMAZINE AND RELATED PHENOTHIAZINES OF THE VASORELAXANT ACTIONS OF LEVCROMAKALIM

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Besides the antidiabetic sulphonylureas, a variety of chemically diverse compounds have been shown to inhibit ATP-sensitive K (K_{ATP}) channels (Edwards & Weston, 1993). Müller *et al.* (1991) recently reported that chlorpromazine and related phenothiazines (PHZs) block K_{ATP} channels in insulinoma cells. The latter finding may have clinical significance because recent evidence suggests that functional K_{ATP} channels occur in the brain and that they may be coupled to dopamine D_2 and $GABA_B$ receptors in the substantia nigra (Roeper *et al.*, 1990). In the present study, we sought to establish whether these drugs exerted a similar K_{ATP} channel blocking action in vascular smooth muscle by comparing their ability with that of the specific K_{ATP} channel blocker, glibenclamide, to antagonise levcromakalim-induced relaxations in rat isolated aorta and portal vein.

Portal veins and rings of thoracic aortae (3-4 mm; endothelium denuded) were removed from Wistar rats (250-400 g), mounted under 1 g and 2 g resting tension, respectively, in a 10 ml bath for isometric tension measurement, and superfused at 37°C with aerated (95% O_2/5% CO_2) Krebs-Henseleit solution. For the aortic ring studies, the Krebs-Henseleit solution contained indomethacin (10^{-5} M) and labetalol ($0.5\text{-}1\times10^{-5}$ M) or prazosin (10^{-5} M). The rings were precontracted to $PGF_{2\alpha}$ ($2\text{-}4\times10^{-6}$ M) either in the absence of, or following, a 20 min pretreatment with glibenclamide (10^{-6}-10^{-5} M) or phenothiazine (chlorpromazine, thioridazine or trifluoperazine, 10^{-6}-10^{-5} M), and subsequently relaxed by cumulative addition of levcromakalim (10^{-8}-10^{-5} M), sodium nitroprusside (SNP, 10^{-11}-10^{-8} M) or isoprenaline (ISOP, 10^{-8}-10^{-4} M). For the portal vein studies, the tissues were pretreated with phenoxybenzamine (10^{-6} M) for 20 min, followed by periodic washout for 1 h. Relaxation responses to levcromakalim (10^{-8}-10^{-5} M) were then evaluated in the absence of, or following, a 20 min pretreatment with glibenclamide, thioridazine or chlorpromazine (3×10^{-6}-10^{-5} M). Mean data were compared by repeated measures ANOVA followed by Dunnett's test.

Levcromakalim-induced relaxation of precontracted aortic rings was antagonised in a concentration-dependent fashion by glibenclamide and by each of the PHZs. Thus, pretreatment with glibenclamide (10^{-5} M) caused a shift in the pEC_{50} of levcromakalim from 7.18± 0.08 to 5.55±0.21 ($P<0.05$; n=5). Similarly, pretreatment with thioridazine, chlorpromazine and trifluoperazine (each at 10^{-5} M) caused a shift in the pEC_{50} of levcromakalim, from 7.59±0.16 to 5.95±0.19 ($P<0.05$; n=6), 6.79±0.15 to 5.56±0.16 ($P<0.05$; n=7) and 7.49±0.08 to 6.82±0.15 ($P<0.05$; n=6), respectively. By contrast, the PHZs failed to antagonise SNP or ISOP-induced relaxations.

Levcromakalim induced concentration-dependent inhibition of the spontaneous phasic contractile activity in portal veins and this effect was also significantly antagonised by glibenclamide and by the PHZs, thioridazine and chlorpromazine. Thus, pretreatment with glibenclamide (10^{-5} M) caused a shift in the pIC_{50} of levcromakalim from 6.93±0.27 to 5.85±0.22 ($P<0.05$; n=4). Similarly, after pretreatment with thioridazine and chlorpromazine (10^{-5} M) the pIC_{50} of levcromakalim was shifted from 6.78±0.06 to 5.71±0.09 ($P<0.05$; n=6) and 6.63±0.09 to 5.94±0.14 ($P<0.05$; n=6), respectively.

These findings suggest that the phenothiazine antipsychotic drugs are blockers of vascular K_{ATP} channels.

This work was supported by The Wellcome Trust. Levcromakalim and glibenclamide were generously supplied by SmithKline Beecham plc & Hoechst UK Ltd, respectively.

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379P ORG 5222 ANTAGONISES THE INHIBITORY EFFECT OF QUINPIROLE ON ADENYLYL CYCLASE ACTIVITY MEDIATED BY THE HUMAN D_{2L} RECEPTOR

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Neurochemical studies have shown that the antipsychotic ORG 5222 is an antagonist at both dopamine D_1 and D_2 receptors and also has pronounced anti-serotonergic properties (De Boer *et al.*, 1990). It has subsequently been shown that ORG 5222 has a high affinity for human dopamine 'D_2-like' receptors including the D_{2L} subtype (Collie *et al.*, this meeting). The aim of the present study was to characterise further the pharmacology of ORG 5222 by examining its effects on cloned human D_{2L} receptor function.

Quinpirole-induced reversal of forskolin-stimulated adenylyl cyclase activity was used as a functional assay for the D_{2L} receptor. Membranes were prepared, from a stable CHO clone expressing the human D_{2L} receptor, by homogenisation in ice cold buffer containing: HEPES (20mM, pH 7.8), sucrose (790mM) and EGTA (1mM). Adenylyl cyclase assays were carried out according to Salomon *et al*, (1974). In addition to assay buffer each tube contained 1μCi of [α-^{32}P]ATP and reaction was started with 60μg of membrane, and incubated at 32°C for 15 min. The ^{32}P-labelled cAMP generated was separated from other nucleotides by sequential chromatography on Dowex resin/aluminium oxide columns.

Forskolin (1μM) stimulated basal adenylyl cyclase activity from 4.84 ± 0.08 to 36.3 ± 3.61 $pmol^{-1}min^{-1}mg^{-1}$ (n=6). Quinpirole (0.1μM - 1mM) produced a concentration-dependent inhibition of forskolin-stimulated adenylyl cyclase activity (47.3 ± 2.1% inhibition at maximum concentration). Haloperidol (100nM), risperidone (100nM), clozapine (300nM) and ORG 5222 (100nM) antagonised the inhibitory effects of quinpirole, all producing parallel rightward shifts in the concentration-response curves. Mean pIC_{50} values ± s.e.m. (n = 3) in the absence and presence of antagonist are shown in Table 1.

Table 1. Effects of ORG 5222 on quinpirole-induced inhibition of adenylyl cyclase

Compound	Control	+ Antagonist	Shift
ORG 5222	6.27 ± 0.03	4.13 ± 0.06	138
Risperidone	6.27 ± 0.03	4.46 ± 0.20	66
Haloperidol	6.12 ± 0.14	4.35 ± 0.21	60
Clozapine	6.16 ± 0.16	5.49 ± 0.21	5

These data confirm that ORG 5222 acts as a very effective antagonist at the human dopamine D_{2L} receptor. This is consistent with the high binding affinity ORG 5222 shows for this receptor (Collie *et al.*, this meeting).

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380P BINDING PROFILE OF ORG 5222 AT CLONED HUMAN DOPAMINE D_2, D_3 AND D_4 RECEPTORS

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ORG 5222 (trans-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenz[2,3:6,7]oxepino-[4,5-c]pyrolidine maleate) is undergoing clinical evaluation as a potentially atypical antipsychotic drug. It has been shown to possess anti-dopaminergic and anti-serotonergic properties and displaced [^{3}H]-spiperone binding in rat brain membranes with high affinity (De Boer *et al.*, 1990; Broekkamp *et al.*, 1990). However, the affinity of ORG 5222 for human dopamine 'D_2-like' receptors has not been examined. The aim of the present study was to determine the binding affinity of ORG 5222 for cloned human dopamine D_{2L}, D_3 and D_4 receptors with reference to known dopamine receptor antagonists.

Stable CHO cell lines expressing human dopamine D_{2L}, D_3 receptors (single cell clones, Janssen Pharmaceutica, Belgium) and D_4 receptors (multiple pooled clones; NV Organon, Holland) were used as a source of receptor protein. Cell membranes were prepared by homogenisation (Potter 'S', 20 strokes, 800/min.) in buffer A (50mM Tris HCl, 120mM NaCl, 1mM EDTA) followed by centrifugation (900 × 'g', 10mins.). This was repeated and the resulting supernatants were centrifuged (40000 × 'g', 30mins.,) and the pellets resuspended in buffer A. Each assay tube contained 100µl [^{3}H]-spiperone (107 Ci/mmol, Amersham) 0.2nM final concentration, 100µl buffer/test drug and 300µl membrane and was incubated at 22°C for 2 hours. Incubations were terminated by rapid filtration through GF/C filters which were washed with 5 × 1ml ice-cold buffer A, dried and radioactivity counted (MicroBeta, Wallac). Non-specific binding was determined in the presence of 3µM (+)-butaclamol (D_{2L}/D_3) or 10µM clozapine (D_4).

Saturation binding:	D_{2L}	D_3	D_4
K_d (nM)	0.06	0.27	0.40
B_{max} (fmoles/mg protein)	1973	1786	556
Competition binding:	Mean $pK_i \pm$ s.e. mean (n = 3)		
ORG 5222	9.3 ± 0.22	9.2 ± 0.12	8.6 ± 0.08
Haloperidol	8.7 ± 0.12	8.4 ± 0.11	8.4 ± 0.06
Clozapine	6.7 ± 0.05	6.6 ± 0.07	7.1 ± 0.04
Risperidone	8.5 ± 0.07	8.0 ± 0.09	7.9 ± 0.19
Olanzapine	7.8 ± 0.05	7.5 ± 0.15	7.6 ± 0.06
Raclopride	8.8 ± 0.08	8.9 ± 0.04	5.5 ± 0.03

The binding data for the standard antagonists bear close similarity to published figures (Seeman & Van Tol, 1994). ORG 5222 shows high affinity for human D_{2L}, D_3 and D_4 receptors, having the highest affinity at the D_4 receptor of the compounds tested. These data confirm that ORG 5222 acts as a high affinity ligand at human 'D_2-like' receptors.

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381P EFFECT OF CLOZAPINE, ACETYLCHOLINE AND ATROPINE ON cAMP ACCUMULATION IN M_4 RECEPTOR (HUMAN) TRANSFECTED CHO CELLS

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Clozapine is an atypical antipsychotic which has affinity for muscarinic receptors (Bolden *et al.*, 1991). Clozapine has been shown to be an antagonist at M_1, M_2, M_3 and M_5 receptors. However its efficacy at M_4 receptors is uncertain. Recent studies have indicated that clozapine may have agonist characteristics at the M_4 subtype (Zorn *et al.*, 1994). We have investigated this in more detail.

Clozapine's efficacy at human M_4 receptors was compared with that of acetylcholine and atropine by examining their ability to modulate intracellular cAMP concentration. M_4 transfected CHO cells were grown in 80 cm^2 flasks until 80% confluency was reached. Cells were harvested and resuspended in fresh growth medium and seeded at 10^5 cells/well in 12 well plates, 24 hours before the assay. The cells were washed twice with serum free medium, then medium containing 0.5 mM IBMX was added to each well with or without test compound (antagonist studies). After a 30 minute incubation, test compound (agonist studies) with or without 1.0 µM forskolin was added. After incubation at 37 ℃ for 30 minutes the medium was aspirated and 1 ml of ethanol was added. 200 µl of the lysate was removed and the ethanol was evaporated from the sample. The cAMP sample was then redissolved in 1 ml assay buffer. cAMP concentration was determined by an antibody-coated tube radioimmunoassay (Immunotech, France).

In the presence of forskolin, acetylcholine inhibited cAMP accumulation (pEC_{50} of 8.7). At concentrations greater than 10^{-7}M acetylcholine stimulated cAMP accumulation. Clozapine also inhibited cAMP accumulation (pEC_{50} of 8.9), but did not cause a stimulation of cAMP accumulation at concentrations higher than 10^{-7}M. Atropine (10^{-7}M) alone increased cAMP accumulation 2 fold and was also able to block the inhibitions produced by clozapine and acetylcholine. In the absence of forskolin only the acetylcholine evoked stimulation of cAMP was observed, whilst clozapine had no effect on cAMP levels.

These findings suggest that clozapine has agonist properties at M_4 receptors. Unlike acetylcholine, clozapine does not act as a full agonist, as it is able to activate only one of the signalling pathways available to M_4 receptors (forskolin-dependent inhibition of cAMP accumulation).

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382P SCOPOLAMINE DOES NOT INFLUENCE THE ABILITY OF CLOZAPINE TO INCREASE DOPAMINE EFFLUX IN THE STRIATUM OF WISTAR RATS

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Clozapine has been demonstrated to possess agonist activity at M_4 muscarinic acetylcholine receptors *in vitro* (Zorn *et* al., 1994). Furthermore, Meltzer *et al.*, (1994) have demonstrated the ability of the muscarinic antagonist scopolamine to inhibit the ability of clozapine to increase dopamine (DA) in the striatum of awake rats, whilst scopolamine had no effect on the ability of haloperidol or thioridazine to increase dopamine release in the striatum. The composition of striatal muscarinic receptors are M_1 (77.8%) M_4 (44.2%) and $M_{2/3}$ (2.7%) (Hersch & Levey, 1995). The present study investigated the effect of a muscarinic antagonist on the ability of clozapine and other antipsychotics to enhance dopamine efflux in anaesthetised and awake Wistar rats.

In choral hydrate anaesthetized rats (290-325g), concentric microdialysis probes (3mm; Harvard) were stereotaxically implanted into the right dorsolateral striatum (coordinates (mm) from bregma: AP +0.7, L -3.2, V 6.0). The probes were perfused continuously (2 µl/min) with artificial ECF. Dopamine in the perfusate (20µl) was measured by HPLC-EC (Zetterström, *et al.*, 1985). Basal output of DA in striatal perfusate (0.10 ± 0.02 pmol/20µl, n=16) was stable for several hours. Perfusion of the striatum with clozapine (1mM) for 2 hours induced an immediate and long lasting (120 minutes) increase in DA in striatal perfusates (max. effect +350 ± 69%, n=4). Similar results were found with carbachol (100µM; max. effect +215 ± 36%). Perfusion with 100µM scopolamine abolished the increase in DA in striatal perfusates induced by carbachol. The calcium dependency of the system was tested by replacing the calcium (2.4mM) with magnesium. Under these conditions the clozapine-induced increase in DA efflux was reduced by approximately 50%. When clozapine and scopolamine (100µM) were co-perfused into the striatum an immediate and long lasting (120 minutes) increase in DA in striatal perfusates (max. effect +398 ± 101%, n=4) was noted.

In awake rats (microdialysis striatal guide canula implanted 7 days prior to dialysis experiment) haloperidol (1mgkg^{-1};s.c.) or clozapine (20mgkg^{-1};s.c.) induced a >350% increase in DA in striatal perfusates 40 min after administration. Pretreatment with scopolamine (1mgkg^{-1};s.c.) did not alter the increase in dopamine efflux by haloperidol or clozapine (n=4).

In summary the present results confirm the ability of antipsychotics to increase the efflux of DA in the striatum, but appear not to support the view that clozapine produces any agonist effect at striatal muscarinic acetylcholine receptors.

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383P BARAKOL INHIBITS STRIATAL DOPAMINE RELEASE *IN VITRO*

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Barakol is a biological active constituent of *Cassia siamea*, a plant widely used for various medical purposes in Thailand which contains the unusual tricyclic 3a,4-dihydro-3a,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene structure. In a previous study we have shown that this compound has an anxiolytic profile in the rat elevated plus maze; but the mechanisms involved are unknown (Thongsaard *et al.*, 1995). Barakol has dopamine agonist properties and the present study examined the effect of this compound on the *in vitro* dopamine release from striatal slices.

Experiments were performed using striata from male hooded Lister rats (200 - 250 g). The tissue was carefully sliced using a manually-operated brain tissue slicer and dopamine release measured using a fixed volume technique involving incubation in Krebs medium and subsequent elution from Sephadex G-10 columns (Ebstein *et al.*, 1982). Dopamine in the eluate was measured using HPLC with EC detection. Release was induced by high K$^+$(30mM) Krebs and % change in dopamine release compared between K$^+$- induced release and basal release using normal Krebs. The study consisted of two experiments. Firstly, Ca^{++} dependence of the K$^+$ stimulated release was demonstrated by replacing Krebs containing Ca^{++} with Ca^{++}- free Krebs. The second experiment compared the effects of two D_2 receptor agonists (apomorphine 10µM, quinelorane 1 µM) with barakol (0.1, 1, 10, 100 µM) in the presence of high K$^+$ (30 mM).

Dopamine release was stimulated by high K$^+$ (basal release = 0.67 ± 0.08, high K$^+$ = 3.44 ± 0.83 pmoles/µl tissue n=4 in each) and this effect was significantly reduced by removal of Ca^{++} from the incubation medium containing high K$^+$ (basal = 0.67 ± 0.25, K$^+$ but no Ca^{++} = 1.30 ± 0.39 pmoles/µl tissue n=4 in each $p<0.05$ Mann Whitney U test). The dopamine agonists also significantly decreased the K$^+$ induced release (apomorphine -43.0% n=13, $p<0.05$, quinelorane -72.7% n=12 $p<0.001$) compared to K$^+$ stimulation in the absence of drugs. Barakol also markedly decreased the response to high K$^+$ at all the concentrations tested (Figure 1).

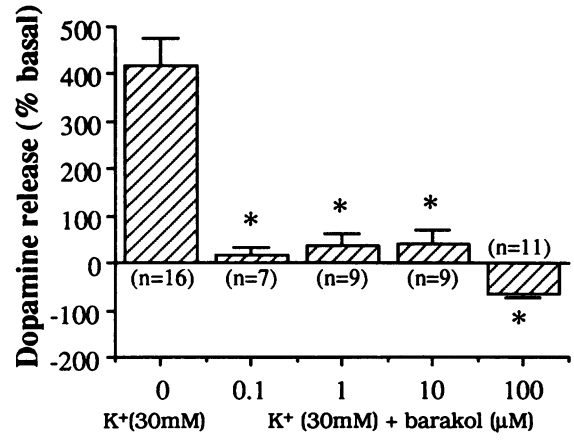

Figure 1. Effect of barakol on K$^+$ stimulated release from striatal slices *in vitro*. *p<0.001 Mann Whitney U test.

The results demonstrate that barakol is a potent inhibitor of K$^+$ stimulated dopamine release *in vitro*. Barakol may cause inhibition by a dopamine D_2 receptor action similar to apomorphine and quinelorane. However as the degree of inhibition was almost complete over a wide concentration range (0.1, 1, 10, 100 µM) another mechanism may be involved.

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384P THE INFLUENCE OF 5-HT ON DOPAMINE SECRETION FROM SLICES PREPARED FROM RAT NUCLEUS ACCUMBENS

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Previous studies suggest that the secretion of dopamine (DA) from terminals in the caudate/putamen (CP) of the rat can be stimulated by 5-hydroxytryptamine (5-HT) acting at presynaptic 5-HT_2 and 5-HT_3 receptors (Chen et al 1991, Ugedo et al 1989). The primary objective of this study was to explore the putative role of these receptors in 5-HT secretion from DA terminals in the nucleus accumbens (NAc).

Brain slices (0.3mm x 0.3mm) were prepared from the NAc and, in comparative experiments, the CP of male Sprague Dawley rats, (~350g), and incubated in HEPES-buffered Krebs solution (pH 7.4) for 90 minutes with [^{3}H]DA (5 x 10^{-8}M). The slices were then superfused with Krebs solution using a procedure similar that described by Winch and Balfour (1988). After a 40 minute washout period, 15 x 2 min samples were collected and assayed for tritium. The first 6 samples were used to measure baseline release; 5-HT or KCl (20mM) was added during the collection of samples 7-10. The addition of KCl increased tritium release in a Ca^{2+} dependent way from both CP and NAc slices ($F(14,84)=14.4$; $P<0.001$ and $F(14,84)= 34.3$; $P<0.001$ (n=4) respectively). The addition of 5-HT (10^{-8} to 10^{-6}M) also increased tritium overflow from slices from CP ($F(42,168)=7.5$; $P<0.001$) and NAc ($F(42,168)=13.5$; $P<0.001$). In both areas of the brain, the maximum response was observed following exposure to 10^{-7}M 5-HT, the peak response, when expressed as a percentage of the mean baseline release being 1478 ± 378% for CP slices and 1639 ± 103% for slices prepared from the NAc. Further studies performed with slices prepared from the NAc showed that the response to 5-HT (10^{-7}M) was abolished if the experiments were performed in Ca^{2+}-free medium containing EGTA (2.25mM). The effects of 5-HT on DA overflow from NAc slices was unaffected by the inclusion in the superfusion medium of a compound, nomifensine (10^{-5}M), which competes for the substrate binding site on the presynaptic DA transporter. The response to 10^{-8}M 5-HT, however, was reduced ($P<0.05$) from a peak increase of 1286 ± 120% to 806 ± 141% (n=4) by the inclusion of granisetron (10^{-7}M) and from 1556 ± 218% to 944 ± 133% ($P<0.001$; n=6) by the inclusion of ketanserin (10^{-6}M) in the medium used to superfuse the slices. A higher concentration of granisetron (10^{-6}M) had no significant effects on the increase in DA secretion evoked by 5-HT. When tested together, the inclusion of granisetron (10^{-7}M) and ketanserin (10^{-6}M) in the superfusion medium seem to exert an additive effect resulting in total abolition of the response to 5-HT (10^{-8}M). The results suggest that DA secretion from terminals in NAc of rat brain can be stimulated by 5-HT acting, possibly synergistically, through presynaptic 5-HT_2 and 5-HT_3 receptors.

The authors gratefully acknowledge the financial support of the Wellcome Trust and the gift of granisetron from SmithKline Beecham Pharmaceuticals

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385P MATERNAL COCAINE ADMINISTRATION IN THE RAT: ALTERED DOPAMINE FUNCTION IN THE OFFSPRING

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Recent literature suggests that cocaine abuse during pregnancy can complicate pregnancy and adversely alter the course of fetal development in animals and humans (Woods *et al.* 1987; Chasnoff *et al.* 1992), with consequential behavioural alterations. The mesolimbic dopamine system is a critical neurobiological substrate for drugs of abuse and we have previously shown (Giustino and Marsden, 1995) that the response to cocaine in the young-adult rat was reduced by maternal cocaine treatment. In the present study we have further investigated the effects of perinatal cocaine treatment on mesolimbic DA function following K^+ as well as physiological (tail pinch) stimulation. The responsiveness of the DA system was assessed using *in vivo* microdialysis to monitor extracellular DA in perinatally treated offspring.

Primiparous female rats (Lister Hooded) weighing 200-230 g, were paired with adult male rats and day 1 of gestation (GD1) was designated as day of copulatory plug detection. Gravid dams were randomly assigned to either the saline or cocaine group and given 0.9% saline (vehicle) or cocaine (20 mg/kg/sc) on GD10 and then every other day until the weaning age of the pups (25 days old) when only male rats were selected and housed in group of 5 pups per cage. 3 to 4 weeks after weaning pups of both maternal saline and cocaine pretreated rats were implanted stereotaxically with a microdialysis probe into the right nucleus accumbens under halothane anaesthesia. The animals were then returned to their home cage for at least 20 hrs with food and water available and the probe continuously perfused with artificial CSF (aCSF) at 1ul/min. The next day basal samples were collected before the probe was perfused with aCSF containing 60 mMK^+ and a further 5 samples collected. In another group of rats basal samples were collected before a paper clip was applied for 3 min to the tip of the rat's tail and further samples dialysis collected. Extracellular DA was determined by high performance liquid chromatography with electrochemical detection and the amount of DA released was expressed as fmols/10ul.

The results show a significant ($p<0.05$ Duncan's test) difference in the basal extracellular DA levels between rats given perinatal saline (45.3 ± 1.6, n=12) or cocaine (32.4 ± 1, n=12). There was also a significant difference in DA release following K^+ stimulation. In the offspring of mothers given saline DA increased from 42.2 ± 2.2 to 99.9 ± 8.9 ($p<0.05$ Duncan's test) while in the cocaine group the increase was from 27.25 ± 0.99 to 65.61 ± 4.14 ($p<0.05$ Duncan's test) but there was a significant difference between the response between the two groups ($F=6.96$; $p<0.05$ ANOVA). Application of tail pinch, to activate mesolimbic DA function, significantly increased DA release compared to basal levels ($p< 0.05$ Duncan's test) in both saline and cocaine pretreated rats and there was no significant difference in the degree of the response between the two groups ($F=0.78$ $p=0.37$ ANOVA).

The results demonstrate that maternal cocaine causes a reduction in basal and K^+ stimulated DA release in the young-adult offspring indicating a prolonged change in pre-synaptic mesolimbic dopaminergic function. In contrast the increase in DA release produced by tail pinch was not altered by maternal cocaine. It remains to be determined if this represents a functional difference in response between the two forms of stimulation or an alteration in some other function, such as pain sensitivity, influencing the tail pinch response. The results however support the view that maternal cocaine can produce long-term changes in DA function in the offspring.

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386P COMPARISON OF 'TYPICAL' AND 'ATYPICAL' ANTIPSYCHOTICS IN THEIR ABILITY TO INHIBIT CHLORPROMAZINE-INDUCED CATALEPSY IN MALE WISTAR RATS

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We have compared, olanzapine, risperidone, seroquel and AJ 76 with 8-OHDPAT, clozapine, haloperidol, ketanserin, (-)-sulpiride, and thioridazine in their ability to inhibit catalepsy, induced by chlorpromazine, in rats.

Male Wistar rats (100-130 g) were injected s.c. with test compound or its vehicle, 10 min. prior to an injection of chlorpromazine (14 $mg.kg^{-1}$ s.c.). Catalepsy was assessed 2 hours after the injection of chlorpromazine by a battery of six tests: as described by Broekkamp *et al.* (1988). This gave an arbitrary score with a maximum of 6.0. Inhibition of catalepsy was tested for statistical significance by means of a 2 way ANOVA followed by Newman Keuls.

Chlorpromazine caused a robust cataleptic score of 3.52±0.05 (mean±s.e. mean, n=20). When compounds were tested for their ability to induce catalepsy, only haloperidol at the highest dose tested, (1 $mg.kg^{-1}$), induced a catalepsy score comparable to that for chlorpromazine. No other compound produced catalepsy at the doses tested. Haloperidol (0.02-1 $mg.kg^{-1}$), ketanserin (1-10 $mg.kg^{-1}$), and (-)-sulpiride (0.1-5 $mg.kg^{-1}$) did not significantly inhibit the chlorpromazine induced catalepsy at any of the doses tested. AJ 76 (1-10 $mg.kg^{-1}$), significantly inhibited chlorpromazine induced catalepsy at all doses tested. Thioridazine (10-30 $mg.kg^{-1}$) significantly inhibited the catalepsy at 10 and 20 $mg.kg^{-1}$ but failed to prevent catalepsy at higher doses. 8-OHDPAT (0.05-5 $mg.kg^{-1}$) inhibited catalepsy at all doses up to 0.5 $mg.kg^{-1}$ but did not inhibit it at higher doses. Olanzapine (0.02-5 $mg.kg^{-1}$) and risperidone (0.022-0.8 $mg.kg^{-1}$) only inhibited catalepsy significantly within a narrow dose range (0.16-0.25 and 0.16-0.48 $mg.kg^{-1}$ respectively). Clozapine (2.2-60 $mg.kg^{-1}$) and seroquel (10-150 $mg.kg^{-1}$) significantly inhibited catalepsy at 12-60 and 30-150 $mg.kg^{-1}$ respectively. In all cases the compounds inhibited chlorpromazine induced catalepsy to a similar degree (70-80% maximum), however the dose range at which this occurred varied markedly between compounds.

These findings suggest that the inhibition of catalepsy is a more powerful tool for predicting 'atypical' antipsychotic activity than testing for the induction of catalepsy alone. Although both risperidone and clozapine did not induce catalepsy in rats, clozapine inhibited chlorpromazine induced catalepsy over a wider dose range than risperidone. Unlike clozapine, risperidone has been found to induce dystonias in primate studies (Casey, 1993) and in man (Marder & Meibach, 1994). Such activity may have been predicted using the rat paradigm outlined above.

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387P INHIBITION OF TRANSMITTER RELEASE BY INHIBITORY G PROTEIN-COUPLED RECEPTOR AGONISTS IN THE HIPPOCAMPAL CA1 REGION IS REDUCED BY TEA

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Adenosine A1, $GABA_B$, and μ opioid receptor activation inhibits transmitter release from both excitatory and inhibitory nerve terminals (Thompson *et al,* 1993). However, the mechanism by which this occurs is as yet unclear. Inhibitory G protein-coupled receptors are known to affect a number of ionic conductances, including activation of several potassium currents (North, 1989) and inhibition of calcium currents (Dolphin, 1995). We have used tetraethylammonium (TEA) to examine the possible role of potassium currents in inhibition of transmitter release by adenosine, $[met]^5$-enkephalin (met-enkephalin) and the $GABA_B$ receptor agonist baclofen.

We used adult male Wistar rats. Recordings were made from the hippocampal CA1 region in an *in vitro* slice preparation at 30°C. Excitatory postsynaptic potentials (EPSP's) evoked by focal stimulation of the Schaffer collateral-commisural pathway were recorded using extracellular DC recordings from stratum radiatum. Inhibitory postsynaptic currents (IPSC's) evoked by direct focal stimulation of interneurones were recorded in the presence of CNQX (10μM) and AP5 (30μM) using whole-cell patch clamp.

The fast, CNQX-sensitive, EPSP was depressed by 67±1% by a submaximal concentration of adenosine (30μM, n=12). Previous investigations have shown the site of action of adenosine in this region to be presynaptic (Thompson *et al,* 1993). TEA (10mM) significantly reduced the adenosine-induced inhibition of the fast EPSP (Table 1). Baclofen (10μM) similarly inhibited the EPSP by 61±3% (n=9), and this effect was also sensitive to TEA (10mM, Table 1). The fast, bicuculline-sensitive, IPSC was depressed by 87±2% by baclofen (10μM, n=3), and depressed by 67±4% by met-enkephalin (10μM, n=4). Again, the effect of both of these agonists was inhibited by TEA (10mM, Table 1).

These data suggest that inhibition of both excitatory and inhibitory transmitter release in the hippocampal CA1 region is at least partially mediated by a TEA-sensitive mechanism. It is not, however, sensitive to extracellular barium (see Thompson *et al*, 1993). We suggest that activation of a potassium current may be involved in inhibition of transmitter release by G protein-coupled receptor activation.

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Table 1: % reduction of agonist effect by TEA

EPSP Adenosine (30μM)	EPSP Baclofen (10μM)	IPSC Baclofen (10μM)	IPSC Met-enkephalin (10μM)
55±5 (4)*	74±9 (4)*	31±2 (3)*	38±1 (4)*

Data are mean ±standard error of the mean. * indicates a significant difference from control at $p<0.01$ (paired Student's t test)

388P KAINATE MAY INDUCE HIPPOCAMPAL PURINE RELEASE VIA NON-NMDA RECEPTORS AND FREE RADICALS

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Since activation of A1 adenosine receptors can reduce the neuronal damage produced by kainic acid (MacGregor *et al.* 1993) we have tried to determine whether kainate will itself induce adenosine release *in vivo*.

Using *in vivo* microdialysis in the hippocampus of male Wistar rats, anaesthetised with urethane, the concentration of extracellular adenosine, analysed by h.p.l.c., was estimated to be 1μM during the first two hours after insertion of the dialysis probe.

Kainic acid (0.1-25mM) in the perfusate (artificial csf), at a flow rate of 2μl/min, evoked a concentration-dependent release of adenosine with an EC_{50} of 0.94mM. A 5min pulse of 1mM kainic acid in the perfusate, during a sampling period of one hour, increased the 20μl dialysate levels from 3.68 ± 0.21 to 7.66 ± 0.82 pmol (mean ± sem, n=10). A second stimulation (S2) 3hrs after the first (S1) also induced adenosine release with an S2:S1 ratio of 0.52 ± 0.03, n=9. Kainate-evoked release of adenosine probably involves the production of action potentials since tetrodotoxin (10μM) significantly reduced the S2/S1 ratio to 0.24±0.05 (by 54%, n=4, p<0.01 ANOVA, Dunnett's t-test). The release was reduced by incorporation into the perfusate of CNQX 4.5μM (6-cyano-7-nitroquinoxaline-2,3-dione), a non-NMDA receptor blocker (S2/S1=0.04±0.03, n=4, p<0.01), but not by the NMDA receptor blockers dizocilpine (100μM) or (±)-AP-5 ((±)-2-amino- 5- phosphonopentanoic acid (1mM), indicating a non-NMDA receptor mediated process. The kappa opioid agonist, U50,488H (100μM), significantly reduced the S2/S1 ratio to 0.23±0.02 (by 56%, n=5, p<0.01). Release was reduced significantly by 44% by ascorbic acid (10mM) (an antioxidant, S2/S1=0.29±0.04, n=4, p<0.05), 48% by glutathione (10mM) (a scavenger of hydroperoxides, S2/S1=0.27±0.06, n=4, p<0.05) and 71% by oxypurinol (1mM) (a xanthine oxidase inhibitor, S2/S1=0.15±0.04, n=4, p<0.01) suggesting the involvement of free radicals in kainate-evoked adenosine release. Neither the adenosine A1 receptor antagonist CPT (100μM) (8-cyclopentyl-1,3-dimethylxanthine) nor the A1 receptor agonist R-PIA (10μM) (R(-) N^6-(2-phenylisopropyl) adenosine) affected kainate-evoked release of adenosine. This indicates that activation of A1 receptors, by endogenous adenosine or an agonist, does not inhibit kainate-evoked release of adenosine. A 5min pulse of 100mM KCl elevated dialysate adenosine levels from 3.6 ± 0.2 to 6.26 ± 0.58 pmol/20μl. Potassium-evoked release was not affected by CNQX (4.5μM), indicating that potassium-evoked release of adenosine is not mediated by activation of non-NMDA receptors.

It is concluded that kainic acid does induce release of adenosine from the hippocampus *in vivo*, an effect mediated by non-NMDA receptors. The mechanism of release may involve the formation of free radicals.

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This work was funded by The Scottish Home and Health Dept.

389P PURINE EFFECTS ON THE PROLIFERATION OF TM4 CELLS IN CULTURE

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Sertoli cells are considered to be the main targets of follicle stimulating hormone, and they in turn are able to modify the responsiveness of Leydig cells to human chorionic gonadotrophin 1. In view of recent evidence that purines are able to affect the growth rate and development of cells in a variety of tissues, we have now examined the effects of adenosine, and A1 receptor selective agonists and antagonists, on cells of the TM4 line (derived from mouse Sertoli cells) in culture.

TM4 cells were taken through a series of passages at 6 day intervals, using Dulbecco's modified Eagles medium and Hams F12 medium (1:1 v/v) supplemented with 7.5% foetal calf serum and 0.01% gentamicin. Cells were resuspended by trypsinisation and plated at a density of approximately 10,000 cells ml^{-1}. Once attached, the cells were washed by replacing the culture medium after at least 4 hours with either control or drug-containing medium. The cultures were maintained at 37° C in an atmosphere of 95% O_2 / 5% CO_2. Cells were counted using a haemocytometer. Data are presented as mean ± s.e.mean and were subjected to analysis of variance followed by a Student-Newman-Keuls test.

By 6 days after plating, adenosine inhibited the proliferation of TM4 cells by 32% ± 2.2 at 1μM, 49% ± 2.7 at 10μM, 74% ± 1.6 at 100μM and 92% ± 0.3 at 1mM (n=4 plates in each case, P<0.001). Caffeine, at the same range of concentrations, had no effect on cell proliferation, but when present in equimolar amounts, it prevented the inhibitory action of adenosine. The A1 receptor selective agonist cyclohexyladenosine (CHA) inhibited cell proliferation by 35.9% ± 0.8 at 1 μM, and 84.7% ± 1.0 at 1mM (n=4; P<0.001) after 6 days, as did the A2 receptor agonist N^6-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)adenosine (DPMA) (inhibition by 21.2% ± 1.3 at 1μM and by 76% ± 1.9 at 1mM; n=4, P<0.001).

Treatments of the cultures with equimolar concentrations of 8-cyclopentyltheophylline or 3,7-dimethyl-1-propargylxanthine were both able to prevent the inhibitory activity of adenosine.

It is concluded that activation of either A1 or A2 purine receptors are able to suppress proliferation of TM4 cells in culture.

390P LAMOTRIGINE FAILS TO ALTER VERATRIDINE OR K^+-EVOKED DOPAMINE AND 5-HT RELEASE IN THE RAT VENTRAL HIPPOCAMPUS AND STRIATUM *IN VIVO*

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A decrease in brain amine content has been shown to lower seizure thresholds, indicating the importance of central neuronal catecholaminergic activity in clinical epilepsy (Barsa & Kline, 1955). Lamotrigine (LTG) is a novel anticonvulsant believed to inhibit Type IIa Na^+-channels in a voltage- and use-dependent manner (Xie *et al*, 1994), and consequently is highly effective at blocking glutamate and aspartate release predominantly (Meldrum & Leach, 1994). In view of the involvement of aminergic systems in the pathophysiology of epilepsy, surprisingly little is known of the effects of LTG on these systems.

In the present study we have addressed this issue using *in vivo* microdialysis to monitor extracellular monoamine levels under depolarizing conditions following LTG administration. Male Wistar rats (250-300g) were implanted with microdialysis probes in the ventral hippocampus (H) or striatum (S), and dialysis was performed the following day. 50μM veratridine or 100mM K^+ (S1) was infused via the probes for 30min following the collection of 4x30min basal samples. At t=180min, LTG (isethionate salt, 20 & 40mg.kg^{-1}) or vehicle was injected i.p.. At the higher dose the animals became ataxic. Then at t=270min, veratridine/K^+ (S2) was infused for a second 30min, before collecting a final 4x30min samples. Samples were analysed for DA and 5-HT content by HPLC. Basal concentrations were:54.7±9.4(H), 298±18.3(S) (DA) and 103±13.2(H), 37.8±6.80(S) (5-HT) fmols.10μl^{-1} dialysate ± s.e.mean. The results indicate that LTG failed to significantly alter evoked release of DA or 5-HT (Table 1).

The lack of an effect observed here on monoamines is additional evidence supporting a selective action for LTG on amino acids.

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SA holds a BBSRC CASE Studentship with Glaxo-Wellcome.

Table 1: The effect of LTG on extracellular DA and 5-HT release. Figures represent ratio of % of basal release (Sample 12/Sample 6).

TREATMENT		HIPPOCAMPUS		STRIATUM	
		DA	5-HT	DA	5-HT
Veratridine -	(Control)	0.74±0.14	0.59±0.10	0.85±0.26	0.25±0.03
+LTG	(20mg.kg^{-1})	0.80±0.07	0.60±0.06	0.65±0.31	0.28±0.06
	(40mg.kg^{-1})	0.78±0.07	0.63±0.17	0.71±0.13	0.21±0.08
K^+-	(Control)	0.86±0.20	0.69±0.15	0.93±0.14	0.45±0.06
+LTG	(20mg.kg^{-1})	0.75±0.10	0.67±0.36	0.89±0.29	0.41±0.08
	(40mg.kg^{-1})	0.84±0.23	0.73±0.15	0.84±0.23	0.43±0.10

S2/S1 ratio (mean ± s.e.mean; n=6)

391P THE EFFECT OF 2-, 8- AND 21-DAY TREATMENT WITH GABA-T INHIBITORS ON $GABA_A$, $GABA_B$ AND FLUNITRAZEPAM BINDING TO RAT CRUDE SYNAPTIC MEMBRANES

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Administration of Vigabatrin (GVG) or ethanolamine-O-sulphate (EOS), mechanism based inhibitors of GABA-transaminase (GABA-T), leads to increases in brain GABA content and it is thought that this is the basis of their anticonvulsant activity. Chronic alterations in neurotransmitter content within the mammalian brain have been reported to produce changes in receptor binding (Creese and Sibley, 1981). The effect of 2, 8 and 21 day GVG and EOS administration on $GABA_A$, $GABA_B$ and flunitrazepam (FNZ) binding has been studied to determine any changes in binding occurring due to enhanced receptor stimulation following an elevation in brain GABA.

27 male Wistar rats (273 ± 10g) were divided into 3 groups, receiving in their drinking water: 3g/l GVG, 3g/l EOS, with controls receiving the vehicle (1g/l sucrose). At each time point, 3 animals from each group were sacrificed and whole brain crude synaptic membranes prepared (Zukin *et al.*, 1974) and frozen. The samples were thawed, washed thoroughly and resuspended in appropriate buffer. Total and non-specific $GABA_A$ and $GABA_B$ binding was determined by incubation with 5nM ^3H GABA using isoguvacine or (-)baclofen as displacing ligands, and total and non-specific FNZ binding by incubation with 1nM ^3H FNZ in the presence or absence of 10μM FNZ.

Animals dosed with GVG and EOS received 172±30 and 274±25mg/kg/day respectively. Specific $GABA_A$ and FNZ binding was unaltered compared with control throughout the time period (2 day control values (mean ± SEM): $GABA_A$ 807 ± 179fmol/mg prot and FNZ 683 ± 112fmol/mg prot). $GABA_B$ binding was unaltered from control values following 2 and 8 day treatment. However 21 day treatment with GVG led to a significant ($p<0.05$) reduction of 50% in $GABA_B$ binding compared with control (218 ± 27fmol/mg), with no change occurring following EOS treatment.

These results indicate that perhaps $GABA_B$ receptors may be involved in the development of the tolerance to chronic (17 days treatment) GVG administration to experimental animals reported by various authors (Loscher and Frey, 1987; Neal and Shah, 1990).

We acknowledge the MRC for research funding and are grateful to Merrell Dow for the gift of GVG.

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392P MUSCIMOL-INDUCED LONG-TERM DEPRESSION AND ITS REVERSAL IN HIPPOCAMPAL SLICES

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Application of the $GABA_A$ agonist muscimol to hippocampal slices induces a form of long-term depression (LTD) which is not dependent on the activation of glutamate receptors, but which is reversed by stimulation at 1Hz (Akhondzadeh & Stone, 1995). We have now examined the need for calcium in this phenomenon.

Hippocampal slices, 450μm thick were prepared from adult male Wistar rats (170-220g) and maintained in artificial CSF of composition (mM) NaCl 115; KCl 2; KH_2PO_4 2.2; $MgCl_2$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25; D-glucose 10, gassed with 95%O_2/5%CO_2. Individual slices were transferred to a recording chamber and superfused with medium at 30°C. Stimulation was effected in stratum radiatum (0.2ms, 50-200μA, 0.01Hz), with recordings being made from stratum pyramidale.

Muscimol at 10μM for 10min induced LTD even when applied during superfusion with nominally calcium-free medium (4 slices) and with the additional presence of EDTA 1mM (4 slices). The depressions were reversed by stimulation at 1Hz as in control slices. Addition of kainic acid or N-methyl-D-aspartate at 5 μM were both able to reverse muscimol-induced LTD, as were additions of potassium (5mM) or veratrine (4μM) (4 slices for each protocol). LTD was not reversed by raising the calcium content of the perfusing medium to 10mM, but was reversed slowly by the ionophore A23187. Raising the potassium level led to the reversal of LTD even when introduced in the absence of calcium (4 slices each).

The results confirm the ability of muscimol to induce LTD in hippocampal slices but reveal that, in contrast to several previous reports of glutamate-receptor-dependent LTD, this form of depression does not appear to require an influx of calcium from the extracellular space. The reversal of muscimol-induced LTD by amino acids and other agents exclude the need for activation of a specific receptor, but suggest reversal can be accounted for by a common mechanism of depolarisation accompanied by an influx of sodium or calcium. The failure of high calcium to induce reversal would suggest that calcium influx for reversal would be through voltage-dependent channels.

S.A. is grateful to the secretary for Health and Medical Education I.R.Iran for support.

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393P INFLUENCE OF $GABA_B$ RECEPTOR ANTAGONISTS ON PENTYLENETETRAZOL-INDUCED KINDLING IN MICE

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Pentylenetetrazol (PTZ) administered chronically in rodents induces kindling which is a recognized model of chronic epilepsy (Lothman and Williamson, 1994). This procedure also impairs shuttle-box learning indicating a possible modulation of memory storage (Becker *et al.*, 1994). It has recently been reported that $GABA_B$ receptor antagonists can improve cognitive performance in rodents and primates (Mondadori *et al.*, 1993). Thus, as a preliminary to examining the effects of these drugs on learning behaviour in kindling we have examined them on the induction of PTZ kindling in mice. PTZ at a subconvulsive dose (40 mg/kg s.c) was injected into male mice (20g) at 48 hours intervals for 8 weeks. Prior to each PTZ injection (40 min) the mice were injected i.p. with CGP 36742 (3-amino-propyl-n-butyl-phosphinic acid 10 mg/kg), CGP 56433 ([3-{1-(S)-[{3-(cyclohexylmethyl) hydroxy phosphinyl]-2-(S) hydroxy propyl] amino] ethyl]benzoic acid 1 mg/kg), CGP 61334 ([3-{[3-[(diethoxymethyl)hydroxy phosphinyl] propyl] amino}methyl]-benzoic acid 1 mg/kg) or saline (0.1 ml/10g) (8 mice per group). The behaviour of each animal was observed for 60 min following the PTZ injection at weekly intervals and the seizure intensity evaluated using a scale from 0 to 5 (Becker *et al.* 1994). Mean values were compared by ANOVA.

Seizure intensity increased progressively in control mice reaching by 8 weeks a mean (± s.e. mean) score of 3.3+0.2 which corresponded to clonic seizures. The $GABA_B$ antagonists suppressed kindling during the first 4 weeks with mean score all below 50% of control values. By week 4 when the control group score was 1.7+0.2, the mean values for the antagonists group were below 0.9. However, by week 5 the levels of kindling in all groups were identical and during the subsequent weeks the antagonist groups all tended to score higher than the controls. At week 6, 7 and 8 the CGP 56433 group scored significantly higher ($p<0.05$) than the saline group and by week 8 the CGP 61334 group was also exhibiting a significantly higher value than control.

The mechanism(s) underlying this biphasic effect is not clear. We have previously observed an up-regulation in $GABA_B$ receptors in rat olfactory cortex and spinal cord following 3 weeks chronic administration of $GABA_B$ antagonists (Malcangio *et al.*, 1995). This might be responsible for the reduction in kindling observed during the first 4 weeks in the present study. However, the status of $GABA_B$ receptors after 6 weeks treatment is not known. If the level of kindling reflects the degree of learning impairment then we might expect to observe a biphasic pattern in learning behaviour during the 8 week period of $GABA_B$ antagonist administration.

D. Getova is a Wellcome Travel Fellow.

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394P EFFECTS OF WITHDRAWAL FROM CHRONIC ETHANOL TREATMENT ON SPONTANEOUS FIRING IN RAT VENTRAL TEGMENTAL AREA SLICES

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The mesolimbic dopaminergic pathway is involved in reward mechanisms of drugs of abuse, including alcohol (Samson & Harris, 1992). However, the involvement of this pathway in the hyperexcitability syndrome that results from withdrawal from chronic ethanol intake is unclear. In the present study, single unit recordings were made from neurones in ventral tegmental area (VTA) slices from rats which were made physically dependent on ethanol.

Male Wistar rats (100-150 g) were fed a liquid diet of 2 days control diet, 2 days diet with 3.5 % ethanol, and 10 days diet with 7 % ethanol. A parallel control group was fed isocalorifical control liquid diet. At the end of the ethanol treatment coronal slices of the ventral midbrain were prepared; the time of preparation (at the end of the dark period of the daily light/dark cycle) was taken as the time of withdrawal from ethanol. The slices were placed in an interface chamber and superfused with artificial celebrospinal fluid of the following composition (in mM): NaCl 124, KCl 3.25, KH_2PO_4 1.25, D-glucose 10, $NaHCO_3$ 20, $CaCl_2$, 2, $MgSO_4$ 2, gassed with 95 % O_2 / 5 % CO_2. Recordings were made from dopamine-sensitive neurons using glass micropipettes filled with 2 M NaCl, resistance 2 to 5 MOhm. Dopaminergic cells were identified using the following criteria: spike duration > 2.5 ms, spiking frequency < 3.3 Hz and responsivity to bath-applied dopamine. Interspike intervals (ISI's) were taken from one minute recordings. Variance of firing was the standard deviation of ISI's devided by the mean ISI. Student's t-test was used for statistical comparison.

Table 1 shows that at 1-3h after withdrawal from ethanol, firing rate was significantly decreased and variance in spike intervals significantly increased, compared with controls. These results demonstrate that in dopamine-sensitive neurones decreases in overall rate and increases in variability of firing can be seen in vitro during the early phase of withdrawal from chronic ethanol treatment. The observed changes could explain the decrease in dopamine release recorded in the nucleus accumbens during withdrawal from chronic ethanol treatment (Rossetti *et al.*, 1992).

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Table 1. The effect of withdrawal from chronic ethanol treatment on VTA neurone spiking. Data are mean ± s.e.mean. n = 5 to 13. Asterisks indicate significant difference (P < 0.05) from control.

time from withdrawal (h)		1-3	3-5	5-7
firing rate (Hz)	control	1.71 ± 0.34	2.42 ± 0.27	1.81 ± 0.40
	ethanol	0.92 ± 0.18 *	1.59 ± 0.31	1.38 ± 0.35
variance	control	0.25 ± 0.02	0.33 ± 0.09	0.57 ± 0.17
	ethanol	0.65 ± 0.13 *	0.73 ± 0.27	0.82 ± 0.25

395P DOTHIEPIN PREVENTS THE ACQUISITION OF PLACE AVERSION TO NALOXONE PRECIPITATED OPIATE WITHDRAWAL

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We have previously shown a capacity of tricyclic antidepressant compounds (TCAs) to attenuate the physical manifestations of opiate withdrawal behaviour (Gray & Sewell, 1991; Gray *et* al., 1992a,b). Whilst this reduction in the severity of the withdrawal syndrome is of obvious importance, the above studies do not directly explore changes in the psychological profile of the withdrawal response. Recently however, we have shown that paroxetine and fluvoxamine prevent place aversion to naloxone-precipitated morphine withdrawal (Rafieian-Kopaei, 1995).

The present study investigates the effect of dothiepin, a tricyclic antidepressant, upon the negative reinforcement resulting from the naloxone precipitated opiate withdrawal syndrome using a place preference (aversion) paradigm . The method of Higgins *et al.*, (1991) was followed employing a 900 second test period in a two-compartment place preference chamber and inducing dependence by morphine depot s.c.

We have demonstrated that both naloxone alone and precipitated opiate withdrawal produce place aversion in the two-compartment chamber utilised for this study. Additionally, Table 1 shows that pretreatment with dothiepin (30 min prior to naloxone) prevents the acquisition of place aversion following naloxone precipitated opiate withdrawal. Furthermore, the dothiepin dose used has minimal effects on physical withdrawal behaviours (Gray, *et al.*, 1992a). It is unlikely that this finding is contingent upon an anxiolytic effect since whilst dothiepin is anxiolytic in the elevated plus maze, amitriptyline does not produce an anxiolytic response in this test (Luscombe *et al.*, 1990) and yet produces similar results to dothiepin (not shown) in our place aversion paradigm.

Table 1. Dothiepin decrement in acquisition of naloxone precipitated morphine withdrawal place aversion. Mean compartment time (seconds ± s.e. mean) spent by rats (n=8) following withdrawal aversion pairing versus habituation alone (mean test value over 3 consecutive days)

Drug treatment ($mgkg^{-1}$)	Initially preferred side	Least preferred side
Habituation alone	750.4±12	100.4±12
Dothiepin 10 (i.p.)	735.2±13	116.2±10
Naloxone 5 (i.p.;Nlx) alone	522.4±12*	319.7±10
Morphine 150 + Nlx	110.6±8**	782.6±9
Morphine+Nlx+Dothiepin	702.4±15	175.4±6

** P<0.05; P<0.01, one-way Anova, Dunnetts vs habituation.*

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396P ISOLATION REARING REDUCES THE AMNESIAC EFFECT OF SCOPOLAMINE IN THE MORRIS WATER MAZE

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Social isolation has been reported to produce behavioural and neurochemical effects (Jones et al, 1992; Wongwitdecha & Marsden, 1995a,b). Impaired learning has been shown as a consequence of isolation rearing (Holson, 1986) although in certain situations learning in isolation reared rats can be enhanced or unimpaired compared to socially reared rats (Jones et al, 1991). The aims of the present experiments were to investigate the effects of isolation rearing on place navigation in the Morris water maze, and to compare the effects of scopolamine on a spatial learning task in isolation and socially reared rats. Two complementary paradigms were studied: isolation and socially reared rats were exposed to the water maze either without drug pretreatment or following systemic administration of scopolamine. Two conditions were examined: place learning and reversal learning (when the hidden platform was placed in the quadrant diagonally opposite to the previous location).

Male Lister Hooded rats were either housed singly (isolation reared) or in group of four (socially reared) from weaning at 21 days of age. Six weeks later spatial learning was determined using the method described by Morris (1984). The time taken to locate the submerged island (escape latency; sec) was used as measure of learning ability. Data are expressed as mean±SEM and analysed using an analysis of variance (ANOVA). Post hoc comparisons were assessed using Tukey's method.

The results show that isolation and socially reared rats did not differ in their initial place learning (day 1) and reversal learning (day 6). However, place learning on day 4 and reversal learning on day 7 were significantly enhanced in the isolation reared rats compared to socially reared rats ($P<0.05$; n=12 rats/group). Pretreatment with scopolamine (0.3-0.5 mg/kg i.p.) for 5 days produced a dose-related cognitive deficit as shown by increased escape latencies in the socially reared rats (saline = 17.8 ± 3.8; scopolamine 0.3 mg/kg = 43.8 ± 6.5; scopolamine 0.5 mg/kg = 51.5 ± 6.7; n=8 rats/group). Scopolamine (0.3 mg/kg i.p.) significantly impaired both place and reversal learning in the socially reared rats ($P<0.05$ on day 5 and day 8), but produced no significant learning deficit in the isolation reared rats (Figure 1).

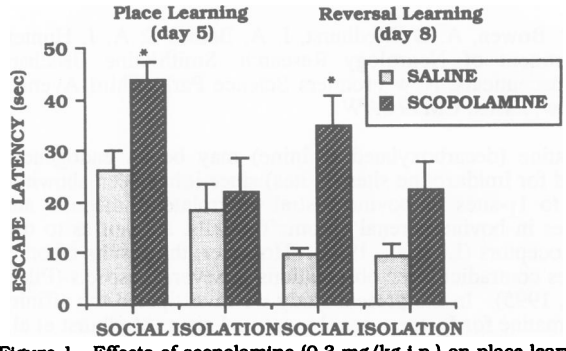

Figure 1. Effects of scopolamine (0.3 mg/kg i.p.) on place learning (day 5) and reversal learning (day 8) in socially and isolation reared rats. Data represent mean ± SEM of the escape latency (n=8 rats/group). *P<0.05 significantly different from saline.

These results demonstrate that isolation rearing enhances spatial learning and decreases the amnesiac effect of scopolamine. The mechanisms involved in these observations are not known but isolation rearing has been shown to produce significant changes in the function of specific amine neurotransmitters and the present results indicate that cholinergic function may also be influenced by rearing conditions.

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397P EXPRESSION OF α_1, α_4 AND γ_2 $GABA_A$ RECEPTOR SUBUNIT mRNAs IN RAT BRAIN AFTER CHRONIC LOW DOSE DIAZEPAM TREATMENT

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The neurochemical basis of benzodiazepine tolerance and dependence remain equivocal. In previous studies using 2-deoxyglucose & $GABA_A$ receptor autoradiography, we have identified the Papez circuit of the limbic system and an habenula-accumbens circuit as being important in diazepam tolerance and withdrawal (Pratt, 1991; Brett and Pratt 1994). The present studies were aimed at determining whether changes in the expression of $GABA_A$ receptor subunits could underlie the changes in these identified circuits.

Groups of male rats received diazepam either for one day or 21 days via subcutaneous capsules containing diazepam (90 mg) according to Gallager *et al.*, (1985). Control animals received empty capsules. The expression of mRNAs for the α_1, α_4 and γ_2 subunits of the $GABA_A$ receptor were determined using *in situ* hybridisation (Laurie *et al.*, 1992). Forebrain tissue not used for *in situ* hybridisation, was assayed for tissue diazepam levels (Gallager *et al.*, 1985). These were 11.8 ± 3.3, 395 ± 38 and 556 ± 37 ng g^{-1} in the control, acute and chronic groups respectively.

There were no changes in mRNA levels for these subunits in the Papez circuit after chronic diazepam treatment (Table 1). Despite moderate amounts of binding to components of the $GABA_A$ receptor in the nucleus accumbens (Brett and Pratt 1994) low levels of mRNA were detected in this structure for the α1 and γ2 subunits in control brains. Chronic treatment did not alter mRNA levels for the α4 subunit in the accumbens. Interestingly, mRNA for the α1 subunit was increased in the ventral pallidum after diazepam treatment (control 73±5; acute 107±16* and chronic 110±10* nCi g^{-1}; *$p<0.05$ compared to controls). These data suggest that is is unlikely that changes in the expression of these subunits are responsible for the changes in functional activity and in GABA-benzodiazepine recepter coupling that we have previously observed following chronic low dose diazepam treatment.

We thank SHERT for the provision of laboratory facilities.

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Table 1 Expression of α1, α4 and γ2 mRNA levels in the Papez circuit of rat brain after acute and chronic diazepam treatment

	α1			α4			γ2		
	Control	Acute diazepam	Chronic diazepam	Control	Acute diazepam	Chronic diazepam	Control	Acute diazepam	Chronic diazepam
Cingulate cortex	188±16	169±25	189±15	36±1	37±1	39±3	27±3	24±3	25±3
Anteroventral thalamus	74±6	85±7	98±7	137±11	140±28	150±5		N.D.	
Mammillary body	82±9	67±11	87±4	57±3	54±5	54±3		N.D.	

Values are expressed as means ± s.e. mean (nCi/g); n=3-4 per group. N.D = values not appreciably greater than background.

398P AGMATINE, A PUTATIVE ENDOGENOUS LIGAND FOR IMIDAZOLINE SITES (I-SITES) HAS VERY LOW AFFINITY FOR I-SITES IN RABBIT, DOG AND RAT TISSUES

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Agmatine (decarboxylated arginine) may be an endogenous ligand for imidazoline sites (I-sites) since it has been shown to bind to I_1-sites in bovine rostral ventrolateral medulla and I_2-sites in bovine adrenal chromaffin cells, as well as to α_2-adrenoceptors (Li et al., 1994). However, the results of other studies contradict these observations in several respects (Piletz et al., 1995). In the present study we investigated the affinity of agmatine for I_2-sites, novel putative I-sites (Medhurst et al., 1995) and α_2-adrenoceptors in a variety of tissues and species.

Membranes were prepared from rabbit cerebral cortex and rabbit, rat and dog kidney cortex based on the methods of Bricca et al. (1993). [^{3}H]-Idazoxan (1 nM), [^{3}H]-RX 821002 (2 nM) and [^{3}H]-clonidine (6 nM, in presence of 10 uM noradrenaline) were used to label I_2-sites, α_2-adrenoceptors and novel I-sites respectively. Membranes (100 ug rabbit cerebral and kidney cortex, 200 ug rat kidney cortex, 300 ug dog kidney cortex), [^{3}H]-ligand and inhibiting drugs were incubated in duplicate to equilibrium (45-60 min, room temperature). Non-specific binding was defined with 100 uM idazoxan (rabbit and dog kidney cortex, rabbit cerebral cortex I_2-sites), 100 uM clonidine (rat kidney cortex) and 100 uM oxymetazoline (rabbit cerebral cortex α_2-adrenoceptors). Bound radioligand was separated from free by filtration. Mean -log IC50 (pIC50 values) ± s. e. mean are shown in Table 1.

Agmatine (up to 100 uM) failed to inhibit [^{3}H]-idazoxan binding in rabbit cerebral cortex suggesting a very low affinity for I_2-sites. Agmatine also had very low affinity for the novel putative I-sites labelled with [^{3}H]-clonidine in rabbit, rat and dog kidney cortex and α_2-adrenoceptors labelled with [^{3}H]-RX 821002 in rabbit cerebral cortex.

Table 1. Mean pIC50 values ± s. e. mean (n = 3-6).

Tissue	Radioligand	Agmatine pIC50
Rabbit cerebral cortex	[^{3}H]-idazoxan	< 4
Rabbit cerebral cortex	[^{3}H]-RX 821002	4.14 ± 0.06
Rabbit kidney cortex	[^{3}H]-clonidine	4.25 ± 0.14
Rat kidney cortex	[^{3}H]-clonidine	4.17 ± 0.03
Dog kidney Cortex	[^{3}H]-clonidine	4.79 ± 0.11

These results show that agmatine, a proposed endogenous ligand for I-sites, has very low affinity for I_2-sites in rabbit cerebral cortex and for novel putative I-sites labelled with [^{3}H]-clonidine in rabbit, rat and dog kidney cortex. Agmatine also had lower affinity for α_2-adrenoceptors in rabbit cerebral cortex than previously reported in rat cerebral cortex (Li et al., 1994). Based on its very low affinity, it is questionable whether agmatine is an endogenous ligand for I-sites in rabbit cerebral cortex and rabbit, rat and dog kidney cortex.

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399P THE EFFECT OF ONDANSETRON AND CP-99,994 ON EMESIS INDUCED BY ROLIPRAM IN CONSCIOUS FERRETS

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Phosphodiesterase (PDE) type IV inhibitors are currently being evaluated as potential anti-asthma drugs. However, it has previously been shown that a highly selective PDE type IV inhibitor, rolipram, causes emesis after oral administration to man (Zeller *et al.*, 1984). Non-selective PDE inhibitors have also been shown to cause emesis in the ferret (Howell *et al.*, 1990). In the present study, we have evaluated the emetogenic potential of roliipram in conscious ferrets, and have investigated the effects of two classes of anti-emetic agent, the 5-HT_3 receptor antagonist, ondansetron, and the tachykinin NK_1 receptor antagonist, CP-99,994, (+)-(2S,3S)-3-(2-methoxy benzylamino)-2-phenylpiperidine (Bountra *et al.*, 1993).

Adult male ferrets (0.7-1.2kg) were pre-treated with ondansetron (1mg kg^{-1} s.c.) and CP-99,994 (3mg kg^{-1} s.c.), either alone or in combination, or with vehicle (saline and dimethylsulphoxide respectively, 0.5ml kg^{-1} s.c.) 15 min prior to oral administration of rolipram (10mg kg^{-1} in 0.1M sodium hydroxide containing 18% ethanol and 9% polyethylene glycol 400). Ferrets were observed for 3h, with any emesis (retching or vomiting) or other behavioural changes being recorded.

The rolipram vehicle (1ml kg^{-1} p.o.) did not cause emesis or any other behavioural changes (n=4). However, in ferrets pre-treated with the antagonist vehicle, rolipram caused marked emesis (Table 1) and a range of other behavioural effects, including ataxia, hyperventilation, flat posture, clawing at the mouth and salivation. The emesis was reduced by pre-treatment with ondansetron, whereas it was almost abolished with CP-99,994. Pre-treatment with a combination of CP-99,994 and ondansetron abolished retching but not vomiting (Table 1). The other behavioural changes induced by rolipram did not appear to be affected by any of the pre-treatments.

Table 1. Effect of ondansetron and CP-99,994 on emesis induced by rolipram in conscious ferrets.

treatment	responders/tested	retches[1]	vomits[1]
vehicle (1ml kg^{-1})	6/6	86±21	22±2
ondansetron (1mg kg^{-1})	5/5	40±10*	22±3
CP-99,994 (3mg kg^{-1})	4/6	2±1**	4±2**
ondansetron + CP-99,994	5/6	0**	6±3**

[1]mean ± s.e.m. *p <0.05 **p <0.01 vs vehicle (unpaired t-test)

These data demonstrate that in ferrets, emesis, but not the other behavioural effects, induced by rolipram is mediated by both 5-HT_3 and NK_1 receptors.

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400P PERTUSSIS TOXIN SENSITIVITY OF HISTAMINE H_3 AGONIST-STIMULATED [^{35}S]GTPγ[S] BINDING IN RAT CEREBRAL CORTICAL MEMBRANES

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We have previously shown (in radioligand binding studies), that [^{3}H]N$^\alpha$-methylhistamine binding to histamine H_3-receptors can be regulated by guanylnucleotides, implying that histamine H_3-receptors are coupled to their effector system via a guanine nucleotide binding protein (G-protein) (Clark & Hill, 1995). In addition, Endou *et al.*.(1993), reported that the histamine H_3-receptor modulation of noradrenaline release from sympathetic nerve endings in guinea-pig myocardium was attenuated by pertussis toxin pre-treatment, suggesting that histamine H_3-receptors may couple to a G_i/G_o protein. In this communication we report that, in rat cerebral cortical membranes, histamine H_3-agonist stimulated specific [^{35}S]-guanosine 5'-o-(γ-thio)triphosphate ([^{35}S]GTPγ[S]) binding is sensitive to thioperamide and pertussis toxin (PTX).

Binding studies were performed in homogenates of rat cerebral corticies (pre-treated with adenosine deaminase 1U/ml), in 1ml of 50mM Tris. HCl buffer containing 100mM NaCl, 10mM $MgCl_2$, 10μM GDP and 0.1nM [^{35}S]GTPγ[S], pH7.4 (30 min, 25^{o}C). Non-specific binding was determined in the presence of 10μM non-radioactive GTPγS. Membranes were pre-treated with PTX as previously described (Sweeney & Dolphin, 1995). All experiments were performed in the presence of 0.1μM mepyramine and 10μM tiotidine.

The histamine H_3-receptor agonists N$^\alpha$-methylhistamine (EC_{50} = 0.25 ±0.06nM, maximal stimulation = 116.0 ± 1.2%; basal = 100%, n = 5) and R$^\alpha$-methylhistamine (EC50 = 0.42 ± 0.12nM, maximal stimulation = 117.8 ± 2.5%; basal = 100%, n = 14) produced a concentration-dependent stimulation of specific [^{35}S]GTPγ[S] binding. Histamine H_3-agonist stimulated binding was attenuated in the presence of the selective H_3-antagonist, thioperamide (1μM), and abolished in membranes pre-treated with PTX (3μg/mg protein). However, basal [^{35}S]GTPγ[S] binding was unaffected.

These results provide the first direct evidence that histamine H_3-receptors can couple to a G_i/G_o protein in mammalian brain.

Supported by MRC and Glaxo/Wellcome Group Research.

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401P EFFECTS OF K$^+$ CHANNEL BLOCKING DRUGS ON TENSION AND MEMBRANE CURRENTS IN RABBIT AORTIC SMOOTH MUSCLE

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Using the whole-cell patch-clamp technique, the effects of several K$^+$ channel-blocking drugs on K$^+$ currents recorded from isolated rabbit aortic smooth muscle cells were investigated. The cell isolation procedure and recording conditions were as previously described (Clapp & Gurney, 1991). Depolarising steps from a holding potential of -80mV induced outward current at potentials above -50mV. We have previously shown (Halliday *et al*, 1994) that the cells contain a transient current (I_t), which is sensitive to block by 1mM 4-aminopyridine (4-AP) and resembles the neuronal A current. They also display a 4-AP sensitive, voltage-activated delayed rectifier K$^+$ current ($I_{K(V)}$), which accounts for approximately 30% of the total outward current measured at +50mV. A tetraethylammonium (TEA)-sensitive Ca^{2+}-activated K$^+$ current ($I_{K(Ca)}$) accounts for around 40%.

In order to improve the separation of individual K$^+$ currents and to investigate their roles in tension regulation, we have investigated a number of other K$^+$ channel-blocking drugs for their effects on rabbit aortic smooth muscle. Phencyclidine (PCP), imipramine, amitryptiline and sotalol all failed to selectively block either I_t, $I_{K(Ca)}$ or $I_{K(V)}$ (the latter two currents collectively referred to as the sustained current, I_{sus}) at either 10 or 100μM. The drugs had similar actions, the most potent being imipramine, which reduced I_t by 51±7% and I_{sus} by 62±7% (mean±s.e.m, n=4) at 100μM. Outward currents were also examined in the presence of TEA (1mM) in order to block $I_{K(Ca)}$, so that the influence of drugs on $I_{K(V)}$ and I_t could be compared. Again, the drugs all caused a non-selective but concentration-dependent block. At 100μM, PCP caused 66±3% and 63±4% block of I_t and $I_{K(V)}$ respectively; imipramine reduced I_t by 71±3% and $I_{K(V)}$ by 49±8%; amitriptyline reduced I_t by 56±7% and $I_{K(V)}$ by 53±3%, while sotalol was less effective, reducing I_t and $I_{K(V)}$ by 32±3% and 29±4% respectively (n=4 for each drug). Clofilium (10 μM), which selectively blocks delayed rectifier currents in cardiac myocytes (Arena & Kass, 1988), appeared to show selective block of $I_{K(V)}$ (49±5%, n=3) compared to I_t (18±2%) in the presence of 1 mM TEA, but only at low stimulation frequency (0.07Hz). At 1Hz, both I_t and $I_{K(V)}$ were reduced to a similar extent (58±10% and 76±6% respectively, n=3). Thus, the blocking action of clofilium was use-dependent.

The effects of all of the blockers on isometric tension were examined using strips of rabbit aorta, bathed in physiological saline at 37°C. The drugs found to non-selectively reduce K$^+$ current caused only minor increases in basal tension even at 100 μM. TEA (1mM) and 4-AP (1mM) were both effective in causing contraction, 4-AP sometimes causing oscillations in tension. When applied together, TEA and 4-AP caused near maximal contractions (89±22% of the tension generated by 50mM K$^+$ solution, n=3). The lack of effect of clofilium, PCP and imipramine may be due to an inhibitory action on Ca^{2+} influx, since at 100 μM, all these drugs suppressed the contractile response to 50mM K$^+$ (78±6%,n=4; 39±2%, n=3; 88±5%, n=4), an action that was not seen with 4-AP or TEA. Thus, there was no correlation between the actions of the drugs on the K$^+$ currents investigated in isolated myocytes, and their effects on tension in intact muscle. It is clear that identification of the specific K channels involved in regulating the tone of vascular smooth muscle will require several experimental approaches and more selective drugs.

This work was supported by the British Heart Foundation

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402P CALCIUM-INDEPENDENCE OF THE RELAXANT EFFECT OF OESTROGENS ON ISOLATED RAT AORTA

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Epidemiological studies indicate that oestrogens may have protective effects on cardiovascular morbidity and mortality (Henderson and Paganini Hill, 1991). 17β-oestradiol (β-EST) was suggested to relax rabbit aorta and coronary arteries (Gisclard *et al.* 1988; Jiang *et al.* 1991) and dilate rat tail artery (Shan *et al.*, 1994) by mechanisms involving calcium antagonism or inhibition of calcium influx. The present work compares the ability of various oestrogens to relax rat aorta and the possible calcium dependence of the relaxation.

Rings of aorta 3-5 mm wide were prepared from male Hooded Lister rats (250-400g, Bradford strain) and placed in Krebs' solution containing 10μM indomethacin under 2g tension (37°C; 95% O_2, 5% CO_2). Rings were contracted with $PGF_{2\alpha}$ (10μM) an approximately EC_{80} concentration giving a response of 2.3 ± 0.06 g. Relaxation was expressed as % reversal of contraction. When contraction was stable, β-EST, 17α–oestradiol (α-EST), oestrone (ESTRO) or diethylstilboestrol (DEST) was applied for 40 minutes. The oestrogens caused concentration-related gradual relaxation in all tissues. Comparison of the relaxation produced by the oestrogens (20μM) is shown in Table 1. (n = 6-10).

Oestrogen (20μM)	% relaxation in normal Krebs'	% relaxation in Ca^{++}-free Krebs'
β-OESTRADIOL	57.4 ± 5.5	54.4 ± 4.4
α-OESTRADIOL	15.5 ± 1.7	16.6 ± 2.7
OESTRONE	12.9 ± 2.1	15.8 ± 2.4
DIETHYLSTILBOESTROL	100	90.0 ± 2.1

Table 1 Oestrogen-induced relaxation of contraction produced by $PGF_{2\alpha}$ (10μM) in normal or $PGF_{2\alpha}$ (50μM) in Ca^{++}-free Krebs' expressed as % reversal of contraction.

No vehicle effects were observed.

To investigate possible calcium antagonism by the oestrogens, aortic rings were incubated for 2 hours with Ca^{++}-free solution. KCl (80mM) was added and after 15 mins a cumulative Ca^{++} concentration-response curve constructed in the absence or presence of β-EST, α-EST, ESTRO (20 μM) or DEST (10μM) or the calcium channel blocker, nifedipine (NIF, 100nM) (n = 4-8.) β-EST, DEST and NIF caused large rightward displacements of the calcium concentration-response curve, significantly decreasing E_{max}, α-EST and ESTRO were ineffective. Thus the actions of β-EST and DEST appeared to resemble calcium channel blockade while α-EST and ESTRO had no such action.

Further experiments examined the calcium-dependence of the oestrogen-induced relaxation. Rings were incubated (2hr) in Ca^{++}-free Krebs' containing 100μM EDTA and contracted with $PGF_{2\alpha}$ (50μM) which produced a small contraction in this solution (0.9 ± 0.1g). β-EST, α-EST, ESTRO and DEST (20μM) caused relaxation similar to that observed in normal Krebs' (Table 1), in a solution which eliminated possible calcium antagonism or inhibition of calcium influx (n = 5-12).

We conclude that the various natural and synthetic oestrogens relax vascular muscle by a mechanism which is independent of extracellular calcium.

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403P EFFECTS OF HYDRALAZINE ON β-ESCIN SKINNED RABBIT AORTA

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Hydralazine has been proposed (Gurney & Allam, 1995) to produce vasodilation by interfering with Ca^{2+} release from the sarcoplasmic reticulum (SR). This study investigated the influence of hydralazine on rabbit aorta, chemically skinned with ß-escin to allow the influence of hydralazine on intracellular pathways to be studied directly. Male New Zealand rabbits (2-3 kg) were killed with sodium pentobarbitone (80mg/kg i.v.). The thoracic aorta was removed, cleaned and cut into strips (3mm x 10mm), which were mounted in an organ bath to measure isometric tension. The tissue was bathed in solution of the following composition (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11 and $CaCl_2$ 2.4. Following control contractions to 1μM phenylephrine (PE), the aortic strips were placed for 30 min in skinning solution (SS) containing (mM): creatine phosphate (Na_2CP) 3, Na_2ATP 6, EGTA 10, BES buffer 100, reduced glutathione 5, phenylmethyl-sulfonyl fluoride (to inhibit intracellular proteolysis) 0.1 and 40μM ß-escin with $CaCl_2$ and $MgCl_2$ added to give $[Ca^{2+}]$ and $[Mg^{2+}]$ of 50nM and 1mM respectively. The tissue was then washed in relaxing solution (RS), which consisted of SS without ß-escin, for 20 min and then placed in RS containing 1μM free Ca^{2+} to fill the SR. After 20 min washing in RS to relax the tissue, 10μM PE was applied. This resulted in contraction of amplitude 46 ± 7% (mean ± s. e. mean, n=6) less than those induced by 1μM PE in intact tissue.

After exposure of the strips to hydralazine for 20 min, contractions to 10μM PE were reduced in a dose-dependent manner with 51% inhibition at 10μM (n=2) and 74 ±4% (n=3) at 100μM hydralazine. This compares to 81 ± 8% (n=15) inhibition of PE contractions by 100μM hydralazine in intact tissue.

Raising the free $[Ca^{2+}]$ produced a concentration-dependent increase in tissue tone, with maximum tension achieved at around 2μM and 50% maximum tension at 0.4μM. After incubating the tissue with 10-100μM hydralazine for 20 min, increases in the free $[Ca^{2+}]$ continued to induce contraction. As shown previously (DeFeo and Morgan, 1989), at free $[Ca^{2+}]$ greater than 0.1μM, hydralazine did not significantly affect the $[Ca^{2+}]$-tension relationship. Hydralazine did however, appear to influence the response to low levels of free Ca^{2+} and to increase the time taken to reach maximum contraction.

In conclusion, hydralazine reduces PE induced contractions of skinned rabbit aorta with a similar potency to that seen in intact aorta, indicating that the action of hydralazine does not require an intact cell membrane. The lack of effect of hydralazine on the $[Ca^{2+}]$-force relationship implies that its mechanism is to cause a reduction in cytosolic free $[Ca^{2+}]$ rather than to reduce the effect of Ca^{2+}. The present results are consistent with an inhibitory effect of hydralazine on IP_3 -induced Ca^{2+} release as recently suggested (Gurney & Allam, 1995).

Supported by the British Heart Foundation. We thank Drs J. Kentish & S. Palmer for help with skinning techniques.

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404P OXIDANT STRESS AND TOLERANCE TO GLYCERYL TRINITRATE IN THE ISOLATED RAT AORTA

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Tolerance to organic nitrates involves reduced bioactivation by organic nitrate converting enzyme (ONCE) and may result from oxidant stress, especially by superoxide anion (Yeates & Schmid, 1992; Münzel *et al.*, 1995). Oxidant stress could signal the genome to give a tolerant phenotype in a nitrate shock scenario. We assessed the effects of an intracellular superoxide anion scavenger, tiron, an inhibitor of oxidant stress-activated nuclear factor κB (NF-κB), pyrrolidine dithiocarbamate (PDTC) and a putative inhibitor of oxidant stress-induced gene expression, dexamethasone (DEX) (Rees *et al.*, 1990), on tolerance to glyceryl trinitrate (GTN) in rat aortic smooth muscle *in vitro*.

Male Wistar rats (250-300g) were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), sacrificed and thoracic aortic rings prepared and mounted under isometric conditions in Krebs' solution (composition in mM: NaCl, 133; KCl, 4.7; NaH_2PO_4, 16.3; $MgSO_4$, 0.61; $CaCl_2$, 2.52; glucose, 7.8) bubbled with carbogen and warmed to 37°C. Tolerance was induced by incubating with GTN (*Nitronal*, 30 or 100μM) for 30min followed by washout over 30min. Control, non-tolerant rings were exposed to vehicle alone (0.9% w/v saline). Rings were then precontracted submaximally with noradrenaline (100nM) and cumulative relaxation to GTN assessed. Tiron (10mM) was added 10min prior to the tolerance protocol and was not re-added while PDTC (50μM) or DEX (1μM) was preincubated for 1h and was present throughout the experiment. GTN (0.01-300μM) relaxed rat aorta in a biphasic manner (pD_2 first phase=7.29±0.03, n=8; pD_2 second phase=5.11±0.08, n=8). Area under the GTN relaxation curve (AUC) was reduced to 74.7±3.6% ($p<0.01$, n=8) and 51±5.6% ($p<0.01$, n=8) in untreated 30μM and 100μM GTN-tolerant rings, respectively (non-tolerant AUC=100%). Relaxation in 30μM and 100 μM GTN-tolerant rings was 69.2±8.8% and 55.8±6.3% (n=5), respectively, in the presence of tiron (10mM) (NS); 80.7±7.2% and 52.5±4.3% (n=5), respectively, in the presence of PDTC (50μM) (NS); and 80.7±3.5% and 54.8±5.0% (n=6), respectively, in the presence of DEX (1μM) (NS). PDTC (50μM) was found to depress AUC for GTN *per se* in non-tolerant rings by 39% from 340±18 (n=8) to 206±14 (n=5, $p<0.01$).

In conclusion, the data suggest that, unlike the case in the rabbit aorta (Münzel *et al.*, 1995), superoxide anion is not a mediator of organic nitrate tolerance in the rat aorta. Furthermore, oxidant stress-induced gene expression, including the activation of NF-κB, is unlikely to account for tolerance in our model. Finally, PDTC inhibits relaxation to GTN in rat aorta.

This work was funded by ONO Pharmaceutical Co., Ltd, Osaka, Japan.

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405P DESMOSINE AND ISODESMOSINE CONTENTS OF THE AORTIC WALL IN A RAT MODEL OF ELASTOCALCINOSIS

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We have developed a rat model of isolated systolic hypertension produced by rigidification of compliance arteries; the latter was induced by hypervitaminosis D and nicotine treatment (model VDN; Atkinson *et al.*, 1994; Tatchum-Talom *et al.*, 1995). Calcium is deposited on elastic fibres (Henrion *et al.*, 1991) leading to a decrease in the relative proportion of elastic fibres in the media determined by histomorphometry (Atkinson *et al.*, 1994). In order to determine whether changes also occur in the chemical structure of elastin, a new technique of desmosine (DES) and isodesmosine (IDE) measurement was used to evaluate the effect of calcification on the amount of cross-linking amino acids. Arterial calcium overload was induced by treating 2-month old rats with vitamin D_3 (300,000 iu/kg, im) plus nicotine (2x25 mg/kg, po) (n=5). The control group (2-month old rats, controls, n=7) received 0.15 M NaCl im and distilled water (5 ml/kg po). Forty days later one subgroup of rats was fitted with carotid and femoral artery cannula in order to determine systolic arterial pressure and aortic pulse wave velocity (Tatchum-Talom *et al.*, 1995). In a second subgroup abdominal aorta fragments were excised. Total calcium content was measured by atomic absorption spectrometry. Hydrolysis of arterial tissue was performed (HCl 6 M, 105°C, 24h) and DES and IDE were analyzed by capillary zone electrophoresis (Giummelly *et al.*, 1995). Results (Table 1) are expressed as means ± s.e.mean (* $P<0.05$ *versus* controls; Student's t test).

In parallel with the development of isolated systolic hypertension and the increase in pulse wave velocity, a decrease in the level of the cross-linking amino acids was observed in the calcified aortic wall of the VDN rat.

Table 1. Systolic arterial pressure, pulse wave velocity, calcium, DES and IDE contents in arterial walls of VDN rats and controls.

	VDN	Controls
Systolic arterial pressure (mmHg)	173 ± 3*	147 ± 2
Pulse wave velocity (cm/s)	800 ± 64*	556 ± 22
Calcium (μmol/g dry weight)	158 ± 36*	8 ± 1
DES (μg/g dry weight)	157 ± 13*	457 ± 35
IDE (μg/g dry weight)	124 ± 12*	339 ± 12

In VDN rats, calcium overload produced elastocalcinosis, a decrease in arterial compliance and isolated systolic hypertension. The structural modifications of the aortic wall were accompanied by a decrease in the number of inter or intra-chain bridges. This change in the chemical composition of elastin could be linked to the modification of the mechanical properties of the aortic wall.

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This laboratory is a member of the EUC Biomed project "EureCa" BMHI-CP94-1375.

406P IDENTIFICATION OF THE IONIC CURRENTS IN HUMAN INTRAPULMONARY ARTERY MYOCYTES

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Channels selectively permeable to K^+ contribute to the resting membrane potential and inhibit excitation of vascular muscle,while calcium entry through voltage-gated Ca channels promotes contraction (Gurney, 1994). We have investigated the electrophysiological properties of adult human pulmonary artery smooth muscle cells (PASMC), enzymatically isolated from intrapulmonary artery of a 45 year old female, after surgery to remove a bronchial carcinoma. Experiments employed the whole-cell patch-clamp technique, with extracellular solution (mM): NaCl 112, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, HEPES 21 (pH 7.3, NaOH) and with pipettes filled with (mM): KCl 130, $MgCl_2$ 1, EGTA 1, Na_3GTP 0.5, HEPES 20 (pH 7.3, KOH). The resting membrane potential (RMP), measured as zero-current potential, was -58 ± 2 mV (mean ± SEM; n=3). Membrane input resistance was 69 ± 9 GΩ (n=7) and capacitance 16 ± 1 pF (n=7). Voltage steps to potentials more positive than -30 mV activated outward current (n=5, current density at 40 mV = 34 ± 6 pA/pF), which contained at least 2 components; one blocked by teraethylammonium (TEA,10 mM), and the other sensitive to quinine (100 μM). In the presence of TEA, steps to potentials above -10 mV also induced an inward current (n=2), most likely carried by L-type Ca current (I_{Ca}). The outward current displayed voltage-sensitive inactivation. However, after clamping the cells at 0 mV for >5 min,a component of non-inactivated current remained (3.1 ± 0.6 pA/pF, n=3), which rectificated at potentials negative to -60 mV, as revealed by voltage ramps from +60 mV to -100 mV (Fig.1). The non-inactivating current was therefore voltage-gated and likely due to activation of channels that closed at negative potentials. This current was little influenced by TEA but was suppressed partially by 82 ± 1 %, (n=4) by 100 μM quinine. In the presence of TEA the RMP (-52 ± 4 mV, n=3) was similar to control, but quinine evoked depolarisation to -23 ± 10 mV (n=3). Human PASMC displayed at least four ionic conductances. I_{Ca}, TEA-sensitive outward current, reflecting Ca-activated K channels, quinine-sensitive outward current, probably delayed rectifier K current and a voltage-gated,non-inactivating K conductance, similar to one described recently in rabbit PASMC (Evans et al., 1995).

Supported by the British Heart Foundation.

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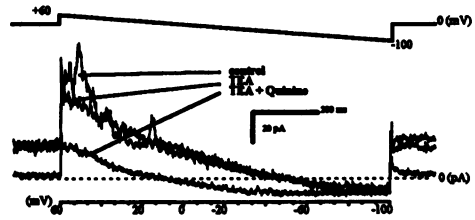

Fig.1 Effects of TEA(10 mM) and quinine (100 μM) on current during a voltage ramp from +60 to -100 mV, after clamping the cell for 5 min at 0 mV.

407P MAGNESIUM PROLONGS THE DURATION OF THROMBIN-INDUCED SHAPE CHANGES IN BOVINE PLATELETS

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Platelets are hyperactive in diseases affecting the cardiovascular system such as essential hypertension or diabetes mellitus; in both these diseases, cellular Mg^{2+} is reduced in platelets and other blood cells (Touyz, *et al.*, 1992; Nadler, *et al.*, 1992). Although Mg^{2+} reduces *in vitro* platelet responsiveness when added to human platelets *ex vivo*, (Hwang, *et al.*, 1992), the mechanism of action of Mg^{2+} is unknown, but it may be that Mg^{2+} competes with Ca^{2+} for entry into the platelet. Platelet shape change is an early response to some agonists, and is not dependent on external Ca^{2+} (Seiss, 1989). We have studied the effects of varying the external concentration of Mg^{2+} *in vitro*, together with Ca^{2+} removal, and attenuation of Ca^{2+} influx by manganese (Mn^{2+}, an inhibitor of receptor-activated Ca-channels in some cell types - Hallam, *et al.*, 1984) on thrombin-induced shape-change in washed, bovine platelets.

Platelet shape-change was recorded using a Chrono-log turbidometric aggregometer (Born, 1962). Fresh cattle blood was collected at an abattoir into acid citrate dextrose (ACD - citric acid 8g/l; trisodium citrate 22g/l; glucose 20g/l; indomethacin 30 μM) in a blood:ACD ratio of 5:1. The blood was diluted with HEPES-buffered Tyrode (blood:buffer ratio 3:1) containing no added Mg^{2+} or Ca^{2+}, then centrifuged for 30 min at 200 g at room temperature to obtain platelet-rich plasma (PRP). Prostacyclin (20 nM final concentration) was added to the PRP to aid resuspension. PRP was centrifuged for 20 min at 840 g at room temperature, the platelets resuspended in modified Tyrode containing ethylene glycol-bis(β-aminoethylether)N,N,N',N'-tetraacetic acid (EGTA; 500 μM) and centrifuged for 15 min at 840 g at room temperature. The pellet was resuspended in modified Tyrode to give 2 x 10^8 platelets/ml. Thrombin (0.08 U/ml) was used as the stimulating agent. Experiments were performed in the presence of 1-20 mM $MgCl_2$ added 5 min before thrombin, with a) 1 mM $CaCl_2$, or b) 500 μM EGTA, or c) 1 mM $CaCl_2$, and 5 mM $MnCl_2$. The duration of the shape-change (i.e. the time taken for the trace to recover to the unstimulated level) was expressed as a % relative to the control (1 mM Ca^{2+}, 1 mM Mg^{2+}) from each experiment. The results (Table 1) are means±SEM, with the number of observations in brackets. The curves were analysed by 2-way ANOVA, and individual points by Student's paired *t* test vs. the controls.

Table 1

$[Mg^{2+}]_o$	a) Ca	b) EGTA	c) Ca + Mn
1	100.0±10.7(7)	150.5±18.3(4)	637.5±110.2(4)
2	**165.2±11.5(7)	180.6±30.5(4)	1098.6±291.9(4)
5	*181.6±28.3(7)	236.6±27.3(4)	990.3±500.6(4)
10	*281.5±61.8(7)	497.3±83.7(4)	1680.6±257.2(4)
20	**712.8±101.1(7)	897.3±143.3(4)	2349.5±1125.1(4)

* = P<0.05; **=P<0.002

Increasing external Mg^{2+} caused a significant rise in the duration of the shape-change both with (P<0.001) and without external Ca^{2+} (P<0.05). Increasing external Mg^{2+} had no significant effects when Ca^{2+} and Mn^{2+} were present together. When Ca^{2+}, but not Mg^{2+} was present, the duration of the shape-change was less than the control (57.9±16.6% vs. 100±44.3%; P<0.05; n=8). These results show that in bovine platelets, Mg^{2+} prolongs the thrombin-induced shape-change by a mechanism which is not dependent on external Ca^{2+}.

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408P SDZ NVI 085, AN α_{1A} ADRENOCEPTOR AGONIST WITH 5-HT$_2$ RECEPTOR ANTAGONIST PROPERTIES

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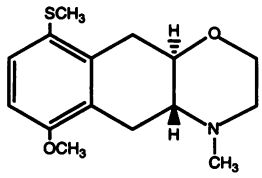
The α adrenoceptor agonist, (±)SDZ NVI 085 (3,4,4a5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth [2,3-b]-1,4-oxazine hydrochloride) (Figure 1), a rigid structural analog of methoxamine, has shown selectivity for the α_{1A}-adrenoceptor subtype (Eltze and Boer, 1992; Buscher et al., 1994). The present study examines the effect of (±)SDZ NVI 085 on serotonin (5-HT) mediated contraction of the caudal artery of rat.

Figure 1. Structure of SDZ NVI 085



Caudal arteries from CO_2 asphyxiated male Sprague-Dawley rats were endothelium-denuded and mounted as helical strips (0.5 - 1.0 cm) at 4 mN in waterjacketed tissue baths (37°C) for measurement of isometric tension in oxygenated (95% O_2, 5% CO_2) Krebs' solution.

In the presence of prazosin (1 μM) and idazoxan (0.3 μM), (±)SDZ NVI 085 inhibited the contractile response to 1 μM 5-HT with a pIC_{50} of 7.2 ± 0.1 (mean ± s.e.mean; 13 tissues from 3 animals). Responses were unaffected by 1 μM cyanopindolol. This inhibitory effect appeared specific for SDZ NVI 085 as phenylephrine and NA did not cause significant inhibition of the contractile response to 5-HT. Methoxamine elicited a relaxant effect at high concentrations only (IC_{50}>300 μM). Finally, (±)SDZ NVI 085 failed to cause a significant relaxant effect in tissues precontracted with U46619 or vasopressin.

In order to study the nature of the inhibitory effect of (±)SDZ NVI 085 toward 5-HT, monophasic concentration-effect (E/[A]) curves were produced by cumulative additions of 5-HT. (±)SDZ NVI 085 (30 to 3000 nM) caused surmountable parallel rightward displacement of E/[A] curves to 5-HT in a concentration-dependent manner. The corresponding Schild regression revealed a pA_2 estimate of 8.1 (slope=1.0; 95% CL = 0.84 - 1.16).

Ketanserin (a reference 5-HT$_2$ antagonist) also elicited parallel dextral shifts of the E/[A] curves to 5-HT without a decrease in the maximal response. Schild analysis yielded a pA_2 estimate of 9.0 (slope=1.2; 95% CL = 0.97 - 1.45). Resultant analysis with (±)SDZ NVI 085 (100 and 300 nM) gave parallel, concentration-dependent dextral shifts in the Schild regression to ketanserin. The displacement of the Schild regressions as a function of the (±)SDZ NVI 085 concentrations yielded a resultant regression with a pK_B of 7.7. The estimates of affinity for (±)SDZ NVI 085 from Schild and resultant analyses agree with the pK_B estimated from inhibition curves toward 1 μM 5-HT (pK_B = 7.6 ± 0.1; mean ± s.e.mean).

It is concluded that (±)SDZ NVI 085, in addition to its α_{1A}-adrenoceptor agonist properties, behaves as a reversible competitive 5-HT$_2$ receptor antagonist.

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409P PRELIMINARY CHARACTERISATION OF AN ENDOTHELIAL 5-HT RECEPTOR WHICH MEDIATES RELAXATION IN A PREPARATION OF DOG ISOLATED VENA CAVA

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Endothelial 5-HT receptors that mediate relaxation in preparations of rat and cat jugular vein resemble the 5-HT$_{2B}$ subtype (Ellis *et al.*, 1995a; 1995b). In this paper, we have used compounds with known activity at the 5-HT$_{2B}$ receptor to examine the characteristics of a relaxant endothelial 5-HT receptor in dog isolated vena cava.

Rings (3mm in length) of isolated vena cava from dogs of either sex (10-16kg) were suspended under a load of 1g in Krebs solution, gassed with 95% O_2 and 5% CO_2 at 37°C, containing ketanserin (1μM). Tissues were initially equilibrated with pargyline (100μM) for 30min. Following washout, agonist-evoked relaxations were measured isometrically in tissues pre-contracted with U46619 (10nM), and responses were expressed as a percentage of the U46619-evoked contraction. Only one cumulative agonist concentration-response curve was prepared in each tissue. In experiments using the selective 5-HT$_{2B}$ receptor antagonist, SB 204,741 (pA_2 7.4-8.0, see Baxter *et al.*, 1994 & Ellis *et al.*, 1995b), tissues were equilibrated with the compound for ≥1h, prior to the construction of an agonist concentration-response curve. In experiments with endothelium-denuded tissues, the functional integrity of endothelial and vascular smooth muscle cells was assessed by use of acetylcholine (ACh, 1μM) and glyceryl trinitrate (GTN, 1μM), respectively.

In agonist studies, the 5-HT$_{2B}$ receptor agonists BW 723C86 (100pM-3μM) and α-Me-5-HT (10pM-100nM) caused monophasic concentration-dependent relaxations, whilst 5-HT (100pM-10μM) evoked a biphasic relaxant response (see Table).

Agonist	pEC_{50} [95% CL]	Emax (%)	n
BW723C86	7.6 [7.5-7.7]	41±5	14
5-HT (first phase)	8.5 [8.3-8.7]	53±10	5
α-Me-5-HT	8.9 [8.4-9.4]	35±6	7

In endothelium-denuded preparations, relaxations to BW 723C86 and ACh were abolished, but relaxations to GTN remained unaltered. The 5-HT$_{2B}$ receptor antagonist SB 204,741 (30nM-300nM) caused a concentration-dependent rightward displacement of the concentration-response curve to BW 723C86 (pK_B [95% CL], 7.5 [7.3-7.7], n=13). SB 204,741 (300nM) also antagonised relaxations to α-Me-5-HT (apparent pA_2 [95% CL], 7.5 [6.7-8.4], n=3).

In summary, we have shown that relaxations to BW 723C86 in preparations of dog vena cava are mediated *via* the activation of an endothelial 5-HT receptor. Given the high agonist potency of 5-HT, α-Me-5-HT and BW 723C86, combined with the antagonist action of SB 204,741, we conclude that the endothelial 5-HT receptor in preparations of dog vena cava has properties which resemble the 5-HT$_{2B}$ subtype.

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410P EFFECT OF DEVELOPMENTAL AGE ON NORADRENALINE- AND ACETYLCHOLINE-EVOKED RESPONSES IN RABBIT ISOLATED PULMONARY RESISTANCE ARTERIES

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Following birth there are structural and functional alterations in the pulmonary circulation, with a marked reduction in the pulmonary vascular resistance. We determined how the responsiveness of the small pulmonary resistance arteries (PRAs) to adrenergic and cholinergic stimulation alters with developmental age. Foetal, neonatal and adult rabbits were killed with sodium pentobarbitone. Small PRAs (I.D. ~250μM) were dissected out and mounted as ring preparations (2mm length) on a wire myograph (under ~125mg tension) in Krebs bubbled with 3% O_2/6% CO_2/ balance N_2 for the foetal vessels and 16% O_2 for all others. Initially, the vessels were contracted twice with 50mM KCl. NA (μM) was then added and upon the induced tone, 1μM ACh was added to evoke relaxation.

The results for NA are shown in Table 1. Contraction to NA was observed in PRAs from foetal, 0-24 hours and 4 day rabbits. However, this was absent in 70% of the 7 day vessels and was non-existent in those obtained from adult rabbits. The greatest response to adrenoceptor stimulation was obtained in 0-24 hour PRAs. This implies that the functional population of adrenoceptors of the PRAs is at it's greatest at birth and then begins to decline with increasing age. Alterations in the responses to ACh was also observed at different age points (Table 1); as has previously been shown in porcine intra-pulmonary arteries (Liu *et al.*, 1992). The greatest relaxation was seen at 4 days, with the poorest being obtained in PRAs from 0-24 hour animals. This is indicative of low levels of nitric oxide (NO) at birth and may account for the marked contraction observed to NA as potentiation of NA-evoked responses by L-NAME is seen in the larger branches of the rabbit PA (MacLean *et al*, 1993). In rabbit PRAs therefore, the contractile response to NA is inversely related to the ability of the tissue to relax to ACh and this relaxation is relatively small in 0-24 hour neonates.

C. Docherty holds a MRC PhD Studentship. This work was also funded by The Wellcome Trust and Tenovus, Scotland. This laboratory is a member of the EUC Biomed project "EureCa" BMHI-CP94-1375.

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Age	Contractile response to 1μM NA (as % 50mM KCl)	n/n	Relaxatory response to 1μM ACh (as % 1μM NA)	n/n
Foetus	26 ± 6*	15/15	99 ± 9 *	17/17
0-24 h	81 ± 21	12/12	40 ± 22	11/11
4 days	22 ± 7*	8/7	122 ± 13 **	11/10
7 days	2 ± 1**	6/6		
Adult	0 ± 0**	12/6		

Table 1. Alterations in the response to NA and ACh with developmental age. n/n=number of vessels/number of animals. Statistical difference from 0-24 hours response, Students unpaired t-test, *p<0.05, **p<0.01.

411P EFFECTS OF PROPRANOLOL AND L-NAME ON B-ADRENOCEPTOR-MEDIATED RELAXATION IN BOVINE PULMONARY ARTERY *IN VITRO*

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β-Adrenoceptor-mediated vasodilation is generally recognized to involve the activation of adenylate cyclase in smooth muscle, although Gray & Marshall (1992) have shown endothelium dependence in rat aorta. Atypical β-adrenoceptors (β_3-adrenoceptors) may also be present in some blood vessels (Oriowo, 1994, McLean *et al.*, 1995). The present experiments were carried out to investigate further the properties of β-adrenoceptors in the bovine pulmonary artery.

Bovine lungs obtained from the abattoir were transported to the laboratory in a sealed bag containing ice cold physiological salt solution (PSS). Ring preparations (4 mm) of the pulmonary artery were suspended in organ baths under 1g of tension. The PSS was maintained at 37°C, gassed with 95% O_2/5% CO_2 and contained EDTA (30 μM) and ascorbic acid (30 μM) to prevent oxidation of isoprenaline. In order to minimise phasic contractions to vasoconstrictor agents, the arteries were first constricted with depolarising solution (120 mM KCl). After washing, the artery rings were constricted with a sub-maximal concentration of the thromboxane-mimetic, U46619 (1 μM), and cumulative concentration curves to β-agonists constructed. In experiments with propranolol, tissues were equilibrated with propranolol (1 μM) for 30 minutes before addition of U46619, untreated tissues acting as controls. In experiments with the nitric oxide synthase inhibitor, L-NAME, two CRCs were performed, the second CRC in the presence of L-NAME. Paired tissues acted as time controls. In some experiments endothelium was removed by gentle rubbing. Endothelial cell denudation was confirmed by lack of relaxation to acetylcholine (1 μM) after phenylephrine-induced constriction. Tissues with intact endothelium were used as controls. Results are expressed as means ± s.e.mean with the number of observations in parentheses. The significance of differences was determined using unpaired Student's *t* test.

Isoprenaline produced a concentration-dependent relaxation of the U46619-constricted vessels. Propranolol (1 μM) shifted the isoprenaline concentration-response curve, with an estimated pA_2 of 9.2 (-logEC30 without and with propranolol: 8.5±0.1 (7), 5.3±0.3 (7), $P < 0.001$). Neither the β_3-adrenoceptor agonist BRL 37344 nor partial agonist CGP 12177A produced relaxation of the arteries at concentrations up to 30 μM, while ZD2079, also a β_3-adrenoceptor agonist (Grant *et al.*, 1994), produced limited relaxation at high concentrations (50% relaxation at 30 μM).

L-NAME (100 μM) produced a 3-fold rightward shift of the Isoprenaline CRC (pD_2 before and after L-NAME: 7.4±0.05 (4), 6.9±0.1 (4), $P < 0.05$). Removal of endothelium by rubbing produced a similar shift (pD_2s with and without endothelium: 7.3±0.05 (3), 6.8±0.02 (3), $P < 0.01$).

The sensitivity to propranolol and lack of response to β_3-adrenoceptor agonists suggests that relaxations to isoprenaline in bovine pulmonary artery are mediated by classical β-adrenoceptors with no contribution to the response from atypical β-, or β_3-, adrenoceptors. A small component of the response to isoprenaline appears to be endothelial-dependent, involving nitric oxide.

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412P SIMILARITY OF RESPONSE TO VASOCONSTRICTORS IN PORCINE HEPATIC AND HUMAN HEPATIC AND MESENTERIC ARTERIES *IN VITRO*

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Vascular hyporesponsiveness to vasoconstrictors has been reported in patients with hepatic cirrhosis (MacGilchrist and Reid, 1990). Investigation of this phenomenon using the human hepatic artery *in vitro* is restricted by difficulties encountered in obtaining donor hepatic artery to act as a control. The aim of this study was to determine whether porcine hepatic and human mesenteric arteries would be suitable alternative controls in such investigations.

Porcine hepatic arteries were obtained from an abattoir immediately following sacrifice. Human mesenteric and hepatic arteries were obtained at hepatic transplantation. 2mm rings of each artery were mounted in organ baths for measurement of isometric force. Optimum resting tension (4g; n=3-6) was determined by adding 80mM KCl to the vessels as the resting force was increased. Concentration response curves to phenylephrine (PE; 10^{-9} - $3x10^{-4}$M), noradrenaline (NA; 10^{-9} - $3x10^{-4}$M), angiotensin II (AII; 10^{-11} - $3x10^{-7}$M) and potassium chloride (KCl; 2.5 - 140mM) were obtained. Endothelial cell function was assessed by adding acetylcholine (ACh; 10^{-5}M) and SIN-1 (10^{-5}M) to vessels preconstricted with NA (10^{-5}M) and the endothelium was stained using 0.4% silver nitrate to assess integrity. All vessels produced large contractions to PE, NA and KCl (Table 1) with no significant differences in sensitivity or maximum contraction (determined using oneway analysis of variance with a Tukey multiple range test). AII failed to produce reproducible contractions in the three vessel types. ACh induced mostly contraction in the vessels whereas SIN-1 induced relaxation (Table 1). Staining confirmed endothelial denudation.

Isolation techniques led to endothelial cell denudation in all arteries. Pig hepatic and human mesenteric and hepatic arteries respond in a similar way to PE, NA, KCl and AII. The reasons for the lack of response to AII remain to be elucidated. This work suggests that pig hepatic and human mesenteric arteries are suitable for use as control vessels in investigations of hepatic artery smooth muscle hyporesponsiveness in cirrhosis.

This work was funded by MRC Grant G9332418PA

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Table 1. Maximum contraction and -logEC_{50} responses to vasoconstrictors and maximum relaxation values to vasodilators.

	Maximum Contraction (g)			-LogEC_{50}				Maximum relaxation (% reversal of tone)		
	PH	HH	HM	PH	HH	HM		PH	HH	HM
PE	8.8±2.1 (5)	7.8±1.6 (7)	12.6±2.1 (7)	5.52±0.45 (5)	5.84±0.17 (7)	5.49±0.17 (7)	ACh	-90.6±43.4 (7)	-12.9±10.5 (5)	11.7±8.6 (5)
NA	12.7±1.2 (9)	6.4±1.5 (5)	11.9±2.3 (8)	5.64±0.13 (9)	6.20±0.18 (5)	5.88±0.28 (8)	SIN-1	73.3±12.0 (6)	51.1±29.8 (4)	63.3±20.8 (5)
KCl	10.6±0.8 (9)	6.6±1.8 (7)	11.5±2.2 (6)	1.59±0.03 (9)	1.55±0.09 (7)	1.55±0.07 (7)				

Results are given as mean ± s.e.mean, n numbers (of animals) in brackets. PH = pig hepatic, HH = human hepatic, HM= human mesenteric arteries. A negative value indicates a contraction in response to the vasodilator agents.

413P CONSERVATION OF RESPONSE TO VASOCONSTRICTOR COMPOUNDS IN RAT MESENTERIC ARTERY STORED IN PHYSIOLOGICAL SALT SOLUTION (PSS) AT 4 °C

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Resistance arteries obtained from human subcutaneous fat biopsies are regularly used in functional investigations, using small vessel myography (Aalkjaer et al., 1986). Due to the scarcity of human tissue it would be advantageous to utilise all vessels obtained. Whilst it is accepted that vascular tissue can be stored overnight in PSS at 4°C without loss of viability, there are little data to show whether storage can be continued for longer periods. This investigation aimed to determine whether rat mesenteric arteries (a readily available source) show altered responsiveness to vasoconstrictors when stored in PSS for up to 4 days to indicate potential use with human tissue.

Mesenteric arteries (80-200μm diameter) were dissected from the mesenteric bed of male Cob rats. These were divided into six 5mm sections, one of which was used immediately whilst the others were stored in PSS at 4°C. Isometric force was measured on the day of sacrifice and on each of the subsequent four days by mounting the vessels in a wire myograph and equilibrating them at their optimum resting force ($0.9L_{100}$ - see Alkjaer et al., 1986). Concentration response curves were obtained to phenylephrine (PE; 10^{-9} - $3x10^{-5}$M), noradrenaline (NA; 10^{-9} - $3x10^{-5}$M), potassium chloride (KCl; 2.5-140mM) and endothelin-1 (ET-1; 10^{-11} - $3x10^{-7}$M).

Arteries continued to contract strongly in response to each of the vasoconstrictors even after four days in PSS. Vessel storage caused a trend towards increased sensitivity to all four vasoconstrictors which was significant when vessels constricted with NA and analysed on day 3 and day 4 were compared with those analysed on day 1 (Table 1). These data fail to demonstrate a significant change in maximum response during storage (Table 1).

These results indicate that small resistance vessels will remain viable if stored for up to 4 days in PSS at 4°C. The time-dependent increase in sensitivity may be a result of gradual endothelial dysfunction but more work is required to verify this. These results suggest that vessels may be stored for up to 2 days for analysis of functional response to vasoconstrictors; after this time sensitivity will be altered.

This work was funded by MRC Grant G9332418PA

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Table 1. Maximum contraction and -logEC_{50} responses to vasoconstrictors.

	Maximum contraction (mN/mm)					-LogEC_{50}				
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
PE	2.3±0.3 (7)	2.4±0.5 (6)	4.1±1.0 (8)	2.0±0.6 (6)	2.5±0.5 (6)	5.34±0.14 (7)	5.54±0.11 (6)	5.83±0.09 (8)	5.84±0.23 (6)	5.92±0.28 (6)
NA	2.8±0.5 (8)	2.4±0.5 (6)	4.4±0.9 (8)	3.0±0.8 (6)	3.7±0.9 (4)	5.99±0.10 (7)	5.68±0.16 (6)	6.06±0.10 (8)	6.30±0.16* (6)	6.54±0.20* (4)
KCl	1.8±0.2 (10)	1.9±0.3 (6)	3.0±0.4 (11)	2.2±0.4 (6)	2.3±0.5 (6)	1.64±0.05 (10)	1.62±0.06 (6)	1.65±0.05 (11)	1.71±0.13 (6)	1.83±0.14 (6)
ET-1	3.7±0.5 (6)	4.2±1.4 (6)	5.6±0.9 (10)	2.8±0.4 (6)	4.2±1.0 (4)	8.18±0.13 (6)	7.79±0.95 (6)	8.46±0.17 (10)	8.43±0.08 (6)	8.58±0.18 (4)

Results are given as mean ± s.e. mean, n numbers in brackets.*P<0.05 when compared with -LogEC_{50} value for day 1, using oneway analysis of variance with a Tukey multiple range test.

414P THE EFFECT OF ALLYLAMINE ON THE TENSION RESPONSES OF CERVINE DIGITAL ARTERIES *IN VITRO* TO NORADRENALINE

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The unsaturated aliphatic amine, allylamine, is highly toxic to rats, through production of necrotic lesions of the myocardium and arterial smooth muscle as well as smooth muscle cell proliferation in the intima of damaged vessels (Boor & Hysmith, 1987; Hysmith & Boor, 1987). The causative agent is the α,β-unsaturated aldehyde, acrolein, produced *in vivo* and *in vitro* by the action of semicarbazide-sensitive amine oxidase (SSAO; EC 1.4.3.6; Boor *et al.*,1990). Other xenobiotic and endogenous amine substrates such as methylamine and aminoacetone have also been suggested to produce potentially toxic aldehydes, which may have clinical relevance in ruminants as well as in human subjects (see Callingham *et al.*, 1995).

In order to establish an *in vitro* preparation from a ruminant species, digital arteries were rapidly dissected from fallow deer (*Dama dama*) at slaughter, placed in cold aerated physiological salt solution and transported to the laboratory. Artery segments were mounted at 40 mN tension in organ baths for isometric recording. Responses to noradrenaline (0.01-30 μM) were recorded in the absence or presence of allylamine (1-100μM), added 1, 2 or 4 h previously. The observations were repeated after incubation with semicarbazide (SC; 100 μM, to inhibit SSAO) for 1 h before addition of allylamine. Each experiment was performed 6 times on two rings from each of 6 deer (n = 6). A tension response relation was obtained for each ring at 0, 1, 2 and 4 h. Fresh rings were used for each concentration of allylamine with or without SC. The response was expressed for each ring as percent of its maximum tension at the start of the experiment (0 h), before addition of allylamine.

Following incubation with allylamine for 1, 2, or 4 h significant reductions in vascular reactivity to noradrenaline were seen, which were prevented by the addition of SC.

Table 1: The effect of exposure to allylamine for 2 h, in the presence and absence of semicarbazide, on the tension developed by deer digital artery in response to noradrenaline.

Drug treatment	EC_{50} (μM ± s.e.mean)	max. tension (% ± s.e.mean)
none (control)	0.59 ± 0.03	99.5 ± 1.4
allylamine (10 μM)	1.45 ± 0.1 ***	57.2 ± 1.2***
allylamine, (30 μM)	7.51 ± 0.01***	9.10 ± 0.01***
allylamine (10 μM) + SC	0.54 ± 0.06	96.3 ± 2.3
allylamine (30 μM) + SC	0.62 ± 0.08	96.5 ± 2.8

*** = significantly different from control ($P < 0.001$)

These results indicate that exposure of deer digital arteries to allylamine (10 or 30 μM) for 2 h *in vitro* significantly reduced the maximum response to noradrenaline with a significant increase in EC_{50}. These effects could be prevented by prior incubation with SC, suggesting that allylamine acts through being deaminated to its toxic aldehyde by vascular SSAO, as in the rat. SSAO inhibitors *in vivo* could reduce the toxicity of this and other amines when ingested or produced in excess.

E.U. Human Capital Programme No: ERBCHRXCT930256 supported this work and F.F. (Siena) and L.C. (Florence)

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415P DIFFERENCES IN CONTRACTILE AND RELAXANT RESPONSES OF AORTAE FROM STREPTOZOTOCIN-INDUCED DIABETIC AND OBESE ZUCKER RATS *IN VITRO*

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The obese Zucker rat represents an animal model of non-insulin-dependent (type II) diabetes mellitus (Kurtz *et al.*, 1989) the aortae from which have been shown to be hyperreactive to the spasmogen 5-hydroxytryptamine (5-HT) (Cox and Kikta, 1992; Growcott *et al.*, 1995) whilst relaxation to acetylcholine (ACh) and glyceryl trinitrate (GTN) was impaired (Growcott *et al.*, 1995). This study evaluates these agents in aortae from an animal model of insulin-dependent (type I) diabetes mellitus, *ie* the streptozotocin (STZ)-dosed rat. In addition, effects of Ca^{++} manipulation have been assessed in aortae from both types of rat.

Aortic rings from male obese and lean Zucker rats (5 months old) and from Alderley Park male Wistar rats 21 weeks after treatment either with a single dose of citrate buffer (pH 4.5) or STZ (45 mg/kg ip) were set up in physiological salt solution (PSS) at 37°C, under 2g tension, to record isometric tension changes. Cumulative additions of 5-HT (10nM-100μM) were made in the absence and presence of verapamil (1μM) and relaxations to cumulative additions of ACh and GTN in 5-HT (10μM) pre-tensioned aortae. Contractile effects of 5-HT (10μM, an EC_{90}) were also assessed in normal PSS and in PSS where Ca^{++} had been omitted and EGTA (1mM) added. Mean pD_2 values with 95% confidence limits and maximum tensions (±s.e.mean) were calculated. $P<0.05$ represents statistical significance.

Aortae from STZ rats were more responsive to 5-HT than their age-matched controls (maximum tension 0.42±0.04 and 0.29±0.03 g/mg, respectively, n=4-6, $P<0.01$). However, sensitivities to 5-HT were identical (pD_2's: 5.9 [6.4-6.7] and 6.0 [6.1-5.9] in aortae from STZ and control rats respectively, n=4-6).

In normal PSS the response of the aortae from STZ rats to 5-HT (10μM) was significantly greater than that observed in aortae from obese Zucker rats (0.34±0.02 and 0.24±0.06 g/mg, respectively, n=4, $P<0.01$). In PSS where Ca^{++} was omitted, responses to 5-HT (10μM) were significantly reduced but were not different from each other (0.03±0.007 and 0.02± 0.002 g/mg in aortae from STZ and obese Zucker rats, respectively, n=4-6, $P<0.001$). Verapamil (1μM) caused a similar degree of inhibition of the responses to 5-HT in the aortae from STZ and obese Zucker rats (concn. ratios 15 [8-126] and 10 [2-11] respectively, n=4) and maximal responses to 5-HT were reduced by 40±10 and 22±7%, respectively.

Although sensitivities of aortae from control and STZ rats to ACh were identical (pD_2's: 8.0 [8.1-7.9] and 8.1 [8.2-7.9], respectively, n=4), these values were significantly greater ($P<0.01$) than those obtained previously in the aortae from the obese and lean Zucker rats (*ie*, pD_2's: 6.5 and 6.9, respectively). Similarly, although sensitivities to GTN of aortae from controls and STZ rats were identical (pD_2's: 8.0 [8.2-7.8] and 8.0 [9.2-7.7] respectively, n=4) these values were significantly greater ($P<0.01$) than those we have previously reported in aortae from obese Zucker rats (*ie*, pD_2: 6.3).

Hence, the aortae from STZ rats exhibited hyperresponsiveness to 5-HT which appears to be unrelated to either an impairment of relaxation - unlike the aortae from obese Zucker rats - or a modification in Ca^{++} mobilization. These differences may be as a consequence of the insulin-sensitive and insulin-resistant states that prevail in tissues from STZ-treated and obese Zucker rats, respectively.

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416P AIRWAY AND CARDIOVASCULAR EFFECTS OF SUBSTANCE P IN THE ANAESTHETISED MINI-PIG

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Substance P (SP) is a less potent bronchoconstrictor than neurokinin A in a variety of species. However, SP is reported to be more potent than neurokinin A in the piglet (Dreshaj et al., 1994). Because of its potential utility to further investigate SP antagonists, we sought to characterise the airway and cardiovascular effects of SP in the *adult* mini-pig.

Male Göttinger mini-pigs (10- 15 kg) were anaesthetised (pentobarbital) and ventilated. Transpulmonary pressure and tracheal air flow (pneumotachograph) were measured to determine tidal volume, and loops constructed to determine lung compliance (C_{dyn}) and resistance (R_L). Arterial blood pressure (ABP), left ventricular (LV) pressure (Millar) and LV volume (conductance catheter) were recorded and indices of LV preload (end diastolic volume; EDV), afterload (arterial elastance; E_a) and contractility (end systolic elastance; E_{es}) calculated, from pressure-volume loops. SP (0.01- 10 nmol/kg; n= 6) or vehicle (saline; n= 5) was administered i.v. as a 2 min infusion, with 20 min recovery between each dose.

Table 1: Airway and cardiovascular effects of SP

	baseline	0.1	1.0	10 nmol/kg
C_{dyn} (ml/cm H_2O)	11.3 ± 0.8	10.5 ± 1.0	6.8 ± 1.5*	2.3 ± 0.3***
R_L (cm H_2O/L/s)	10.2 ± 1.3	10.5 ± 1.2	33.4 ± 14.4	74.6 ± 15.6*
ABP (mmHg)	113 ± 4	83 ± 12**	59 ± 3**	51 ± 2**
EDV (ml)	28.2 ± 3.5	22.4 ± 3.2	16.8 ± 1.9***	15.5 ± 2.4***
E_a (mmHg/ml)	12.8 ± 1.6	10.2 ± 2.5	6.5 ± 0.8***	6.5 ± 0.7***
E_{es} (mmHg/ml)	6.6 ± 1.0	8.3 ± 0.9	10.1 ± 1.1*	9.5 ± 1.3**

mean ± s.e.mean;
* P< 0.05, ** P< 0.01 *** P< 0.001, vs. vehicle control (ANCOVA)

All measured parameters of the vehicle control group (data not shown) were stable over the protocol. SP, at doses exceeding 0.1 nmol/kg, elicited dose-dependent effects on airway smooth muscle tone (Table 1 & Fig 1ab) manifested as an increase in R_L as well as a decrease in C_{dyn}. Cardiovascular effects (Table 1 & Fig. 1c) of SP were evident at the lowest dose with a marked fall in ABP secondary to a decrease in LV preload (EDV) and afterload (E_a), suggesting that SP is both an arterial and venous dilator. There was also a small increase in LV contractility, which may be of reflex origin.

In conclusion, SP had potent airway (bronchoconstriction) and cardiovascular effects (arterial and venous dilation). The mini-pig may therefore be a useful model to investigate the pharmacological profile of SP antagonists.

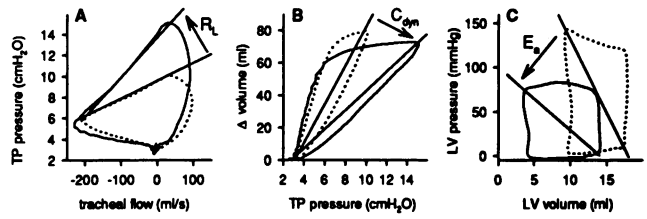

Fig 1: Representative airway (a,b) and cardiovascular (c) loops, recorded before (···) and after (—) 10 nmol/kg SP from one expt.

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417P EFFECTS OF VANADATE IN HUMAN ISOLATED BRONCHUS

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Occupational inhalation of vanadium compounds, particularly vanadate, causes bronchial asthma (Musk & Tees, 1982). We have studied the characteristics of vanadate-induced contraction of human isolated bronchus (3-4 mm i.d.; isometric recording; resting tension 2 g; data are mean±s.e.mean).

Vanadate (0.1μM-1mM) produced sustained, concentration-related, contractions of bronchial muscle with pEC_{50}=3.78±0.05 (n=9) and a maximal effect of 94.8±5.9% of the contraction elicited by acetylcholine (1mM). Vanadate (200μM ≈ EC_{50})-induced contraction of human bronchus was independent of the presence of epithelium, and it was not inhibited by indomethacin (2.8μM) or by a mixture of atropine, mepyramine and phentolamine (each at 1μM) nor after mast cell depletion by compound 48/80 (two consecutive challenges of 100μg/ml), indicating that contractions were not mediated by a number of the known endogenous mediators of epithelial, inflammatory or neural origin.

Vanadate (200μM)-induced contractions were not changed after 30 min incubation in Ca^{2+}-free (EGTA 0.1mM) solution nor after verapamil or nifedipine (each at 1μM), indicating that contraction to vanadate was not reliant on influx of extracellular Ca^{2+}. Incubation in Ca^{2+}-free (EGTA 0.1mM), solution containing ryanodine (10μM) reduced vanadate-induced contraction (57±5% absence vs. 36±3% presence of ryanodine, n=4, P<0.05), indicating the contribution of the intracellular sources of Ca^{2+} in the action of vanadate.

Incubation with ouabain (10μM) or in K^+-free solution did not alter the bronchial contraction to vanadate (200μM) suggesting that inhibition of the Na^+/K^+-ATPase was not involved in the contractile action of vanadate. This was further supported by (1) the finding that ouabain (10μM) but not vanadate (200μM) abolished the K^+-induced relaxation (0.60±0.02 g for KCl 30mM) of human bronchus bathed in K^+-free medium; and (2) Na content (modified Li method, atomic absorption spectrophotometry) was not increased in vanadate (200μM)-treated tissues (5.9±1.5 nmol/mg in control vs. 6.3±1.8 nmol/mg in treated tissues, n=4). Incubation with a low Na^+ (25mM) medium or amiloride (0.1mM) markedly reduced (about 75% inhibition) the constrictor effect of vanadate (200μM) thus suggesting a role for Na/Ca exchange in the contractile action of vanadate. The protein kinase C inhibitor staurosporine (1μM) had no effect on vanadate-induced contraction. Vanadate (200μM) did not change the concentration-response curve of acetylcholine (1nM-1mM) or KCl (0.1-100mM) but depressed that of histamine (1nM-1mM; E_{max}=1.26±0.40 g in control vs. 0.47±0.05 g in treated tissues, n=7, P<0.05).

In conclusion, vanadate, at micromolar concentrations, produced a sustained contraction of human isolated bronchus. This action appears related to intracellular Ca^{2+} mobilization and did not cause in vitro hyperreactivity to spasmogens.

Supported by SAF93-132 & SAF94-838 from CICYT (Spain)

Musk, A.W. & Tees, J.G. (1982) Med. J. Aust., 1, 183-184.

418P REVERSAL OF METHACHOLINE-INDUCED CONTRACTION IN ISOLATED BRONCHIAL RINGS FROM CHRONICALLY HYPOXIC AND CONTROL RATS

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Sustained alveolar hypoxia can be a complication of airway diseases such as chronic obstructive pulmonary disease. The hypoxic/hypobaric chamber creates a state of chronic global hypoxia in experimental animals and may therefore be a useful model for such diseases. We have previously shown (Clayton *et al.*, 1994) that chronic exposure to hypoxia significantly attenuates responses evoked by the spasmogens methacholine and endothelin-1 in isolated rat bronchi. It is not known, however, if responses to bronchodilators are also altered. This study compared responses evoked by the dilators atrial natriuretic peptide (ANP), salbutamol and sodium nitroprusside (SNP) in isolated bronchi from control and chronically hypoxic rats. 28-30 day old male Wistar rats were reared in a hypoxic/hypobaric chamber for 14 days under a pressure of 500-550millibars, giving effective gas concentrations of 10% O_2, 0.03% CO_2 with the balance N_2. Rats were killed by sodium pentobarbital overdose and the primary bronchi excised. In Krebs-Henseleit solution of standard composition gassed with 95% O_2, 5% CO_2 and maintained at 37°C, responses evoked in rings of primary bronchi were measured isometrically. Tissues were challenged with methacholine ($3x10^{-7}$M) and once the contractions had reached a plateau, cumulative concentration-response curves were obtained for ANP (10^{-9}-10^{-6}M, in the presence of phosphoramidon $3.7x10^{-5}$M to prevent hydrolysis), salbutamol (10^{-9}-10^{-5}M) and SNP (10^{-9}-10^{-5}M) (n=6 in each case). Responses are expressed as the mean ± s.e.mean. Significance between data sets was analysed by two-way analysis of variance (ANOVA) while significance between data points was analysed by Student's t-test.

In bronchi from both control and chronically hypoxic rats, salbutamol, ANP and SNP each reversed methacholine-induced tone in a concentration dependent manner. ANP was the most potent agent used in this study, initiating relaxation at concentrations of $3x10^{-8}$M in control bronchi and 10^{-9}M in hypoxic rat bronchi. Chronic exposure to hypoxia did not alter responses evoked by salbutamol, however responses to both ANP and SNP were significantly enhanced ($p<0.01$ and $p<0.001$, respectively), across the whole data set, in bronchi from hypoxic rats compared to controls. The highest concentration of ANP we were able to achieve was 10^{-6}M and at this concentration, ANP reversed 25.75±4.53% of the induced tone in control rat bronchi and 41.01±9.26% in hypoxic rat bronchi. Salbutamol, at the 10^{-6}M level, reversed 24.55±6.00% of the methacholine-induced tone in control rat bronchi and 28.08±6.34% in hypoxic rats. At a concentration of 10^{-6}M, SNP was significantly ($p<0.05$) more effective in hypoxic rat bronchi, reversing 53.24±5.65% of the induced tone, compared to 28.04±6.11% in control bronchi. These results suggest that while chronic exposure to hypoxia attenuates responses to the spasmogens methacholine and endothelin-1 in isolated rat bronchi, it enhances the responses to some, but not all dilators.

Clayton, R.A., Nally, J.E., MacLean, M.R., Thomson, N.C. & McGrath, J.C. (1994). *Br. J. Pharmacol.*, **113**, 163P.

This work was supported by the Chest Heart and Stroke Assoc. (Scotland) and the National Asthma Campaign.

419P A SIMPLE METHOD FOR THE EVALUATION OF CENTRAL AORTIC STIFFNESS IN AWAKE RATS

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Measurement of arterial stiffness in small animals with a rapid heart rate is hampered by the difficulties involved in the assessment of impedance based on arterial dynamics and the small vessel diameter. As a method for the measurement of aortic input impedance applicable to non anaesthetized intact rats is lacking, we describe a simple method based on the determination of aortic pulse wave velocity in awake rats. Adult, male Wistar rats were chronically cannulated with either PE50/PE50 cannula (polyethylene, 0.96 mm od, 0.58 mm id) under halothane anaesthesia in both the thoracic and the abdominal aorta (n=8) or a PE50/PE50 cannula in the thoracic aorta and a PE50/PE10 cannula (0.61 mm od, 0.28 mm id) in the abdominal aorta (n=7). The smaller diameter abdominal aorta cannula was tested as this is easier to implant and often used in rats for the determination of abdominal aortic blood pressure. Aortic thoracic and abdominal pressures were measured in awake rats and transit time was determined on-line by a computer algorithm which brought forward the waveform of the distal pressure signal until it could be superimposed on that of the proximal pressure signal (method based on that proposed by McDonald, 1968). The goodness of fit of superimposition of the 2 pressure waveforms was estimated by least squares analysis. Pulse wave velocity (cm/s) was calculated as the distance between the 2 cannula tips (measured following post-mortem dissection) divided by the transit time. Aortic pressure wave amplification (abdominal / thoracic aortic pulse pressure) and elastic modulus ($E = 2*PWV^2*R/h$ with PWV=velocity, h=wall thickness and R=internal radius; Bergel, 1961) were calculated following histomorphometry of the thoracic aorta. Results (Table 1) are expressed as means ± s.e.mean.

Table 1. Blood pressure, wave velocity, aortic dimensions and elastic modulus

Diameter intra-aortic cannula	large	small
Thoracic mean arterial pressure (mmHg)	118 ± 4	118 ± 4
Thoracic pulse pressure (mmHg)	39 ± 2	39 ± 2
Abdominal pulse pressure (mmHg)	56 ± 4	38 ± 6*
Pulse wave velocity (cm/s)	507 ± 57	349 ± 30*
Aortic pressure wave amplification	1.5 ± 0.1	1.0 ± 0.1*
Internal diameter (mm)	1.6 ± 0.1	1.5 ± 0.1
Wall thickness (mm)	83 ± 5	80 ± 5
Elastic modulus (10^6 dynes/cm^2)	5.1 ± 0.9	2.4 ± 0.3*

* = $P<0.05$ *versus* large diameter (ANOVA plus Bonferroni test)

Pulse wave velocity, aortic pressure wave amplification and elastic modulus were lower in rats fitted with a small diameter intra-aortic cannula due to damping of the abdominal pressure waveform. In conclusion, we propose a new simple method for the evaluation of central aortic stiffness in awake, unrestrained rats; care should be taken as to the diameter of the intra-aortic cannula used.

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This laboratory is a member of the EUC Biomed project "EureCa" BMHI-CP94-1375.

420P 4-AMINOPYRIDINE-INDUCED RESPONSES IN THE PRE-CONSTRICTED RAT SUPERIOR MESENTERIC VASCULAR BED ARE DEPENDENT ON THE CONSTRICTOR AGENT USED

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Potassium channels are important regulators of membrane potential and contraction in vascular smooth muscle (VSM) (Knot & Nelson 1995). But, when examining the effects of drugs which block potassium channels in whole blood vessels it is important to consider the effects of these agents in the presence of different contractile agents. It is also important to consider possible effects on other cell types which are present in whole blood vessels. The rat superior mesenteric bed not only contains VSM, but also a large endothelial bed capable of producing vasoactive substances *eg* nitric oxide(NO). We have used this preparation to examine the interactions of the potassium channel blocker 4-Aminopyridine(4-AP) with different contractile agents.

Male Wistar rats (300-350g) were anaesthetised with Sagatal prior to cervical disslocation. The mesenteric bed was dissected and perfused ($5ml.minute^{-1}$) with Krebs-Henseleit solution at 37°C. Mesenteric perfusion pressure (MPP) was increased with either 80mM K^+, 3nM endothelin-1(ET-1) or 96μM phenylephrine (PE) and the effects of 5min perfusion with 4-AP(10mM) examined. When required 100μM Nitro-L-Arginine (NOARG) was perfused through for 15 minutes prior to 5 minute application of 4-AP. This was followed by a 10 minute washout of 4-AP. In some experiments, MPP was increased by increasing the flow rate to 14 or $22ml.min^{-1}$. Results expressed as mean ± s.e.m., n=4. Statistical analysis performed using a paired t-test except where stated.

Under basal conditions at the three different flow rates 4-AP produced a flow dependent monophasic reversible increase in MPP from 31±1, 57±2 and 93±1mmHg to 38±2, 82±6 and 126±8mmHg respectively ($p<0.05$).

At $5ml.min^{-1}$, ET-1, K^+ or PE increased MPP to 60±7, 80±6 and 109±8mmHg respectively. When 10mM 4-AP was applied to these preconstricted preparations different results were obtained depending on the vasoconstrictor used.

In the presence of ET-1, 4-AP increased MPP to 143±36mmHg ($p<0.05$). This reversed on washout. In contrast, in K^+ preconstricted preparations 4-AP caused a transient dilation to 68±2mmHg followed by an increase in MPP to 93±7mmHg. On removal of 4-AP, MPP further increased to 112±9mmHg($p<0.05$) before returning to control levels. In PE preconstricted preparations, 4-AP caused a rapid decrease in MPP from 109±8mmHg to 62±5mmHg ($p<0.01$), followed by a recovery to 101±6mmHg after 5 minutes. On removal of 4-AP, MPP showed a rebound increase to 170±8mmHg ($p<0.01$) before returning to control levels.

100μM NOARG did not inhibit the 4-AP-induced vasodilation in the K^+ and PE preconstricted preparations. However it significantly potentiated the increase in MPP associated with the removal of 4-AP in these two experiments ($p<0.05$ TWO WAY ANOVAR).

Our data shows that 4-AP produces distinct vascular effects depending on the contractile agents used and highlights the need to carry out functional experiments using whole blood vessels as well as single cell studies.

D.A.W. is a School of Pharmacy & Pharmacology research student.

Knot, H.J. & Nelson, M.T. (1995). *Am. J. Physiol.*, **269**, H348-H355.

421P ZD9989 IS A POTENT, SELECTIVE β_3-ADRENOCEPTOR AGONIST *IN VITRO*

T.L. Grant, R.M. Mayers, S.P. Quayle, R. Howe, B.S. Rao, J.W. Growcott and B.R. Holloway. ZENECA Pharmaceuticals, Alderley Park, Cheshire, SK10 4TG.

It is now apparent that, in addition to β_1 and β_2-adrenoceptors, there exists a third β-adrenoceptor known as the atypical- or β_3-adrenoceptor (Granneman *et al,* 1992). The aim of this paper is to describe the *in vitro* β-adrenoceptor pharmacology of ZD9989 ((R)-N-(2-[4-(carboxymethyl)phenoxy]ethyl)-N-(β-hydroxy-β-[3-chlorophenyl]ethyl)ammonium chloride), a novel β_3-adrenoceptor agonist.

Adipocytes were prepared from white epididymal fat from male Wister rats, weight range 170-200g. Measurement of free fatty acid release (Quayle *et al,* 1993) revealed that ZD9989 increased lipolysis in white adipocytes with an EC_{50} of 0.04μM (n=2). ZD9989 had a maximal effect (109±4%) equivalent to that evoked by BRL 37344 (104±2%; n=9), a standard β_3-adrenoceptor agonist, and isoprenaline (100%). In carbachol (0.5μM)-precontracted rat ileum (Growcott *et al,* 1993) which had been pretreated with β_1- and β_2-adrenoceptor antagonists (CGP20712A and ICI 118551; both at 1μM), ZD9989 produced a concentration-related relaxation of tone with a EC_{50} value of 0.14μM (95% confidence limits: 0.11-0.17uM) and a maximal effect (127±27%) which was equivalent to that evoked by isoprenaline (100%) (n=4).

The effects of ZD9989 (10μM) in guinea-pig right atria (β_1-adrenoceptors) and tracheal chain (β_2-adrenoceptors) were assessed as described by Growcott *et al* (1993). In atria, ZD9989 (10μM) increased the basal rate by only 12±2% and produced only a 4-fold rightward shift in the concentration-chronotropic response curve to isoprenaline (n=7). In trachea, ZD9989 (10μM) had no significant agonist or antagonist effects (n=4).

In conclusion, ZD9989 is a potent, selective β_3-adrenoceptor agonist. Relative to standard β-adrenoceptor agonists, ZD9989 is a full agonist at the β_3-adrenoceptor in white adipocytes which mediates lipolysis and at the β_3-adrenoceptor in rat ileum which mediates smooth muscle relaxation. ZD9989 had only modest effects at β_1-adrenoceptors and had no activity at β_2-adrenoceptors at the concentration tested. ZD9989 may be a useful tool with which to investigate the physiological and patho-physiological roles of β_3-adrenoceptors.

Granneman, J.G. *et al* (1992). Mol. Pharmacol., 42, 964-970.
Growcott, J.W. *et al* (1993). Br. J. Pharmacol., 109, 1212-1218.
Quayle, S.P. *et al* (1993). Br. J. Pharmacol., 108, 169P.

422P ZD9989 IS A POTENT, SELECTIVE β_3-ADRENOCEPTOR AGONIST *IN VIVO*

T.L. Grant, R.M. Mayers, M.G. Briscoe, R. Howe, B.S. Rao, and B.R. Holloway. ZENECA Pharmaceuticals, Macclesfield, Cheshire, SK10 4TG.

It is now apparent that, in addition to β_1 and β_2-adrenoceptors, there exists a third β-adrenoceptor known as the atypical β-adrenoceptor or β_3-adrenoceptor (Granneman *et al*, 1992). The aim of this paper is to describe the *in vivo* β-adrenoceptor pharmacology of ZD9989 ((R)-N-(2-[4-(carboxymethyl)phenoxy]ethyl)-N-(β-hydroxy-β-[3-chlorophenyl]ethyl)ammonium chloride), a novel β_3-adrenoceptor agonist.

ZD9989 produced a dose-related increase in rat brown adipose tissue activity as measured by specific mitochondrial GDP binding (method as described by Holloway *et al*, 1991) (ED_{50}=0.16 mg/kg p.o., 95% confidence limits: 0.12-0.21, n=5). In conscious dogs (Holloway *et al*, 1991), ZD9989 produced a dose dependent increase in oxygen consumption which was maintained for at least 4 hours (ED_{50} = between 0.01-0.1 mg/kg p.o.; n=4).

ZD9989 was also tested in *in vivo* models of β_2-adrenoceptor agonist activity (Holloway *et al*, 1991). In conscious dogs ZD9989, at doses up to 1mg/kg p.o., did not significantly modify blood K^+ levels. ZD9989 was of low potency in a cat soleus model of tremor (Holloway *et al*, 1991): ZD9989 had no activity at doses $\leq$ 7.5mg/kg, i.v. and in additional studies with higher doses had a maximal inhibition of twitch tension of 17±7.3% at a dose of 200mg/kg. In contrast, the standard β_2-adrenoceptor agonist, salbutamol had potent effects in this model (ED_{50}= 0.21μg/kg, i.v.; 95% confidence limits: 0.14-0.32).

In the anaesthetised, syrosingopine-treated dog, β_3-adrenoceptor agonists produce a decrease in hindlimb perfusion pressure (HLPP) which appears to be mediated by the β_3-adrenoceptor (Briscoe *et al*, 1993). ZD9989 had an ED_{50} of 3.09μg/kg i.v. (95% confidence limits: 0.98-9.72, n=4) for effects on HLPP and an ED_{50} for positive chronotropic effects of 365μg/kg (95% confidence limits: 227-587, n=4). Thus ZD9989 is relatively selective for vascular β_3-adrenoceptors in hindlimb compared to cardiac β-adrenoceptors.

In conclusion, ZD9989 is a novel β_3-adrenoceptor agonist and thermogenic agent and has limited agonist or antagonist effects at β_1- or β_2-adrenoceptors *in vivo*.

Briscoe, M.G, *et al* (1993). Br. J. Pharmacol, 108, 181P.
Granneman, J.G. *et al* (1992) Mol. Pharmacol., 42, 964-970.
Holloway, B.R. *et al* (1991). Br. J. Pharmacol, 104, 97-104.

423P THE INVOLVEMENT OF KININS IN THE CARDIOVASCULAR EFFECTS OF *LEIURUS QUINQUESTRIATUS* SCORPION VENOM

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The involvement of kinins in the terminal hypotensive response to scorpion envenoming was suspected from the protective effect obtained with aprotinin, a kallikrein-kinin inhibitor (Ismail *et al.*, 1992). The present study was undertaken to investigate this involvement. Anaesthetised Buskat rabbits (urethane 1.75 g kg^{-1}, i.v.) were prepared for blood pressure, respiration and ECG recording. *Leiurus quinquestriatus quinquestriatus (L.Q.Q.)* venom in a lethal dose (0.5 mg kg^{-1}, i.v.) caused a triphasic effect on mean arterial blood pressure (16 rabbits) consisting of an immediate fall lasting for 1 min (from 109±3 to 68±3 mmHg, P<0.0001, mean ± s.e.m., Mann-Whitney U-test) followed by a pronounced rise (to 136±4 mmHg, P < 0.0001) that reached a peak within 5 min and lasted up to 35 min; blood pressure then declined, reaching its lowest value (10±2 mmHg, P<0.0001) just prior to death of the animals at 89±7 min. Pretreatment of rabbits (n=5) with aprotinin (6000 KIU kg^{-1} i.v. initially, followed by i.v. infusion of 2000 KIU kg^{-1} h^{-1} at a rate of 4.5 ml h^{-1}) 50-60 min prior to venom injection, significantly attenuated (P<0.01) the venom-evoked initial fall (from 92±5 to 71±10 mmHg). The terminal hypotension was also reduced with the lowest value reached being 63±10 mmHg (P=0.0005); four out of five rabbits were alive and well for the duration of the experiment (5 h), whilst one died at 143 min (P<0.0001). The bradykinin receptor (B_2) blocker Hoe 140 (from Dr K Wirth, Hoechst AG, 0.6 mg kg^{-1}, i.v., 30 min before venom) caused a hypotensive effect and enhanced the venom-induced bradycardia; five out of six rabbits subsequently died (49±12 min). Hoe 140 (0.6 mg kg^{-1}, i.v., 30 min before venom) when injected 5-10 min after atropine (2 mg kg^{-1}, i.v.) significantly attenuated (P<0.0001) the venom-evoked initial fall (87±6 to 70±6 mmHg). The fall in blood pressure during the late hypotensive stage was significantly reduced (69±5 mmHg, P=0.0001 vs venom alone). Four out of five rabbits survived longer than 5 h with one dying at 180 min. Atropine itself also attenuated (P < 0.001) the initial fall (100±7 to 90±3, P > 0.05), but did not significantly modify the late phase. Plasma bradykinin concentration was determined by radioimmunoassay (Moshi *et al.*, 1992) in serial blood samples obtained following the injection of the venom into 14 rabbits. A significant increase was obtained upon comparing the mean fluctuation in plasma bradykinin between zero and other sampling times (every 15 min for 2 h) for each individual rabbit in the control and venom-treated groups (3±0.4 vs 14±3 pg respectively, P=0.003). Maximum elevation occurred either at the beginning of the terminal hypotension, or just before death. It is concluded that kinins may be involved in the terminal hypotensive response to scorpion envenoming and might be responsible for the refractoriness to hypertensive therapy in fatal cases following scorpion stings.

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Moshi, M.J., Zeitlin, I.J., Wainwright, C.L. & Parratt, J.R. (1992) *Cardiovasc. Res.* 26, 367-370.

424P COMPARISON OF TWO METHODS OF MYOGRAPHY FOR DETECTION OF CONSTRICTOR ENDOTHELIN ET_B RECEPTORS IN RAT SMALL MESENTERIC ARTERIES

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In vivo evidence implicates a constrictor role for ET_B receptors in resistance beds of the rat (Clozel *et.al.*, 1992) and in man (Haynes *et al.* 1995). However, a role for these receptors in mediating constriction of the small arteries that contribute to determination of vascular resistance has proved more difficult to demonstrate *in vitro*. The majority of studies to date have measured isometric contraction of arterial rings stretched between two wires in the Mulvany wire myograph (WM). A more physiological alternative is to cannulate and pressurise the vessels to levels encountered *in vivo* using the perfusion myograph (PM). The aim of this study was to compare responses to the ET_B selective agonist sarafotoxin S6c (SRTX S6c) obtained in rat mesenteric arteries (diameter 150-350 μm) mounted in either the WM or the PM.

Second or third order branches of the mesenteric artery were dissected from male Wister rats (10-15 weeks old) and mounted either under 0.2g tension in the WM or pressurised to 60 mmHg in the PM. The vessels were constantly bathed in oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution of standard composition, at 37°C, and allowed to equilibrate for 1 hour. In the case of the PM, the solution was constantly superperfused from a reservoir. All vessels were then exposed to 60 mM KCl twice to obtain a maximal contraction. In the PM the endothelium was removed by passing an air bubble through the lumen of the vessel. In both preparations, the integrity of the endothelium was investigated by addition of acetylcholine (ACh, 10^{-6}M) to vessels pre-constricted with phenylephrine (PE, 10^{-5}M). After washing, cumulative concentration-response curves to SRTX S6c (10^{-12}-10^{-7}M; n=17) were obtained. All data is expressed as % of maximal KCl contraction.

In the WM, SRTX S6c failed to induce a significant contraction, even when the vessels were partially depolarised with 20mM KCl. In the PM, SRTX S6c evoked concentration-dependent contraction in 9 out of 17 vessels studied (EC_{50} 3 ± 1 x 10^{-9}M, Emax 19 ± 4 % KCl). Similar concentration-dependent contraction to SRTX S6c in the WM (EC_{50} 3 ± 2 x 10^{-9}M, Emax 20 ± 5%, n = 6) could be exposed by pre-constricting the vessels using the stable thromboxane analogue, U46619 ($3x10^{-8}$M). In this case the level of constriction was strongly influenced by the amount of endothelium present, the less endothelium present, the greater the constriction. U46619 failed to influence constriction in the PM.

These results indicate that constrictor ET_B receptors are present on the smooth muscle of small calibre arteries. However, several factors influence the detection of these receptors, including the presence of endothelium and particularly the level of intrinsic tone. Pressurisation of vessels in the PM is thus a more appropriate technique for *in vitro* investigation of small calibre arteries.

This work was supported by the British Heart Foundation. EJM is a recipient of a Medical Research Council studentship.

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Haynes, WG, Strachan, FE & Webb, DJ *Circulation* in press

425P ATRIAL NATRIURETIC PEPTIDE REVERSES BUT DOES NOT PROTECT AGAINST CHALLENGE WITH ENDOTHELIN-1 IN ISOLATED HUMAN BRONCHI

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The naturally occurring hormone atrial natriuretic peptide (ANP) can reverse and confer protection against methacholine-induced contraction in isolated human and bovine bronchi (Angus *et al.*, 1994) and has been proposed as a possible therapeutic agent in the treatment of asthma. Little is known, however, about the effect of this agent on endothelin-1 (ET-1)-mediated contraction. This present study examined the ability of ANP to relax and protect against challenge with ET-1 in human isolated bronchi.

Responses produced by rings of human bronchi were measured isometrically (n=7 in each case). Contractions were evoked by ET-1 ($3x10^{-8}$M). Once a plateau had been reached, cumulative concentration-response curves were obtained for ANP (10^{-9}-10^{-6}M). Phosphoramidon ($3.7x10^{-5}$M) was pre-incubated 5 minutes prior to addition of ANP to prevent its rapid hydrolysis. In a further series of experiments, the ability of ANP to protect against subsequent challenge with ET-1 was examined. Cumulative concentration-response curves were constructed to ET-1 (10^{-10}-$3x10^{-7}$M) in the presence and absence of ANP (10^{-7}, $3x10^{-7}$ or 10^{-6}M), again in the presence of phosphoramidon. Results are expressed in mg wt and in terms of the -logEC_{400mg} (the -log of the concentration evoking a 400mg wt. contraction). Responses are expressed as mean±s.e.mean. Significance between data sets was analysed by two-way analysis of variance (ANOVA) while significance between data points was analysed by Student's t-test.

ANP relaxed pre-constricted tissue in a concentration-dependent manner, initiating responses at between $3x10^{-9}$ and 10^{-8}M. Due to financial constraints, the highest concentration
of ANP we were able to achieve was 10^{-6}M. At this concentration, ANP reversed 55.62±7.72% of the induced tone.

When ANP was pre-incubated at a concentration of 10^{-7}M, it did not provide any protection against ET-1 challenge (control EC_{400mg}; 7.22±0.19 plus ANP 10^{-7}M; 7.57±0.19). This was despite reversing 34.64±5.13% of the ET-1-induced tone in the previous experiments. In contrast, at concentrations of $3x10^{-7}$ (which reversed 43.14±5.49% of the ET-1-induced tone) and 10^{-6}M, ANP significantly ($p<0.05$ and $p<0.01$ for data sets, respectively) enhanced responses to ET-1, albeit with no significant change in the EC_{400mg} (control EC_{400mg}; 7.76±0.12, plus ANP $3x10^{-7}$M; 7.93±0.11, plus ANP 10^{-6}M; 7.98±0.16). Phosphoramidon ($3.7x10^{-5}$M) alone did not alter responses to ET-1.

This enhancement of Et-1 responses was unexpected, since ANP was effective at reversing ET-1-induced tone as well as providing protection against methacholine challenge (Angus *et al.*, 1994) in this tissue. It appears, therefore, that the ability of ANP to evoke a pre-protective effect may depend on the spasmogen used.

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This work was supported by the Chest Heart and Stroke Assoc. (Scotland) and the National Asthma Campaign.

426P POTENTIATION OF ENDOTHELIN-1-EVOKED CONTRACTIONS BY ANGIOTENSIN II OR HYPOXIA: THE ROLE OF CYCLOOXYGENASE OR 5-LYPOXYGENASE METABOLITES IN BOVINE BRONCHI

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We have previously demonstrated that contractions evoked by endothelin-1 are potentiated by either angiotensin II (Nally *et al.*, 1994) or hypoxia (Nally *et al.*, 1995). The mechanism underlying this hyperreactivity is, however unknown. The present study sought to elucidate the role of metabolites of cyclooxygenase and 5-lipoxygenase in this synergy.

Contractions of rings of bovine bronchi were measured isometrically in vertical 10ml organ baths. Cumulative concentration-response curves were constructed for contractions evoked by endothelin-1 (10^{-10}-$3x10^{-7}$M) in the presence of threshold concentrations of angiotensin II ($3x10^{-7}$M) or hypoxia (4% O_2). Cumulative concentration-response curves were further constructed in the additional presence or absence of indomethacin (10^{-6}M) or nordihydroguaiaretic acid (NDGA, 10^{-5}M). n=6 in each case.

Endothelin-1-mediated concentration-response curves were significantly leftward shifted by angiotensin II ($p<0.001$, pD_2: 8.13±0.2 compared with control 7.7±0.1) with an increased maximum response (48.8% increase at endothelin-1 $3x10^{-7}$M). The presence of the cyclooxygenase inhibitor indomethacin (10^{-6}M) virtually abolished the enhanced response ($p<0.001$). Likewise the presence of the 5-lipoxygenase inhibitor reversed the potentiating effect of angiotensin II ($p<0.001$). Endothelin-1-mediated contractions were also enhanced by the presence of hypoxia (pD_2; 8.11±0.23 compared with control 7.7±0.2). As with angiotensin II, this enhanced response was attenuated by the presence of indomethacin ($p<0.05$). The presence of NDGA under hypoxic conditions likewise evoked reversal of the angiotensin II-potentiated response such that the curve lay midway between control and potentiated values. By contrast, contractions evoked by endothelin-1 alone were unaltered by the presence of either indomethacin or NDGA.

In bovine bronchi, endothelin-1-evoked contractions are not mediated via the release of either cyclooxygenase or 5-lipoxygenase metabolites since neither indomethacin nor NDGA altered contractions evoked by this peptide alone. By contrast, these two pathways each appear to have a role to play in the enhancing effect of both angiotensin II and hypoxia. The ability of an inhibitor of either cyclooxygenase or 5-lipoxygenase to attenuate the potentiated responses suggests that some from of crosstalk occurs between these "limbs" of the arachidonic acid cascade.

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This work was supported by the Chest Heart and Stroke Assoc. (Scotland) and the National Asthma Campaign.

427P ENDOTHELIN-CONVERTING ENZYME INHIBITION *IN VITRO* AND *IN VIVO* BY N-PHOSPHONOMETHYL-LEU-TRP

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Endothelin-Converting Enzyme (ECE) inhibitors could prevent the endogenous production of endothelin-1 (ET-1) from its precursor (big ET-1) and, therefore, might have therapeutic value for the treatment of various pathologies associated with elevated ET-1 levels such as hypertension, asthma, congestive heart failure or cerebral vasospasms (Masaki *et al.*, 1992).

Phosphoramidon (N-L-[α-rhamnopyranosyloxyhydroxyphosphinyl]-L-Leu-Trp), a potent metalloendopeptidase inhibitor, has consistently demonstrated ECE inhibitory activities which are conserved after removal of its sugar moiety, *i.e.*: by N-phosphoryl-Leu-Trp (P-Leu-Trp) (Pollock *et al.*, 1992). However, since we recently observed a reduced *in vivo* activity for P-Leu-Trp compared to phosphoramidon (Bigaud *et al.*, 1994), probably due to a reduced stability of the molecule, we attempted to improve the ECE inhibitory activity of P-Leu-Trp, by designing a more stable molecule, *i.e.*: N-phosphonomethyl-Leu-Trp (P-CH_2-Leu-Trp).

Phosphoramidon and P-CH_2-Leu-Trp were first compared *in vitro*, using a partially purified ECE from bovine lungs, and they both presented similar inhibitory activities, with IC50s of 7 ± 3 µM and 14 ± 5 µM, respectively. Similar IC50s, 6 ± 1 µM and 5 ± 4 µM were also obtained with phosphoramidon and P-CH_2-Leu-Trp, respectively, in a 2nd assay using ET-1-mediated stimulation of phosphatidylinositol (PI) turnover as a marker of ET-1 production by slices of rat striatum incubated with big ET-1. We previously verified that this assay was reflecting the activity of a specific ECE, insensitive to captopril or des-Tyr-Met-enkephalin, and therefore different from angiotensin converting enzyme or neutral endopeptidase 24.11. Furthermore, we confirmed that neither phosphoramidon nor P-CH_2-Leu-Trp could interfere with the stimulation of PI turnover induced by exogenous ET-1. Phosphoramidon, P-Leu-Trp and P-CH_2-Leu-Trp were also compared *in vivo*, on their ability to reduce big ET-1-induced hypertension in pentobarbital-anaesthetised rats (250-300g). The administration of big ET-1 (1 nmol/kg) induced a slowly developing increase in mean arterial pressure reaching 42 ± 4% within 15 min. This response was reduced by about 69%, 60% and 64% after a 5 min pretreatment with, respectively, phosphoramidon, P-Leu-Trp or P-CH_2-Leu-Trp (10 µmol/kg, i.v., bolus). After a 30 min pretreatment, the reduction of big ET-1-mediated effect was maintained to about 67%, in the case of P-CH_2-Leu-Trp, but reduced to about 31% and 24% in the case of phosphoramidon and P-Leu-Trp, respectively. None of the 3 compounds reduced the hypertensive effect of ET-1 (1 nmol/kg, i.v.).

These results present P-CH_2-Leu-Trp as an ECE inhibitor, at least as potent as phosphoramidon, with a longer duration of action *in vivo*. Because of its availability by synthesis, P-CH_2-Leu-Trp appears to be an attractive tool to assess the biological role of ECE.

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428P REDUCED ENDOTHELIN-1 SECRETION BY PULMONARY VASCULAR ENDOTHELIAL CELLS EXPOSED TO HYPOXANTHINE-XANTHINE OXIDASE

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Factors associated with vascular injury have been shown to stimulate endothelin-1 (ET-1) secretion by the vascular endothelium (Maemura et al, 1992). The aim of this study was to examine effects of the hypoxanthine/xanthine oxidase (HX/XO) system (a known generator of injurious reactive oxygen species) on secretion of ET-1 by bovine pulmonary artery endothelial cells (BPAEC).

Endothelial cells isolated from bovine pulmonary artery were subcultured using trypsin/EDTA (0.5%/0.2%) and were used between the 10th and 15th passages. ET-1 secretion was measured by radioimmunoassay, using an antibody to the C-terminal ET[16-21] sequence. ET-1 secretion was measured in confluent monolayers exposed to serum-free medium for the duration of the experiment, except for one set of experiments where the effect of complete medium was investigated. The generation of reactive oxygen species (ROS) by HX/XO was confirmed using the cytochrome C assay (Fridovich, 1970). Data presented are means ± s.e.m. of n observations. Statistical analysis was performed by ANOVA and Bonferoni (B test) or Dunnet (D test) post-tests as appropriate.

BPAEC monolayers secreted ET-1 into the overlying medium in a time-dependent manner with a level of 1248 ± 106 fmol/10^6 cells recorded by 12 hr. Secretion was significantly reduced ($p < 0.001$, B test, n=9) to 669 ± 118 fmol/10^6 cells in cultures exposed to 100 μM phosphoramidon (PA). In the corresponding experiment carried out in complete medium, PA significantly reduced ($p < 0.001$, B test, n=9) secretion from 2932 ± 238 to 1270 ± 35 fmol/10^6 cells. When cells were exposed to two concentrations of HX/XO (see Table) for up to 12 hr, significant reductions (D test) in ET-1 secretion were apparent at both concentrations by 4 hr (n=6). By 12 hr however, a significant reduction was seen only at the higher concentration (n=11). XO alone (at 2.0 mU/ml only) significantly reduced ET-1 secretion at 4 and 12 hr (n=6 and 12 respectively, see Table) in contrast to HX alone which had no effect. The reductions in ET-1 secretion observed were shown not to be due to changes in cell number, total cellular protein (Lowry) or cell viability (trypan blue exclusion).

In conclusion we propose that exposure of BPAEC to the HX/XO system generates injurious ROS which then reduce ET-1 secretion. A reduction in ET-1 secretion following injury would suggest that this peptide may exert beneficial actions in the pulmonary vasculature under physiological conditions.

Supported by the Irish Health Research Board.

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Table 1. Modulation of ET-1 secretion (fmol/10^6 cells) by HX/XO; *p < 0.05, **p < 0.001 w.r.t basal (D test)

Time (hr)	basal	XO (2mU/ml)	HX/XO (0.02mM/2mU/ml)	XO (0.2mU/ml)	HX/XO (0.002mM/0.2mU/ml)
4	440 ± 125	276 ± 121**	270 ± 119**	459 ± 151	395 ± 121*
12	1018 ± 77	572 ± 110**	500 ± 107**	985 ± 101	931 ± 119

429P TRANSIENT ANTECEDENT ISCHAEMIA REVERSES THE BENEFICIAL MECHANICAL AND METABOLIC EFFECTS OF ADENOSINE IN ISOLATED WORKING RAT HEARTS

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Adenosine (Ado) inhibits myocardial glycolysis and enhances the recovery of mechanical function following severe ischaemia (Finegan *et al.*, 1993). A similar cardioprotective effect is achieved by pre-ischaemic glycogen depletion (Wolfe *et al.*, 1993), a process that also limits glycolysis. To examine the interactions between Ado and glycogen depletion, we measured the effects of Ado on glucose metabolism and mechanical function in control hearts and in hearts in which glycogen levels were reduced by transient ischaemia (TI).

Hearts were removed from pentobarbitone-anaesthetized rats, perfused in the working mode at 37°C with Krebs' buffer containing 2.5 mM Ca^{2+}, 1.2 mM palmitate, 11 mM glucose and 100 $\mu U \cdot ml^{-1}$ insulin, and paced at 300 beats.min^{-1}. H^+ production was calculated from glycolysis and glucose oxidation rates measured by the rate of formation of ^3H_2O and $^{14}CO_2$ from [$^3H/^{14}C$]glucose.

In subsets of hearts frozen after 45 min of perfusion, glycogen levels ($\mu mol \cdot g$ dry wt^{-1}) were lower (68±4, n=9, $P<0.05$) in hearts subjected to TI (two 10-min periods of global ischaemia, each followed by 5 min reperfusion) compared with controls (158±9, n=8). After 45 min, hearts were perfused in the absence or presence of Ado (500 μM) or the A_1 agonist, N^6-cyclohexyladenosine (CHA, 500 nM), and subjected to further aerobic perfusion for 30 min (Aerobic Group) or to 30 min global ischaemia and 30 min reperfusion (I/R Group). In the Aerobic Group, left ventricular (LV) work was similar in control and TI hearts and was not affected by Ado or CHA. In control hearts, Ado (n=9) reduced glycolysis by 49% and H^+ production from glucose metabolism by 70% ($P<0.05$). In contrast, in TI hearts, Ado (n=7) increased glycolysis by 74% and H^+ production by 77% ($P<0.05$). CHA had similar effects in both control (n=7) and TI hearts (n=9) and inhibited ($P<0.05$) both glycolysis (by 50% and 61%) and H^+ production (by 62% and 53%).

In the I/R Group, LV work (as a % of pre-ischaemic values) recovered to a similar extent in untreated control (26%, n=32) and untreated TI hearts (43%, n=23). During reperfusion of control hearts, Ado (n=7) reduced glycolysis by 50%, H^+ production from glucose metabolism by 67%, and enhanced the recovery of LV work to 81% ($P<0.05$). However, in TI hearts, Ado (n=8) increased glycolysis by 34%, H^+ production by 54% and depressed further the recovery of LV work to 9% ($P<0.05$). In contrast, in TI hearts, CHA (n=8) inhibited glycolysis by 53%, H^+ production by 53%, and increased the recovery of LV work to 90% ($P<0.05$).

These data indicate that an improved metabolic coupling of glycolysis to glucose oxidation, which decreases H^+ production, will enhance the recovery of LV work during reperfusion. Antecedent ischaemia reverses the effect of Ado, but not that of CHA, on glycolysis. Under these conditions, Ado impairs the coupling of glycolysis to glucose oxidation, increases H^+ production and depresses the recovery of LV work.

Supported by a grant from the MRC (Canada).

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430P EFFECTS OF A PHORBOL ESTER ON ANGIOTENSIN II-EVOKED [^{3}H]NORADRENALINE RELEASE AND $[Ca^{2+}]_i$ IN HUMAN NEUROBLASTOMA (SH-SY5Y) CELLS TRANSFECTED WITH RAT AT_{1A} RECEPTORS

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Regulation of noradrenaline (NA) release from sympathetic neurones is one of the many actions of angiotensin II (AII; Reid, 1992). We have investigated the ability of AII to modulate [^{3}H]NA release and regulate $[Ca^{2+}]_i$ in human neuroblastoma (SH-SY5Y) cells stably expressing the recombinant rat AT_{1A} receptor (Balmforth *et al.*, 1994). These cells are derived from human sympathetic tissue and retain many of the properties of mature sympathetic neurones (Vaughan *et al.*, 1995). [^{3}H]NA release assays and measurements of $[Ca^{2+}]_i$ (using Fura-2 loaded cells) were performed exactly as previously described (Vaughan *et al.*, 1993; McDonald *et al.*, 1994). All values for [^{3}H]NA release are expressed as percentage (± SEM, n=6 experiments) of total tissue [^{3}H] content. Statistical differences were determined using the unpaired Student's t-test.

Exposure of cells for 4 min to 30nM AII stimulated [^{3}H]NA release, from a basal level of 2.9±0.2% to 5.6±0.8% ($P<0.01$). When cells were pretreated for 10min with 100nM 12-O-tetradecanoylphorbol-13-acetate (TPA) to stimulate protein kinase C (see Vaughan *et al.*, 1995), 30nM AII caused significantly greater stimulation of [^{3}H]NA release, to 8.3±0.3% ($P<0.02$). In the absence of Ca^{2+}_o (100μM EGTA added) the effects of 30nM AII were significantly reduced to 3.4±0.2% in non-TPA treated cells and 5.4±0.4% in TPA treated cells i.e. removal of Ca^{2+}_o reduced the effects of AII by approximately 50% in TPA-treated cells, and in non-TPA treated cells the effects of AII were almost completely abolished in the absence of Ca^{2+}_o (taking into account basal release levels of 2.5-3.6%).

Brief applications of AII caused increases in $[Ca^{2+}]_i$ of 100±19nM, 313±27nM and 453±34nM at concentrations of 0.3, 3 and 30nM respectively (n=4 experiments). However, pretreatment of cells for 10 min with 100nM TPA reduced these rises significantly ($P<0.03$ to $p<0.003$), to 17±15nM, 75±22nM and 280±28nM (n=6 experiments) at the same AII concentrations.

Our results indicate that stimulation of recombinant AT_{1A} receptors in SH-SY5Y cells leads to enhanced [^{3}H]NA release and rises of $[Ca^{2+}]_i$, and suggest that these effects of receptor stimulation are differentially regulated by protein kinase C activation, which enhances AII-evoked [^{3}H]NA release but suppresses AII-evoked rises of $[Ca^{2+}]_i$.

Supported by the British Heart Foundation and the Leeds University Research Fund.

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431P A ROLE FOR $p70^{s6K}$ IN THROMBIN-STIMULATED DNA SYNTHESIS IN PULMONARY ARTERY FIBROBLASTS

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Pulmonary artery fibroblasts proliferate intensely during pulmonary hypertension. The stimuli for this response remain elusive (Stenmark *et al.*, 1988). A number of signalling pathways have been implicated in regulating cell growth and division in response to growth factors and G protein-coupled receptor agonists. These include cascades involving mitogen-activated protein (MAP)-kinases and the 70kDa S6 ribosomal protein kinase ($p70^{s6k}$) (Malarkey *et al.*, 1995). We have shown previously that α-thrombin is a strong stimulant for MAP kinase activation and DNA synthesis in bovine pulmonary artery fibroblasts (BPAFs)(Belham *et al.*, 1995). The aim of the present work was to assess the role of $p70^{s6k}$ in thrombin-stimulated DNA synthesis in BPAFs. As phosphatidylinositol (PI) 3-kinase has been shown to be required for $p70^{s6k}$ activity in response to growth factors (Chung *et al.*, 1994) and thrombin stimulates PI 3-kinase activity in platelets (Kucera & Rittenhouse 1990), we have also investigated the role of PI 3-kinase in the signal generated by thrombin.

Confluent BPAFs cultured from primary explants were rendered quiescent in serum-deprived media for 48h prior to stimulation. Thrombin-stimulated $p70^{s6k}$ activity was assessed in anti-$p70^{s6k}$ immunoprecipitates by mobility shift bands on Western blots probed with anti-$p70^{s6k}$ antibody (Santa Cruz Biotechnology Inc.) and *in vitro* [γ-^{32}P]ATP phosphorylation of a S40 ribosomal peptide pseudosubstrate (Upstate Biotechnology Inc.). DNA synthesis was measured by incorporation of [^{3}H]thymidine during the final 4h of 24h exposure to thrombin.

Thrombin (T) caused a concentration and time-dependent activation of $p70^{s6k}$ which was maximal at 30 minutes and 300nM. The $p70^{s6k}$ inhibitor rapamycin (RAPA) (50ng/ml) and the PI 3-kinase inhibitor wortmannin (WORT)(100nM) abolished thrombin-stimulated $p70^{s6k}$ activity (control (C): 10569±1968 cpm, T: 36346±11091, RAPA: 4684±1473, T+RAPA: 4604±2148, WORT: 4016±1871, T+WORT: 6680±3601). At the same concentrations, rapamycin and wortmannin caused 33% and 80% inhibition, respectively, of thrombin-stimulated [^{3}H]thymidine incorporation into DNA (C: 265±32 dpm, T: 57580± 2761, RAPA: 185±10, T+RAPA: 38220±3600, WORT: 194±22, T+WORT: 11836±2143). Furthermore, wortmannin did not affect thrombin-induced activation of MAP kinase. Thrombin-stimulated $p70^{s6k}$ activity was not inhibited under conditions where immunodetectable protein kinase C (PKC) α and ε isoforms were downregulated by 48h pretreatment with 1μM 12-O-tetradecanoylphorbol 13-acetate. All values above represent mean± s.e.mean for at least 3 independent experiments.

The data supports a role for a PI 3-kinase-dependent $p70^{s6k}$ pathway in regulating a proportion of thrombin-stimulated DNA synthesis in BPAFs. This pathway does not appear to involve diacylglycerol-dependent PKC isoforms. Other signalling pathways are likely to mediate the $p70^{s6k}$-independent component of thrombin-stimulated growth.

C.M.B. is funded by a Medical Research Council Quota Studentship. This work was funded by the Medical Research Council and British Lung Foundation.

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432P RELEASE OF SUPEROXIDE ANIONS IN CORONARY RESISTANCE VESSELS OF HYPERCHOLESTEROLAEMIC RABBITS

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Endothelium-dependent vasodilation mediated by nitric oxide is impaired in the resistance vessels of hypercholesterolaemic and atherosclerotic rabbits (Simonsen *et al.* 1992) although these vessel have no evidence of atherosclerosis. Minor *et al.* (1990) has found that the production of nitric oxide by aortae from cholesterol-fed rabbits is enhanced although endothelium-dependent relaxation has been impaired. This observation has been explained by the the inactivation of nitric oxide in these vessels by superoxide anions and is suppported by an increased production of these anions in hypercholesterolaemic rabbits (Mugge *et al,* 1994). In this work, the possibility is investigated that a similar mechnism operates in the coronary resistance vessels.

The left circumflex artery (third order branch) coronary vessels were removed from four Watanabe Hereditary Hyperlipidaemic (WHHL) rabbits (total cholesterol, 25 mM) and four age-matched normocholestreolaemic New Zealand White (NZW) rabbits and dissected clean of adherent adipose tissue. 2 mm segments of the artery were equilibrated in Krebs buffer bubbled with 95 % O_2, 5 % CO_2 for 30 min at 37^{o}. Segments were transferred to scintillation vials containing 0.25 mM lucigenin in Krebs Buffer (2 ml) in the absence and after pretreatment with 0.1 mM diethyldithiocarbamic acid (DETC) or 6 µM superoxide dismutase (SOD). Superoxide anion production was measured by lucigenin-enhanced chemiluminescence in a LKB 217 beta counter with a single active photomultiplier tube (in an out-of-coincidence mode) as described by Mugge et al. (1994). Superoxide production is expressed as counts/min/mg tissue (mean ± s.e. mean). Statistical analysis was performed using Student's t-test with $P<0.05$ considered significant.

Superoxide production by the WHHL small coronary vessels (42023 ± 7306 counts/min/mg) were significantly higher (n=4, $P< 0.01$) than that of the NZW coronary vessels (7922 ± 3655 counts/min/mg). The addition of SOD significantly reduced superoxide levels by 88 % (974 ± 662 counts/min/mg; n=4, $P<0.02$) and 46 % (22708 ± 7801 counts/min/mg; n=4; $P<0.05$) in NZW and WHHL rabbits respectively. Pretreatment with 0.1 mM DETC, an inhibitor of superoxide dismutase, increased superoxide production by 16 % in WHHL compared with 48 % in NZW arteries, implying that SOD activity was decreased in hypercholesterolaemia. In conclusion, this study demonstrates that superoxide anion generation is increased by hypercholesterolemia in rabbit coronary resistance arteries and is likely to contribute to the impairment of coronary blood flow during the development of atherosclerosis.

We are grateful to the BHF for their financial support.

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433P THE EFFECTS OF L-ARGININE SUPPLEMENTATION ON MYOCARDIAL INFARCT SIZE AND ET-1 RELEASE IN HEARTS ISOLATED FROM GENETICALLY HYPERLIPIDAEMIC RABBITS

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ET-1 is a potent vasoconstrictor peptide which has been implicated in various pathophysiological conditions of the cardiovascular system. Circulating ET-1 levels and local tissue immunoreactivity have been shown to be elevated in conditions of acute myocardial infarction in both experimental animals (Watanabe et al., 1991) and humans (Rubanyi & Polokoff, 1994), and in patients with hypercholesterolaemia (Lerman et al., 1991). Thus in situations where hyperlipidaemia and myocardial infarction coexist, endothelin may make a significant contribution to the extent of myocardial injury. The release of ET-1 is modulated, in part, by endothelium-dependent nitric oxide (NO; Boulanger & Luscher, 1990). Since NO production may be reduced in hyperlipidaemic and ischaemic states, this may contribute to enhanced ET-1 production, thus aggravating the outcome. The objective of the present study was to determine whether replenishing NO, by administering L-arginine (the substrate for NO) would influence cardiac ET-1 release and decrease infarct size in hearts isolated from genetically hyperlipidaemic rabbits and subjected to acute regional ischaemia and reperfusion.

Twenty age-matched male Froxfield Heritable Hyperlipidaemic (FHH; 3.0-4.0kg) rabbits were split into the following treatment groups: i) Controls (n=8), which received no drug treatment; ii) 2 weeks L-arginine (0.5g kg^{-1} day^{-1} p.o.; n=6) followed by 2 weeks without L-arginine; iii) 4 weeks L-arginine (0.5g kg^{-1} day^{-1} p.o.; n=6). L-arginine was administered in the drinking water. At the end of the treatment period the rabbits were anaesthetized with sodium pentobarbitone (30mgkg^{-1} i.v.) containing heparin (500IUkg^{-1}) and the hearts rapidly excised and mounted on a Langendorff apparatus. The hearts were perfused with modified Krebs Henseleit buffer (37°C), gassed with 95% O_2/5% CO_2, at a constant flow rate (10ml min^{-1} kg^{-1} body weight). Hearts were stabilised for 15 min, after which they were subjected to 30min regional myocardial ischaemia (by occluding the LAD coronary artery) and 180min reperfusion. Heart rate, perfusion pressure and a surface electrocardiogram were monitored continuously throughout the experiment. Samples of perfusate were taken at regular intervals and subsequently analysed for endothelin-1 content using a Biotrak® ET-1 ELISA assay kit. At the end of the experiment the coronary ligature was re-tied, the heart perfused with Evans blue dye (0.5% w/v) to delineate area at risk (AAR), removed from the perfusion apparatus, sliced and incubated with triphenyl tetrazolium chloride (1% w/v) to stain viable tissue. The slices were then fixed, photographed and infarct size (IS) measured by computerised planimetry. Statistical analysis performed using ANOVA/Dunnett tests (haemodynamics) and Mann-Whitney test (Infarct size & ET-1 data).

Baseline haemodynamics did not differ among treatment groups. However, in the group of rabbits given 4 weeks L-arginine, 3 hearts developed irreversible ventricular fibrillation shortly after coronary occlusion. Thus subsequent haemodynamic data, infarct size and endothelin release could not be measured from these hearts. Coronary occlusion in control hearts increased perfusion pressure from 18.6±2.2 mmHg to 38.6±4.7mmHg 30 minutes after occlusion, compared with an increase from 26.2±2.3mmHg to 51.7±8.5mmHg ($P<0.05$) in hearts from rabbits which had received 2 weeks of L-arginine supplementation. Reperfusion restored perfusion pressure to pre-ischaemic values, after which there was a gradual increase during reperfusion which was similar in magnitude in both groups. Infarct size in the hearts from rabbits which had received 2 weeks L-arginine supplementation was significantly smaller (24.7±4.9% of AAR; mean±s.e.m.) than that in the control hearts (54.5±2.7% of AAR; $P<0.05$). In contrast, basal ET-1 release from control hearts (3.63 (2.03-6.45) pg min^{-1} g^{-1}, median (Q_1-Q_3)) was significantly lower than that from L-arginine-treated hearts (32.0 (18.9-49.1) pg min^{-1} g^{-1}; $P<0.05$). Coronary occlusion and reperfusion significantly decreased ET-1 release in both groups. These results show that short-term L-arginine supplementation decreases infarct size, but enhances cardiac endothelin release. The mechanism by which ET-1 release is enhanced by L-arginine requires further investigation.

J Chokkukannan is a recipient of an ORS award

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434P A COMPARISON OF INFARCT SIZE AND ET-1 RELEASE IN HEARTS ISOLATED FROM NORMOCHOLESTEROLAEMIC AND GENETICALLY HYPERLIPIDAEMIC RABBITS

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Hypercholesterolaemia is known to induce a number of changes in the vasculature, such as an impairment of endothelium-dependent vasodilation (Vrints et al., 1990), an effect which may result from reduced nitric oxide formation (Tanner et al., 1991), and increased release of vasoconstrictor substances such as ET-1 (Boulanger et al., 1990). An imbalance between vasodilator and vasoconstrictor substances in the coronary circulation may be expected to increase the severity of myocardial injury which occurs as a consequence of an ischaemic insult. Indeed, dietary induction of acute hyper- cholesterolaemia in rabbits increases infarct size (Golino et al, 1987), although it is not known to what extent this influences the release of vasoactive mediators. Furthermore, there appears to be a difference between the effects of dietary- and genetically-induced hyperlipidaemia on vascular and endothelial function (Greenlees et al., 1995; this meeting). The aim of this study was therefore to perform a comparative study of cardiac release of ET-1 and the consequences of acute regional myocardial ischaemia (infarct size) in normocholesterolaemic and genetically hyperlipidaemic rabbit hearts.

Age-matched male New Zealand White (NZW; 2.0-2.5kg; n=9) and Froxfield Heritable Hyperlipidaemic (FFH; 3.0-4.0kg; n=8) rabbits were anaesthetized with sodium pentobarbitone (30mgkg^{-1}, i.v.) containing heparin (500IUkg^{-1}) and the hearts rapidly excised and mounted on a Langendorff apparatus. The hearts were perfused with modified Krebs Henseleit buffer (37°C), gassed with 95% O_2/5% CO_2, at a constant flow rate (10ml min^{-1} kg^{-1} body weight). Hearts were stabilised for 15 minutes, after which they were subjected to 30min regional myocardial ischaemia (by occluding the LAD coronary artery) and 180min reperfusion. Heart rate, perfusion pressure and surface electrocardiograms were monitored continuously throughout the experiment. Samples of perfusate were taken at regular intervals and subsequently analysed for ET-1 content using a Biotrak ® ET-1 ELISA assay kit. At the end of the experiment the coronary ligature was re-tied, the heart perfused with Evans blue dye (0.5% w/v) to delineate area at risk (AAR), removed from the perfusion apparatus, sliced and incubated with triphenyl tetrazolium chloride (1% w/v) to stain viable tissue. The slices were then fixed, photographed and infarct size (IS) measured by computerised planimetry. Statistical analysis performed using ANOVA/Dunnett tests (haemodynamics) and Mann-Whitney test (Infarct size & ET-1 data).

Baseline heart rates (191±10 and 163±14 beats min^{-1}) and perfusion pressures (20.6±1.9 and 18.6±2.2 mmHg) were similar in hearts from both NZW and FFH rabbits respectively. Coronary occlusion caused a similar increase in coronary perfusion pressure in both groups of hearts (to 44.4±3.8 and 38.6±4.7 mmHg at 30min post-occlusion in NZW and FFH hearts, respectively; P<0.05). Reperfusion in both groups of hearts resulted in a restoration of coronary perfusion pressure. Heart rate was unaffected by the occlusion/reperfusion protocol. IS, expressed as a percentage of AAR, was 58.4±2.7% in the NZW hearts and 54.8±2.7% in the FFH hearts (n.s.). AAR was similar in both groups (73.1±2.5 and 63.6±5.2%; n.s.). Basal ET-1 release in FFH hearts was significantly higher than in NZW hearts (Table 1). Coronary occlusion and reperfusion resulted in a reduction in ET-1 release in the FFH hearts, but in an increase in ET-1 release in NZW hearts.

Table 1 **ET-1 release (pg min^{-1} g^{-1} wet heart weight)**

	NZW	FFH
Pre-ischaemia	0.31 (0.00-0.60)	3.63 (2.03-6.45)*
30min ischaemia	1.20 (0.56-5.10)^b	0.02 (0.00-0.24)^b
180min reperfusion	1.37 (0.63-5.20)^b	0.50 (0.20-0.79)^b

Values are expressed as medians (Q_1-Q_3). *P<0.05 vs NZW; ^{a}P<0.05, ^{b}P<0.01 vs pre-ischaemic value within groups.

These results demonstrate that genetically-induced hyperlipidaemia does not influence the infarct size in hearts subjected to ischaemia/reperfusion. However, there is an enhanced basal release of ET-1 in hyperlipidaemic hearts and the pattern of release during an ischaemia/reperfusion insult is different from that seen in normocholesterolaemic controls.

J Chokkukannan is a recipient of an ORS award.

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435P CURRENT ACTIVATION AND INHIBITION OF L-TYPE Ca^{2+} CHANNEL CURRENTS BY EXTRACELLULAR ATP IN GUINEA-PIG SINGLE SINOATRIAL NODE CELLS

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We have investigated the effects of extracellularly applied ATP on spontaneously active cells isolated from guinea pig sinoatrial node (Anumonwo *et al.*, 1992), using the whole cell patch-clamping technique (Hamill *et al.*, 1981) at room temperature (20-22° C). In normal Tyrode's solution (132 mM $[Na^+]_o$), application of extracellular ATP (1-1000 μM) elicited a concentration-dependent increase in the amplitude of inward currents (holding potential = -60 mV) with a Hill's coefficient of 1.7. This suggests that two molecules of ATP are required for binding to the ATP receptors to induce inward current. At different holding potentials, 200 μM extracellular ATP caused a rectified inward current which was reversed at -8 mV. The extracellular ATP-induced inward current was rapid in onset and desensitized in the continued presence of ATP. In a bathing solution which eliminated Na^+ currents (N-methyl-D-glucamine 132 mM and TTX 10 μM) and K^+ currents (Cs^+), the effects of extracellular ATP on L-type Ca^{2+} channel currents were also studied using 10 mM Ba^{2+} as the charge carrier. The maximal peak potential of the L-type Ca^{2+} channel current was observed at 20 mV and the amplitude was 289 ± 85 pA (holding potential = -40 mV, 24 cells). Using the train protocol (holding potential = -40 mV, test pulse potential = 20 mV for 100 ms, stimulated every 5 s) to measure the rate of onset of block, extracellular ATP caused a concentration-dependent (2-2000 μM) inhibition of the peak amplitude of Ca^{2+} channel currents with an IC_{50} = 80 ± 19 μM (mean ± SEM). The rate of inactivation of L-type Ca^{2+} channel current was dependent on the concentration of extracellular ATP (control (τ) = 205 ± 9 ms, 2 μM ATP = 191 ± 5 ms, 200 μM = 116 ± 6 ms and 1000 μM ATP = 90 ± 5 ms). This inhibitory effect of extracellular ATP on calcium current was rapid and could be completely reversed after washout. These results suggest that extracellular ATP may play a regulatory role in the pacemaker activity of sinoatrial cells. Studies of the underlying mechanisms(s) responsible for the inhibitory effects of L-type calcium channel currents by extracellular ATP and the type(s) of ATP receptors involved in the extracellular ATP-induced inward current are currently underway.

This work was kindly supported by the Croucher Foundation (H.K.).

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436P THE EFFECT OF DIABETES ON CARDIAC α_1-ADRENOCEPTOR SUBTYPES IN THE RAT

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Several studies have demonstrated a reduction in the density of α_1-adrenoceptor binding sites in the hearts of rats following experimentally induced diabetes (Heyliger et al., 1982; Wald et al., 1988). The population of α_1-adrenoceptors in the rat heart is heterogeneous with a predominant population of α_{1B}-adrenoceptors but a minor population (25%) of α_{1A}-adrenoceptors. The present study examines the effects of diabetes on these two populations of cardiac α_1-adrenoceptors. Diabetes was induced in male Wistar rats by a single injection of streptozotocin (65mgKg^{-1}, i.p.). Fourteen days later ventricular membranes were prepared and saturation curves obtained for [^{3}H]prazosin binding (Butterfield & Chess-Williams, 1991). α_{1A}- and α_{1B}-adrenoceptor densities in control and diabetic hearts were assayed by constructing competition curves for the α_{1A}-adrenoceptor selective agent, 5-methylurapidil.

In hearts from diabetic animals, the density of [^{3}H]prazosin binding sites was reduced ($P<0.01$, Student's t-test), from 32.3 ± 2.4 (mean ± SEM, n=7) for control animals to 21.28 ± 1.11 fmoles mg^{-1} protein (n=5) for diabetics. In addition the affinity of binding was increased ($P<0.01$), the [^{3}H]prazosin dissociation constant (K_D) falling from 0.95 ± 0.06nM for controls to 0.65 ± 0.05nM for diabetics.

In membranes from control rats, the displacement data for 5-methylurapidil were best described by a two site fit. The majority of sites (74.6 ± 2.2%) had a low affinity for 5-methylurapidil (α_{1B}) with a K_i of 1.70 ± 0.40 μM and a density of 24.10 ± 1.79 fmoles mg^{-1} protein (n=7). The density of high affinity site (α_{1A}) in normal tissue was 8.2 ± 0.61 fmoles mg^{-1} protein with a K_i of 6.64 ± 1.73 nM for 5-methylurapidil.

In cardiac tissue from diabetic animals two binding sites could again be identified using 5-methylurapidil. The density (7.29 ± 1.11 fmoles mg^{-1} protein) and affinity (K_i = 5.64 ± 1.28nM) of the high affinity binding sites was similar to the values obtained for control tissues. Although the K_i for the low affinity site was similar to that in control tissue (K_i = 2.04 ± 0.40 μM), the density of these sites was reduced to 13.98 ± 0.73 fmoles mg^{-1} protein in diabetic animals ($P<0.01$).

These results confirm the previous finding of a reduction in α_1-adrenoceptor density and an increase in [^{3}H]prazosin binding affinity at cardiac α_1-adrenoceptors in the diabetic heart (Wald et al., 1988). In addition, the results suggest that the reduction in receptor density is due solely to changes in the population of α_{1B}-adrenoceptors without significant effects on the population of cardiac α_{1A}-adrenoceptors.

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437P AN INITIAL IDENTIFICATION OF THE VOLTAGE-SENSITIVE CALCIUM CHANNELS THAT CONTRIBUTE TO THE DORSAL ROOT-EVOKED, POLYSYNAPTIC SPINAL REFLEX OF THE NEONATAL RAT

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Several peptides that were either isolated from the venom of predatory marine snails or whose primary structures were deduced by molecular genetic strategies have been shown to be potent and in some cases, selective, blockers of voltage-sensitive calcium channels (VSCCs; Olivera *et al.*, 1994). We have explored the actions of two such peptides on the long-duration, polysynaptic potential generated in rat spinal motoneurones.

Dorsal root stimulation in an *in vitro*, hemisected spinal cord from neonatal Wistar rats (3-5 days old) elicits a multi-phasic reflex in the corresponding ventral root which comprises both short- and long-latency components (Evans, 1989). Here, the peak amplitude of the longer-latency component from the ventral root of the L3, L4 or L5 segment was measured in response to single stimuli (0.5 ms, 8 x threshold) delivered at 10-min intervals to a dorsal root. Drugs were applied by superfusion at 0.5 ml min^{-1} in a 25°C, Krebs-bicarbonate buffer gassed with a 95% O_2/5% CO_2 mixture and peptides were delivered for 30 min in a carrier of bovine serum albumin (0.5 mg ml^{-1}).

The non-selective VSCC blocker, cadmium, completely eliminated this potential at 100 µM. The selective N-type VSCC blocker from *Conus geographus*, ω-conotoxin GVIA (ω-CgTx GVIA), irreversibly reduced the potential (to 36 ± 9 % of control at 1 µM) with an apparent IC_{50} of 180 ± 2 nM (means ± s.e.means, n=4, Fig. 1). Concentrations of ω-CgTx GVIA up to 3 µM had no further influence. The residual component was, however, consistently abolished by 100 µM Cd^{2+} (Fig. 1).

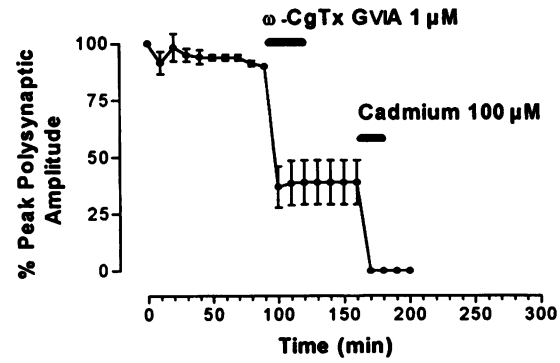

Fig. 1. Influence of VSCC blockade on the polysynaptic spinal reflex. Drugs were applied for the periods indicated by the solid bars (n=4).

The non-selective VSCC-blocking peptide from *Conus magus*, ω-conotoxin MVIIC, has inhibitory actions on Q-, P- and some N-type calcium channels and caused its maximal inhibition of the reflex at 3 µM (to 16 ± 4% of control, IC_{50}=863 ± 2 nM, n=4). The residual potential was again susceptible to 100 µM Cd^{2+}.

Whilst N-type VSCCs are clearly involved in the generation of this polysynaptic reflex, the data imply a similar role for one or more other VSCC subtypes of which the P- and/or Q-type are the most likely candidates.

Supported in part by the MRC and the Royal Society.

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438P NICOTINIC RESPONSES RECORDED FROM SYMPATHETIC PREGANGLIONIC NEURONES IN THE NEONATAL RAT SPINAL CORD SLICE *IN VITRO*

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Sympathetic preganglionic neurones (SPN) are the population of neurones that represent the last stage at which signals from the CNS to the periphery can be modified. Although there is little evidence for the presence of strong descending or ascending cholinergic input to SPN it has been suggested that there are cholinergic neurones intrinsic to the spinal cord (Borges & Iversen, 1986). Previous results have demonstrated inhibitory muscarinic responses upon SPN (Gibson & Logan, 1995). We have utilised the *in vitro* rat spinal cord slice preparation to report here the excitation of SPN via nicotinic receptors and describe the pharmacology of this response.

Briefly, transverse slices, (300μm) were cut from 7-15 day old Sprague Dawley rats of either sex as described previously (Pickering et al., 1993). Slices were maintained at room temperature in an artificial cerebrospinal fluid (ACSF) consisting (in mM) NaCl (127), KCl (1.9), KH_2PO_4 (1.2), $MgSO_4$ (1.3), $CaCl_2$ (2.4), $NaHCO_3$ (26) and D-Glucose (10) pH 7.4 gassed with 95%:5% O_2:CO_2. Both agonists and antagonists were diluted to known concentrations in ACSF and applied to the slice by superfusion. Antagonists were applied for at least 5 minutes before subsequent applications of the agonist. Whole-cell recordings were made from the lateral horn region using pipettes filled with the following solution consisting (in mM) K-gluconate (130), KCl (10), $MgCl_2$ (2), Na_3-ATP (2), HEPES (10), EGTA (11) $CaCl_2$ (1) (final Ca concentration ~100nM) pH 7.4 with 1M KOH. The electrical activity of the SPN was recorded using an Axopatch 1D amplifier, displayed on a Gould 2300S chart recorder and following digitisation (at 22kHz) stored on video tapes.

Whole-cell current-clamp recordings were made from 51 neurones identified as SPN based upon their characteristic electrophysiological properties. Nicotine applied at concentrations between 500nM to 100μM evoked membrane depolarisations (n=45). The mean (±s.e.mean) amplitude of the depolarisation was 5.6 ± 0.5mV (range 2 to 17mV). In 21 SPN the membrane potential remained above that recorded prior to the application of nicotine. The me an amplitude of this maintained depolarisation was 2.8± 0.3mV (range 1 to 5mV). The duration of those nicotine-induced depolarisations that returned to baseline also varied markedly, having a mean value of 343 ± 53s (range 120 to 832s).

Constant application of TTX (500nM) to the slice for 5 minutes blocked nicotinic responses in 2 neurones but had no effect in 10 other SPN. The nicotine-induced membrane depolarisations could be subject to desensitisation as suggested by the reduced response to subsequent applications of the agonist (n=4). Prolonged intervals between applications (>25min) did not reduce the degree of desensitisation in these 4 neurones. The responses were abolished by the nicotinic antagonists tubocurarine (10-20μM; n=5) and hexamethonium (10-20μM; n=4). Modification of the membrane potential by direct current injection down the recording electrode (n=4) and preliminary ion substitution studies (n=3) indicate that the nicotinic response is not due to a reduction of a potassium conductance or chloride conductance. Our results suggest that acetylcholine acting via nicotinic receptors can lead to excitation of SPN. The relative interaction between nicotinic and muscarinic receptor activation has yet to be determined.

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We thank the Wellcome Trust for financial support.

439P BINDING OF THE NOVEL α_2-ADRENOCEPTOR AGONIST [^{3}H]MIVAZEROL: COMPARISON WITH [^{3}H]CLONIDINE

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Mivazerol (UCB 22073) is a novel α_2-adrenoceptor agonist (Noyer *et al.*, 1994). The present studies were undertaken to compare the binding characteristics of [^{3}H]-mivazerol with that of that of the established α_2-adrenoceptor agonist [^{3}H]-clonidine.

Whole brain membranes were prepared from male Wistar rats (250 - 300g) as previously described (Mallard *et al.*, 1992). Membranes were incubated (50mM Tris-HCl buffer, pH 7.4) with [^{3}H]-mivazerol (3nM, UCB Pharma) or [^{3}H]-clonidine (3nM, Amersham Int.) and displacing drug (0.1nM - 100μM) in triplicate to equilibrium (30min, final volume 1ml, 22°C). Bound and free ligand were separated by cold filtration.

Saturation assays suggested that both [^{3}H]-mivazerol and [^{3}H]-clonidine bound to single receptor populations with B_{max} values of 970 ± 80.8 and 134.8 ± 0.7 fmol.mg^{-1} protein and K_D values of 13.4 ± 2.4 and 1.7 ± 0.7nM respectively. Subsequent competition assay demonstrated that [^{3}H]-mivazerol binding was completely displaced by cold mivazerol (K_i=8.1 ± 0.2nM). However, noradrenaline (K_i = 63.7nM ± 1.4nM), idazoxan (K_i = 90.8 ± 8.4nM) and clonidine (K_i = 1.2 ± 0.1μM) were unable to fully displace bound [^{3}H]-mivazerol, producing maximal inhibitions of 43.9 ± 7.5%, 61.7 ± 10.4% and 52.5 ± 4.6% respectively. Likewise, the imidazoline-site preferring ligands moxonidine (I_1-sites, K_i=294.0 ± 20.6nM), and 2-BFI (I_2-sites, K_i=19.2 ± 0.4μM) inhibited [^{3}H]-mivazerol binding by only 54.2 ± 4.5% and 57.2 ± 2.4% respectively. In contrast, [^{3}H]-clonidine binding was totally displaced by 100μM noradrenaline (K_i = 6.0 ± 1.4nM), idazoxan (K_i = 6.0 ± 0.2nM), mivazerol (K_i = 0.8 ± 0.1nM), 2-BFI (K_i=3.0 ± 0.6μM) and moxonidine (K_i =2.3 ± 0.4μM).

The non-adrenoceptor element of [^{3}H]-mivazerol binding was determined in the presence of 10μM rauwolscine, and was virtually abolished by 100μM cold mivazerol (K_i = 45.2 ± 8.8nM). Furthermore the non-adrenoceptor binding of [^{3}H]-mivazerol was potently and fully displaced by the α_2-adrenoceptor ligands dexmedetomidine (K_i = 49.7 ± 6.3nM), laevomedetomidine (K_i = 17.4 ± 1.7nM) and atipamezole (K_i = 12.4 ± 3.5nM).

These data indicate that in addition to α_2-adrenoceptors, [^{3}H]-mivazerol labels a non-adrenoceptor site unrecognised by [^{3}H]-clonidine. This site would appear to be distinct from the previously described I_1- and I_2- binding sites (Ernsberger, 1992), but appears to be similar to the non-adrenoceptor site previously described for the binding of [^{3}H]-atipamezole in rat lung (Sjöholm *et al.*, 1992).

This work was supported by UCB Pharma.

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440P ACTIONS OF CHLORMETHIAZOLE ON VOLTAGE-GATED SODIUM CURRENTS IN MOUSE NEUROBLASTOMA NIE-115 CELLS

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The anticonvulsant, sedative, hypnotic and neuroprotective properties of chlormethiazole are believed to result from an enhancement of the actions of γ-amino butyric acid at the $GABA_A$ ionophore (Cross *et al.*, 1989; Hales & Lambert, 1992; Green & Cross, 1994). However, the possibility that some of the effects of this drug are mediated via inhibition of voltage-gated Na^+ currents has not been discounted. The present experiments were therefore designed to investigate whether such an action of chlormethiazole can be observed in a mouse neuroblastoma clonal cell line. Voltage-dependent Na^+ currents were recorded from N1E-115 cells using the whole-cell voltage clamp technique (see McGivern *et al.*, 1995).

When applied to cells held at -80 mV, chlormethiazole produced a concentration-related reduction of the Na^+ current generated by 10 ms steps to membrane potentials ranging from -70 to +50 mV. The reduction of the peak Na^+ current (± s.e. mean) was 10.4 ± 3.3% (3 cells), 16.7 ± 3.6% (3 cells), 30.1 ± 1.5% (6 cells) and 39.5 ± 6.3% (6 cells) at 0.1, 0.3, 1 and 3 mM chlormethiazole, respectively.

When applied at a concentration of 3 mM, chlormethiazole produced a -3.4 mV shift of the steady-state inactivation curve ($V_{HALF(CONTROL)}$ = -66.7 ± 0.68 mV; $V_{HALF(CHLORMETHIAZOLE)}$ = -70.1 ± 0.34 mV; $P < 0.02$; 4 cells), with no change in slope. Furthermore, the peak current generated in the steady-state inactivation curve (i.e., at a prepulse potential of -120 mV) was reduced by 38.4 ± 7.0%. Analysis of these data using methods developed by Bean *et al.* (1983) yielded affinity estimates of 1.5 mM and 4.7 mM for the *inactivated* and *resting* states of the Na^+ channel, respectively.

In the final series of experiments, 3 mM chlormethiazole was applied to 3 cells held continuously at -120 mV for a 30 s period. At the end of the 30 s, depolarizing steps to 0 mV were initiated and the amplitude of the first current generated was compared to the current developed before application of drug. In this way, chlormethiazole reduced the amplitude of the first current by 30.0 ± 2.8%, suggesting that the inhibitory effect of the drug is probably independent of an interaction with *open* Na^+ channels.

These results demonstrate that chlormethiazole is a weak inhibitor of Na^+ currents in N1E-115 cells and that this inhibition arises from an interaction with inactivated and resting states of the channel. However, it seems unlikely that this action contributes materially to the clinical profile of chlormethiazole given that potentiation of GABA-evoked currents occurs at concentrations at least an order of magnitude lower than those active at the Na^+ channel (Hales & Lambert, 1992).

We are grateful to Dr. A.R. Green (Astra Neuroscience Research Unit, London, U.K.) for the gift of chlormethiazole and to Mr. G.P. Duncan and Miss C. Linton for providing the cell cultures.

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441P LOSS OF THE NEUROPROTECTIVE EFFICACY OF THE ION CHANNEL MODULATOR LIFARIZINE (RS-87476) FOLLOWING HYPOTENSION IN A FOCAL MODEL OF CEREBRAL ISCHAEMIA

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Lifarizine (RS-87476)(1-[(2-(4-methylphenyl)-5-methyl)-1H-imidazol-4-yl-methyl]-4-diphenyl-methyl-piperazine) is an ion channel modulator which has been shown to inhibit both Na^+ and Ca^{2+} channels. It has been shown to have a broad neuroprotective profile in both global (rat 4 vessel occlusion) and focal (cat middle cerebral artery occlusion) models of cerebral ischaemia (Alps, 1992). The aim of the present study was to investigate the efficacy of two dosing regimen of lifarizine in the rat focal photochemical rose Bengal model of cerebral ischaemia(Grome et al.,1988).

The experiments were carried out using male Sprague-Dawley rats (230-350g). Anaesthesia was induced with 5% halothane in oxygen (O_2) and maintained at 2% halothane in O_2. The femoral artery and vein were cannulated to allow blood pressure recording and administration of the histological dye rose Bengal, respectively. A midline head incision was made and the skull exposed. The skull was marked at the bregma and a fibre optic light source (Osram Xenophot 250W lamp, passed through a heat filter, with a 3mm diameter tip) was positioned over the right cerebral cortex at the level of the bregma. Rose Bengal (15mg kg^{-1}) was injected via the femoral vein over 2min. On completion of the rose Bengal administration, the light source was switched on and the skull illuminated for 20min. 5min. post-illumination each rat received an i.v. dose of either 100μg kg^{-1} (n=12) or 1mg kg^{-1} (n=14) lifarizine. Parallel control groups were studied for each dosing regime.

The anaesthetic was discontinued and the rats were allowed to recover consciousness in a warm environment. Each rat was subsequently dosed twice daily for 72h with i.p. injections of either 2ml kg^{-1} vehicle or 500μg kg^{-1} lifarizine. Following this dosing regimen, each rat was killed by an overdose of halothane anaesthesia and decapitated. The brains were rapidly removed and frozen in isopentane at -42°C. Coronal sections, 20μm thick, were cut on a cryostat, fixed in FAM (formol:acetic acid:methanol; 8:1:1) and stained with cresyl violet. The lesioned area on each section is able to be clearly delineated on the image analyzer. Volume of infarct was then calculated for each brain. Following rose Bengal photochemical insult the area of cresyl violet deficit stain, and hence the area of lesion, was evident only in the right hemisphere of the brain. This indicates the focal nature of the lesion, since at no time was there any evidence of damage in either the ipsilateral subcortical or contralateral hemisphere brain regions.

1mg kg^{-1} lifarizine i.v. produced a significant reduction in mean arterial blood pressure (MABP) from 72 ± 3 mmHg to 49 ± 3 mmHg within 5 min, and no neuroprotection was evident (48.0 ± 5.0 to 48.6 ± 5.4 mm^3). 100μg kg^{-1} lifarizine i.v. had no such effect on MABP, and was able to produce a significant reduction in damage from 49.1 ± 5.4 to 32.0 ± 3.5 mm^3 ($P<0.05$, Student's t-test). Loss of efficacy with lifarizine in this model appears to be associated with the hypotensive action of the compound at higher i.v. doses of 1mg kg^{-1} lifarizine.

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442P A MUTATION IN THE PUTATIVE M2 DOMAIN OF A *DROSOPHILA* GABA RECEPTOR SUBUNIT DIFFERENTIALLY AFFECTS ANTAGONIST POTENCY

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The mutation of an alanine to a serine residue in the putative ion channel domain (M2) of a *Drosophila* recombinant GABA subunit greatly reduces the antagonist potency of picrotoxin (ffrench Constant *et al.*, 1993). Structurally diverse convulsants and insecticides, such as tert-butylbicyclophosphorothionate (TBPS) and γ-hexachlorocyclohexane (γ-HCH, lindane) respectively may bind to the picrotoxin site. By contrast to lindane, the δ isomer (δ-HCH) is a positive allosteric modulator of the mammalian $GABA_A$ receptor (Woodward *et al.*, 1992). Whether these actions of δ-HCH result from it binding to the same binding site as γ-HCH and picrotoxin is not known. Here, the actions of picrotoxin, TBPS, heptachlorepoxide, γ-HCH and δ-HCH on GABA-evoked currents recorded from *Xenopus laevis* oocytes expressing a *Drosophila melanogaster* wild type GABA subunit (Chen *et al.*, 1994) and this subunit containing the point mutation of an alanine to a serine residue in M2 have been compared in an attempt to better define the binding site of these agents. Oocytes were injected with the appropriate cRNAs and 2-12 days later, GABA-evoked chloride currents were recorded under voltage-clamp at a holding potential of -60mV. GABA (10^{-5} M - 3 x 10^{-3}M) produced a concentration-dependent inward current with a calculated EC_{50} of 152 ± 10μM (n = 4) and 93 ± 10μM (n = 4) for the wild type and mutant "receptor" respectively. Additionally, although desensitization of the wild type receptor was evident at GABA concentrations > 100μM little or no desensitization occurred with the mutant receptor even at the highest agonist concentration tested (3mM). Utilizing the appropriate EC_{50} concentration of GABA the antagonists inhibited the wild type receptor with the following rank order of potency: heptachlorepoxide > γ-HCH > picrotoxin >> TBPS (Table 1). The alanine to serine mutation decreased the antagonist potency of picrotoxin (74 fold) γ-HCH (~ 9 fold), TBPS (45 fold) and heptachlorepoxide (> 1000 fold), indeed only partial inhibition by the latter two agents was achieved (Table 1). Hence, although this mutation has influenced the actions of all of these structurally diverse antagonists the magnitude of the potency reduction is compound dependent. The δ isomer of lindane produced a concentration-dependent (10^{-7}M - 3 x 10^{-5}M) enhancement of GABA (EC_{10})-evoked currents recorded from oocytes expressing the wild type receptor with a calculated EC_{50} of 3.4 ± 0.1μM (n =4) and a maximal potentiation to 1068 ± 43% of control. Although the antagonist actions of γ-HCH were greatly reduced by the mutation (Table 1) this substitution had no effect on the positive allosteric actions of δ-HCH (EC_{50} = 2.3 ± 0.3 μM, maximal effect = 955 ± 28% of control, n = 3). Hence the negative and positive effects of these isomers of hexachlorocyclohexane are unlikely to be mediated through a common binding locus.

TABLE 1 Antagonist Potency in Wild Type and Mutated Subunits

	Drosophila Wild Type	*Drosophila* ALA→SER
Picrotoxin	46 ± 6nM	3.4 ± 0.3μM
TBPS	2.2 ± 0.2μM	Max inhibition at 100μM = 85±2% of control
Heptachlor-epoxide	9.3 ± 2.6nM	Max inhibition at 10μM = 90 ± 12% of control
γ-HCH	20 ± 2nM	175 ± 42nM

Where appropriate IC_{50} values are given (n = 3-7)

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Acknowledgements
We thank Dr. N. Lan for the *Drosophila* cDNA and Dr. P. Whiting for help with the mutagenesis. Supported by the M.R.C.

443P THE ANAESTHETIC AND GABA MODULATORY ACTIONS OF ORG 21465, A NOVEL WATER SOLUBLE STEROIDAL INTRAVENOUS ANAESTHETIC AGENT

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The i.v. administration of certain pregnane steroids induces rapid hypnosis, an effect that might plausibly be linked to a potent and selective potentiation of inhibitory neurotransmission mediated by the $GABA_A$ receptor (Lambert *et al.*, 1987). In the present study, we describe the hypnotic activity of the water soluble steroid Org 21465 [(2β, 3α, 5α)-3-hydroxy-2-(2,2-dimethylmorpholin-4-yl) pregnane-11,20-dione] and the effect of this agent upon human recombinant $GABA_A$ receptors, composed of α_1, β_2 and γ_{2L} subunits, expressed in *Xenopus laevis* oocytes.

Org 21465 administered i.v. over 10 seconds, induced a rapid loss of the righting reflex in mice, rats, pigs and dogs. In mice, Org 21465 was approximately equipotent to the endogenous pregnane steroid 5α-pregnan-3α-ol-20-one (5α, 3α DHP) and the synthetic anaesthetic steroid alphaxalone (Table 1). All three pregnane steroids were considerably more potent than the intravenous induction agents propofol and thiopentone (Table 1). The therapeutic index of Org 21465, although smaller than alphaxalone, was greater than for the non-steroidal anaesthetics examined. The duration of sleep induced by Org 21465 (at 2x the dose required to produce hypnosis in 50% of animals; *i.e.* HD_{50}), like that for alphaxalone, propofol and thiopentone, was short. In rats, the potency of Org 21465 exceeded that of non-steroids, and was approximately half that found for alphaxalone. In pigs and dogs, Org 21465 acted as a potent, smooth onset, short duration anaesthetic.

In voltage-clamped *Xenopus* oocytes expressing human recombinant $GABA_A$ receptors, Org 21465 enhanced the peak amplitude of the inward current response elicited by GABA, bath applied at the EC_{10}. Potentiation was concentration-dependent and reversible, with threshold effects observable at 10 nM and maximal enhancement (to 91 ± 4%; mean ± s.e.mean; n=3 of the response evoked by a saturating concentration of GABA) occurring with 10 μM of the steroid. The EC_{50} for potentiation by Org 21465 was 2.3 ± 0.2 μM (n = 3). This value compares with those of 177 ± 2 nM, 2.2 ± 0.3 μM and 3.8 ± 0.2 μM (n = 4 each) determined for 5α,3α DHP, alphaxalone and propofol respectively which also acted as positive allosteric regulators of the $GABA_A$ receptor.

In conclusion, the water solubility and profile of Org 21465 in several species suggests that this agent may prove suitable for the induction of anaesthesia in man, an action likely to involve enhancement of central inhibition.

Table 1. The hypnotic activity of Org 21465 in mice in comparison to reference compounds

Compound	HD_{50} (μmol.kg^{-1}) (95% Confidence Limits; n = 8)	Therapeutic Index (LD_{50}/HD_{50})**	Mean Sleep Time at 2x HD_{50} (min ± s.e.mean; n = 10)
5α,3α DHP*	10 (8.2-12.2)	33.2	7.9 ± 1.1
Alphaxalone*	6.3 (5.7-7.2)	23.7	1.7 ± 0.2
Org 21465	9.8 (8.9-10.6)	13	3.7 ± 0.4
Propofol	68.2 (64.1-71.8)	3.6	3.1 ± 0.2
Thiopentone	83.2 (74.5-91.7)	3.7	4.1 ± 0.2

* Dissolved in 20 % hydroxypropyl-β-cyclodextrin **LD_{50} determined by probit analysis, n = 8

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444P TACHYKININ ACTIVATION OF A NON-SELECTIVE CATION CONDUCTANCE IN ND7/23 CELLS

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Tachykinins exert effects upon various ion channel types present in neurones and studies have indicated a depolarizing action on dorsal and trigeminal root ganglia neurones. The novel neuroblastoma-dorsal root ganglion hybrid cell line ND7/23 (Wood *et al.*, 1991) was used as a model for dorsal root ganglion neurones. The actions of the selective tachykinin agonists GR73632 and GR64349 (Hagan *et al.*, 1991) and senktide (Wörmser *et al.*, 1986) were studied in undifferentiated cells and cells in which differentiation was induced by a medium containing FCS 0.5%, NGF 2ng ml^{-1} and 2mM dibutyryl cAMP.

Whole-cell patch-clamp recordings were made from ND7/23 cells. Drugs were applied via the perfusate or by current compensated microiontophoresis from multibarrel micropipettes placed 2 to 5μm upstream from the cell. The contents of the perfusate were (in mM): NaCl 135; KCl 5; $MgCl_2$ 1; D-glucose 11.1; HEPES 10; $CaCl_2$ 1.8 (pH 7.4 NaOH). The primary external cation in the fluid was varied to study flux through channels by replacing NaCl with (in mM): TEA 100; KCl 100; CsCl 100; $CaCl_2$ 110; $BaCl_2$ 110. The patch electrode for whole-cell patch-clamp recordings contained (in mM): K_2HPO_4 100; $MgCl_2$ 2.5; HEPES 5; EGTA 5; Na_2ATP 2 (pH 7.4 KOH). Guanosine 5'-triphosphate (GTP) at 0.3mM was included in the patch electrode in most experiments.

Slow voltage ramps from +50mV to -100mV in 1.5 or 3 seconds were applied every 2.5 or 5 seconds. This induced a current with outward and inward components which varied in amplitude according to the intracellular and extracellular media. This was increased by bath application of tachykinin agonists in differentiated cells. The increase ranged from 0.01 to 1.8nA inward at negative potentials and 0.01 to 1.6nA outward at positive potentials. No such effect was seen if GTP was not included in the patch electrode, nor was it evident in undifferentiated cells. In a four-fold NaCl gradient ($[32mM]_o$, $[127mM]_i$; osmolarity preserved with sucrose) the tachykinin induced current reversed at -36.5 ± 7.5mV (mean ± s.e. mean, n=4), a value similar to that predicted for a perfectly cation-selective channel.

Induced inward components were greatly attenuated when the extracellular perfusate contained only the divalent cations Ca^{2+} or Ba^{2+}, indicating a low conductance for divalent cations. Inward components were induced with Cs^+, K^+, Na^+ and TEA as the major external cation indicating that the induced conductance was non-selective for monovalent cations. The NK_2 receptor agonist GR64349 displayed a greater potency for induction of inward and outward components than the NK_1 agonists GR73632 and substance P, and the NK_3 agonist senktide. With TEA as the major external cation the EC_{50} values for induction of inward current (at -100mV) for the tachykinin agonists were (in μM): for GR64349, 0.046; for GR73632, 0.51; for substance P, 1.1 and for senktide, 2.2. With microiontophoretic agonist application a dose ratio for GR64349 was obtained in the presence of the selective competitive NK_2 antagonist GR94800 (McElroy *et al.*, 1992). The Gaddum equation yielded a pK_B of 5.1.

These data suggest the presence in ND7/23 cells of a tachykinin induced cation conductance displaying some selectivity for monovalent cations. The response is probably mediated via activation of the NK_{2r} receptor type (Regoli *et al.*, 1994).

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445P INVESTIGATION OF THE COUPLING OF THE HUMAN NK_3 RECEPTOR EXPRESSED IN THE CHO CELL TO THE CELLULAR ACIDIFICATION RESPONSE BY THE USE OF THE CYTOSENSOR MICROPHYSIOMETER

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Measurements of the increase in inositol phosphate formation in CHO cells expressing the human NK_3 receptor (Chung *et al.*, 1994), or of the secondary increase in $[Ca^{2+}]_i$ (Pinnock *et al.*, 1994) confirm the importance of phopholipase C as a transducer of receptor activation to cellular response. Our experience with the Cytosensor Microphysiometer (Molecular Devices Corp.) as an alternative method of measuring the cellular acidification rate as a more integrated functional response to activation of the human NK_3 receptor, has pointed to the utility of this technique for pharmacological experiments with isolated cells (Jordan *et al.*, 1994) and we have now been investigating the coupling between receptor activation and response using this technique.

CHO cells (~$0.6x10^6$ cells *per* channel) were perfused at 120μl min^{-1} with bicarbonate-free Hams F-12 (pH 7.4) and the extracellular acidification rate measured at 2min intervals by stopping the perfusion for 15s. Responses were evoked by the serial addition of the NK_3-selective agonist senktide to the perfusion fluid for 2min 42s with a dose-cycle of 30min, and were normalised against the response to a standard addition of senktide 30nM at the start of the experiment. Agents tested for effects on the response were added to the perfusion fluid in one or more channels, with untreated cells run in parallel acting as control.

The results from the present experiments agreed with our previous findings; senktide produced concentration-related increases in acidification rate over baseline (0.15-0.35pH units min^{-1}; 150-350μV sec^{-1}), with the full concentration-response relationship obtained in the range 0.03-1000nM (pEC_{50} 8.24±0.10, n=10).

To investigate the contribution of mobilisation of calcium from IP_3-sensitive stores to the acidification response to senktide, cells were exposed to thapsigargin (1μM, for 10min). Treatment with the Ca^{2+}-ATPase inhibitor did not affect the potency of the agonist (pEC_{50} 8.26±0.14 *versus* 8.09±0.21, n=5), but the the maximum response was reduced by 69.9±3.4%. In contrast to the findings with measurements of $[Ca^{2+}]_i$ in the cells (Pinnock *et al.*, 1994), and inconsistent with the effect of thapsigargin, the acidification response to senktide was largely resitant to block by the phospholipase inhibitor U-73122 (10μM). Interestingly, while the addition of the Na^+-H^+ inhibitor amiloride alone at 1mM also reduced the maximum response to senktide by the same extent as thapsigargin (67.2±1%, n=4), the combination of thapsigargin and amiloride abolished the response to senktide throughout the concentration range.

In conclusion, CHO cells expressing the human NK_3 receptor respond by increasing their metabolism, as evinced by an increased acidification rate response measured in the Cytosensor. At least two thirds of this response can be blocked by amiloride, suggesting an active pumping of protons. In contrast to measurements of $[Ca^{2+}]_i$ in response to activation of the NK_3 receptor, where coupling through phopholipase C can explain all of the observed response, the cellular acidification response seems also to rely on another mechanism.

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446P EFFECT OF 1-(2-TRIFLUOROMETHYLPHENYL)IMIDAZOLE (TRIM) ON NEURONAL, INDUCIBLE AND ENDOTHELIAL ISOFORMS OF NITRIC OXIDE SYNTHASE

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Imidazole and 1-phenylimidazole have been reported to inhibit nitric oxide synthase (NOS) enzyme activity *in vitro* (Wolff *et al.*, 1993). We have now evaluated a novel imidazole derivative, 1-(2-trifluoromethylphenyl) imidazole (TRIM), for ability to inhibit NOS enzyme activity *in vitro* as well as for antinociceptive and vasopressor activity in the mouse *in vivo*.

All methods employed in this study have been described previously (Babbedge *et al.*, 1993). Homogenates (10,000 x g) of mouse cerebella and lungs removed from urethane-anaesthetised (10 g kg^{-1}, i.p.) rats 6 h after i.p. administration of 5 mg kg^{-1} E. Coli endotoxin (serotype: 0127-B8) and bovine aortic endothelial cells were used as the source of nNOS, iNOS and eNOS respectively. NOS enzyme activity was determined as the conversion of [^{3}H] L-arginine to [^{3}H] citrulline. Both mouse cerebellar nNOS and rat lung iNOS were inhibited by TRIM *in vitro* with IC_{50} values of 28.2 ± 0.5 μM and 27.0 ± 0.9 μM respectively (mean ± s.e. mean, n=6). In contrast, TRIM proved to be a poor inhibitor of bovine aortic endothelial eNOS with an IC_{50} of 1057.5 ± 12.8 μM (n=6). Comparable IC_{50} values for L-NAME were 0.66 ± 0.06 μM, 10.6 ± 0.4 μM and 6.5 ± 0.06 μM (n=6) for nNOS, iNOS and eNOS isoforms respectively.

TRIM (10-50 mg kg^{-1}, i.p., 15 min before subplantar injection of 10 μl 5% formalin) inhibited late phase (15-30 min post-formalin injection) hindpaw licking (e.g. 20 mg kg^{-1}: 40.3 ± 10.7 s c.f. 106.6 ± 7.7 s in saline-injected animals, n=12, P<0.05) without influencing the increase in hindpaw weight (e.g. 20 mg kg^{-1}: 61.7 ± 7.9 mg c.f. 56.9 ± 2.6 mg in saline-injected animals, n=12,P>0.05). The ED_{50} for antinociceptive effect was 78.9 μmol kg^{-1}. Pretreatment of mice with L-arginine (50 mg kg^{-1}, i.p.) reversed the late phase antinociceptive effect of TRIM (20 mg kg^{-1}: 102.3 ± 11.8 s, n=14, c.f. 116.9 ± 19.6 s in L-arginine-injected controls, n=5, P>0.05). In urethane (10 g kg^{-1}, i.p.) anaesthetised mice (resting mean arterial pressure, MAP, 52.3 ± 3.3 mm Hg, n=9) administration of TRIM (50 mg kg^{-1}, i.p.) failed to alter MAP (e.g. 45 min: 46.7 ± 6.1 mm Hg, c.f. 42.1 ± 2.7 mm Hg in saline-injected animals, n=4-5, P>0.05).

TRIM exhibited selectivity (37x and 39x) for inhibition of nNOS and iNOS compared with eNOS *in vitro*. This profile of NOS isoform inhibitory effect *in vitro* was reflected *in vivo* by (i) pronounced antinociception in the mouse and (ii) lack of vasopressor activity in the anaesthetised mouse. Thus, TRIM may prove to be a useful experimental tool with which to study the biological roles of nitric oxide.

We thank the ARC for financial support.

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447P EXCITATION OF KNEE JOINT MECHANOCICEPTORS BY A BRADYKININ B_1 RECEPTOR AGONIST IN INTERLEUKIN-1β-TREATED RAT KNEE JOINTS: STUDIES *IN VITRO*

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We have previously validated an *in-vitro* knee joint model for studying the excitatory effects of bradykinin on knee-joint mechanonociceptors (Kelly *et al.*, 1995); in this model the action of bradykinin in normal knee joints is mediated via bradykinin B_2 receptors. In behavioural studies a role for cytokines in the induction of B_1 receptors in inflamed knee joints has been suggested (Davis & Perkins., 1994). We have investigated the effects of a bradykinin (Bk) B_1 agonist desArg^9-Bk on neural discharge in normal and Interleukin 1β (IL 1-β)-treated rat knee joints.

Male wistar rats were anaesthetised using urethane (25% w/v, 0.6ml 100g^{-1} weight i.p.). The abdominal aorta and vena cava were cannulated - the catheters were advanced past the iliac bifurcation to allow perfusion of the left hind limb with modified Krebs solution (95% O_2, 5% CO_2; 30°C; 1ml min^{-1}); the rat was then killed by cardiac puncture. Recording of afferent neural activity from high threshold mechanonociceptors was similar to that described elsewhere (Birrell *et al.*, 1993) except the medial articular nerve was dissected from the saphenous nerve. Experiments were carried out in untreated, boiled (inactivated) IL 1β and IL 1β-treated (100units, intra-articular. 90min prior to perfusion).

All mechanonociceptor units studied were activated by capsaicin (1-3μg, i.a.) and had conduction velocities within the C-fibre range (0.65 ± 0.11ms^{-1}, range 0.31 - 1.0ms^{-1}). The on-going neural discharge was significantly greater in IL 1β-treated knee joints (1.1 ± 0.3 impulses per second[i.p.s.]) when compared to untreated and boiled IL 1β-treated knee joints (0.3 ± 0.1 i.p.s; 0.2 ± 0.1 i.p.s.). DesArg^9-Bk only increased neural discharge in IL 1β treated knee joints. This increase was blocked by the B1 antagonist desArg^{9}leu^8-Bk [1mg kg^{-1}, i.a.](Table 1). Pilot studies showed that desArg^9-Bk also increased neural discharge in arthritic (15 ± 1 days following Freunds Complete adjuvant 0.1mg, intar-articular)knee joints (100μg, i.a. produced an increase of 90 ± 12 impulses above control level).

These data suggest that, in inflamed rat knee joints there is a role for bradykinin B_1 receptors in the activation of peripheral mechanonociceptors. The inflammatory cytokine, IL 1β, may play a role in the induction of this B_1-like activity.

Work is supported by MRC and Sandoz Institute for Medical Research.

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Table 1. Effects of desArg^9-Bk on neural discharge from knee joint afferents *in-vitro* (values shown as mean ± s.e. mean).

	UNTREATED (n=3) *increase in discharge (impulses)*	boiled IL-1β (n=3) *increase in discharge (impulses)*	IL 1-β (n=6) *increase in discharge (impulses)*
10μg	-	-	43 ± 13
30μg	1 ± 6	21 ± 18	141 ± 34*
100μg	7 ± 24	-4 ± 3	115 ± 30*
100μg (desArg^{9}leu^8-Bk)	-	-	47 ± 27†

*p < 0.05 Mann Whitney U-test; compared to boiled IL 1-β. † p < 0.05 paired t-test, compared to desArg^9-Bk 100μg.

448P INFLUENCE OF REARING CONDITIONS ON 5-HT$_{2C}$ RECEPTOR RESPONSIVENESS DURING STRESS IN THE RAT

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Early social isolation rearing can cause changes in behaviour, neurochemistry and alter responsitivity to psychotropic drugs (Wongwitdecha & Marsden, 1995a,b; Fulford *et al.*, 1994). The aims of the present studies were to investigate the effects of isolation rearing on forced swimming behaviour and to compare the effects of acute and chronic treatment with a 5-HT$_{2C}$ receptor agonist, 1-(3-chorophenyl) piperazine (mCPP) on this behaviour in isolation and socially reared rats. Each rat was exposed to the swimming test: 1) without drug pretreatment; 2) following acute systemic mCPP (0.25, 0.5 and 1 mg/kg i.p.) or saline; and 3) following chronic mCPP (0.5 mg/kg i.p.) or saline.

Male Wistar rats were housed from weaning either alone (isolation reared rats) or in groups of six rats/cage (socially reared rats) for 4 weeks. After a pre-exposure to forced swimming (Porsolt et al., 1978) for 15 min the day before, each rat was allowed to swim in a glass cylinder (height 35 cm, diameter 21 cm) containing 17 cm of water maintained at 25±1°C for a 5 min test. Their behaviour was quantified by measuring the time they spent almost motionless (immobility time) or struggling. Data were presented as mean±SEM and analyzed using ANOVA. Differences between groups were assessed using a post hoc Dunnett's test.

The results demonstrate that the isolation reared rats showed significantly less immobility time (isolation=127±12.1 sec; social=204±18.1 sec) and more struggling (isolation=149±14.6 sec; social=86.4±19.2 sec) than the socially reared rats (P<0.01; n=12 rats/group). Pretreatment with mCPP (0.25-1 mg/kg i.p.) in the socially reared rats decreased immobility and increased struggling compared to the controls (Table 1).

Table 1. Effects of mCPP on the total duration of immobility and struggling during 5 min tests (n=6 rats/group). Differences from control were assessed statistically using the Dunnett's test, *P<0.05.

Treatment	Dose (mg/kg, i.p.	Immobility (sec)	Struggling (sec)
Saline	-	124.0±12.8	125.0±15.3
mCPP	0.25	72.8±30.5	204.0±33.4
	0.50	61.7±15.9*	212.0±12.6*
	1.00	57.2±18.4*	222.0±17.4*

The effects of acute mCPP (0.5 mg/kg i.p.) were significantly greater in the isolation reared rats than the socially reared rats (P<0.05; n=6 rats/group). Chronic treatment with mCPP (0.5 mg/kg i.p.) for 7, 14 and 21 days, also significantly reduced immobility time and increased struggling in both the socially and isolation reared rats; however, there was no significant difference between the two groups.

The present results show that isolation rearing enhances the behavioural response to stress and the responsitivity to acutely administered mCPP. However, this difference was not seen after chronic mCPP treatment. The results indicate that isolation rearing causes increased 5-HT$_{2C}$ receptor responsiveness but this effect is only observed after acute mCPP administration.

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449P ARE mCPP-INDUCED BEHAVIOURS 5-HT$_{2C}$ OR 5-HT$_{2B}$ RECEPTOR-MEDIATED?

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m-Chlorophenylpiperazine (mCPP) is a non-selective 5-HT$_{2C/2B}$ receptor agonist (Kennett, 1993). In rats, mCPP causes hypolocomotion, oral dyskinesias, tumescence and hyperthermia as well as anxiety and hypophagia by stimulation of central 5-HT$_{2C/2B}$ sites (Kennett, 1993, Kennett et al., 1994). To examine whether 5-HT$_{2B}$ receptors mediate these, or other, behaviours, the effect of i.c.v. infusion of BW 723C86 (BW), an agonist on the 5-HT$_{2B}$ rat stomach fundus receptor [pEC$_{50}$ 7.9, intrinsic activity (IA) 0.8] with selectivity over the rat caudal artery 5-HT$_{2A}$ (pEC50 6.9, IA 0.3), cloned human 5-HT$_{2C}$ receptor (pEC$_{50}$ 6.3, IA 1.0) and all other receptors tested (pK$_I$ <6, Baxter et al., 1995), was studied.

Male Sprague Dawley rats (200-250 g) were held under a 12 h light cycle (lights on 0700 h) with free access to food and water. They were implanted with guide cannulae in the right lateral ventricle (coordinates; dorsal ventral, +0.64 mm, rostral caudal, -0.08 mm from the bregma and medial lateral, + 0.15 mm Paxinos and Watson, 1986) under medetomidine HCl and fentanyl anaesthesia. After a 5 day recovery period, rats were individually placed in clear perspex observation cages (26 x 26 x 22 cm) with free access to water. After 15 min, a cannula was inserted and 1 µl saline or BW infused over two minutes. After a further two minutes, the cannula was removed and behaviour scored for 15 mins. Rectal temperatures were measured, both immediately prior to BW infusion and 30 min later. Data is cited as means ± s.e.m. n=11-13 per group. Significance was tested by Mann-Whitney U test and Kruskal-Wallis ANOVA or 1 way ANOVA (temperature data).

ICV infusion of BW 723C86 1-30 µg i.c.v. did not affect locomotion (transits; vehicle 29.9 ± 6.6, BW 3 µg 31.7 ± 3.0, 10 µg 24.1 ± 3.6), rears (vehicle 23.0 ± 3.0, BW 3 µg 22.4 ± 3.4, BW 10 µg 20.4 ± 3.5), rectal temperature change (vehicle -0.13 ± 0.4, BW 3 µg + 0.12 ± 0.27, BW 10 µg + 0.06 ± 0.37°C), tumescence or oral function over 1-30 µg. Also, BW did not alter grooming, sniffing, or drinking and no idiosyncratic behaviours were observed. Despite the weak partial agonist properties of BW at the 5-HT2A receptor, no increase in head shake behaviour was observed. Indeed, in a separate study, systemically administered BW antagonised 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI)-induced head shakes (ID50 464 mg/kg s.c.).

Hypolocomotion, tumescence, oral dyskinesias and hyperthermia, behaviours induced by the 5-HT$_{2C/2B}$ agonist mCPP, are thus likely to be 5-HT$_{2C}$ receptor mediated as is mCPP-induced anxiety (Kennett et al., Bright et al., this meeting).

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450P ETHANOL CAN BOTH INHIBIT AND POTENTIATE THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPE α3β4

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We have used voltage clamping to study nicotinic acetylcholine receptors (AChRs) expressed in *Xenopus* oocytes and the molecular basis of the correlation between nicotine and alcohol consumption (Kowzlowski et al., 1993). Ethanol (EtOH) enhances the response of muscle nicotinic AChRs to acetylcholine (ACh) (Arcava et al., 1991). In contrast, the a7 homomeric receptor is inhibited by alcohol, but the effect of alcohol on the a2b2, a3b2 and a4b2 combinations is marginal (De Fiebre et al., 1994). However the b4 subunit found in ganglia and many areas of the brain (Dineley-Miller and Patrick, 1992), often in association with the a3 subunit, has not been studied.

Current responses to 1-10μM ACh or nicotine, either alone or co-applied with various concentrations of EtOH, were obtained from *Xenopus* oocytes injected with cRNA or DNA encoding the α3β4 neuronal nicotinic receptor subunits. The test response obtained in the presence of EtOH was expressed as a % of the average of the prior control and recovery responses. Pipette solutions contained: Current pipette, CsF 0.25M, CsCl 0.25M, 100mM EGTA, pH 7.2; Voltage pipette, 3M KCl; External recording solution in which drugs were applied: 115mM NaCl, 1.8mM $BaCl_2$, 2.5mM KCl, 10mM HEPES (pH 7.2) and 1mM atropine.

The mean inward current obtained with 3μM ACh was 455.1 ± 71.4 nA (n=8). The mean effect (±S.E.M.) of 1 mM EtOH on all inward current responses to either ACh or nicotine (1-10μM) from all oocytes was 103.8 ± 5.6% of the control value (n=6, range: 92.5-133%). In 3mM EtOH the response was 101.8 ± 4.7% (n=13, range: 64.5-146.5%); in 10 mM EtOH, 100.8 ± 3.1% (n=12, range: 71.1-110.1%); in 30mM EtOH, 99.6 ± 4.8 (n=21, range: 26.6-123.5%); in 100mM EtOH, 134.0 ± 4.7% ($p<0.01$, Students' t-test, n=13, range: 100.3-156.7%) and in 300 mM EtOH 208 ± 19.3% ($p<0.01$, n=14, range: 87.5-316.4%). 13 of the 14 responses at 300 mM were potentiations. However at lower concentrations of ethanol the effect is more varied, and a greater proportion of the responses are inhibitions. The results suggest that nicotinic receptors, especially those containing a3 and b4 subunits, could be mediators of the acute synergistic addictive processes involving both nicotine and alcohol.

We wish to acknowledge the support of the M.R.C. (U.K.), The Royal Society, S.H.E.R.T., the Strathclyde Molecular Biology Laboratory and R&D Fund; and the technical contributions of Fiona Kempsill, Dr. Diane Dixon and Angela Garman.

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451P DIMINISHED HYPERALGESIC RESPONSE TO INTRAPLANTAR CARRAGEENAN IN RATS AFTER TWO SUCCESSIVE INTRAPLANTAR INJECTIONS, ONE WEEK APART

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Welsh and Nolan (1994) have reported that hyperalgesia to noxious thermal stimulation after intradermal injection of carrageenan in sheep decreased after repeated injection of the irritant (3 doses, 1 week apart). Hyperalgesia to thermal stimulation in rats after intraplantar injection of an irritant has been demonstrated at sites local and distant to the site of injection (Kaiser & Guilbald, 1987), but responses after repeated administration have not been fully studied. Ferreira *et al.* (1978) reported that in rats, 2 injections of carrageenan given acutely, 2h apart in opposite paws, reduced the time to maximal hyperalgesia in the second paw. This study was designed to identify if tachyphylaxis to evoked hyperalgesia occured in rats when carrageenan was injected on 2 occasions, 1 week apart.

Sprague Dawley male rats were divided randomly into two groups. Group I (GI) animals (n = 8) received two intraplantar saline (0.9%) injections, 100 μl, 1 week apart, and group II (GII) animals (n = 10) received two intraplantar carrageenan (0.9%) injections, 100 μl, 1 week apart, in the same paw. Responses to thermal stimulation were recorded using the tail-flick method, with a water temperature of 51°C and the response time, tail flick latency (TFL), measured in seconds. Measurements were taken immediately before, and 0.5, 1, 2, 4, 6 and 24 h after intraplantar injection.

The area under the time response curve from 0 to 6 h (AUC) was calculated using the trapezoidal rule. AUC data were analysed using a general linear model routine which analysed treatment, week and their interaction. An unpaired t-test was used to compare pretreatment TFL values between GI and GII and a paired t-test was used to compare pretreatment TFL values for GI and GII and 24h after carrageenan injection. Differences were judged to be significant where $P < 0.05$.

The mean (±SEM) TFL before injection in GI (4.4±0.2 sec) and GII (4.1±0.1 sec) were not significantly different. Similarly, there was no difference between the TFL before carrageenan injection in GII on week 1 (4.1±0.1 sec) and week 2 (4.4±0.2 sec). There was a significant week ($P = 0.013$) and treatment ($P < 0.001$) effect and a significant week-treatment interaction ($P = 0.019$). The AUC for GI on week 1 (27.2±0.6 sec.h) was not different from that recorded on week 2 (27.3±0.6 sec.h), but was significantly greater than the AUC for GII on week 1 (20.7±0.4 sec.h). However, AUC for GII on week 1, was significantly less than the AUC for the same group on week 2 (24.4±1.1 sec.h), although this value was less than the saline treated group (GI, week 2). TFL values 24 h after injection of carrageenan were not different from pretreatment values.

These results indicate that the hyperalgesic response to intraplantar carrageenan injection in rats was significantly attenuated when carrageenan administration was repeated one week later and confirm the results recorded in sheep (Welsh & Nolan, 1994). Thus tachyphylaxis to an irritant-induced hyperalgesic response may occur. The nature and mechanism of this effect are under investigation.

Acknowledgement: This work was supported by the Robertson Bequest Fund, Glasgow University.

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452P REGULATION OF MICROTUBULE-ASSOCIATED PROTEINS IN CULTURED BASAL FOREBRAIN NEURONS

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Aging and Alzheimer's Disease are associated with a progressive degeneration of the ascending cholinergic pathway of the basal forebrain. Nerve growth factor (NGF) is essential for the survival and function of these neurons (Hefti *et al.*,1989). PC12 cells treated with NGF respond with increased neurite outgrowth,which may be related to enhanced expression of the microtubule-associated proteins MAP2 and tau. Nitric oxide (NO) has been shown to stimulate MAP2 expression in hippocampal granule cells (Johnston & Morris,1994).The aim of the present work was to investigate the effects of NGF and NO-releasing compounds on tau and MAP2 expression in cultured basal forebrain cholinergic neurons.

The basal forebrain region was dissected from foetal Wistar rats of embryonic age E17.Cells were grown in neurobasal medium with B27 supplement(Gibco).On culture day 4,cells were treated with the following:1) 40ng/ml NGF 4 days, 2) sodium nitroprusside (SNP), S-nitroso-N -acetyl- penicillamine (SNAP), 8bromo-cyclic GMP (8br.) or haemoglobin(Hb) for 24 h. Cells were fixed and taken for immunocytochemistry using monoclonal antibodies to tau and MAP2.Staining intensities were analysed using a computer image analysis program and values after drug treatment expressed as a percentage of control optical density values ±s.e.mean.The results are shown in Table 1.*P<0.05,**P<0.01,***P<0.001 compared to control value by Mann-Whitney U-test, n=6 for all.

Table 1

conc. in μM	tau % control	MAP2 % control
50 SNP	103±2	132±1*
50 SNAP	104±3	140±5 *
20 8br	100± 2	160±4 **
20 Hb	101±3	101±2
Hb+SNAP	102± 2	102±1
NGF(40ng/ml)	101± 2	286±3 ***

Thus,basal forebrain neurons respond to NGF in a similar manner to PC12 cells with increased expression of MAP2.The induction of MAP2 but not tau may indicate a preferential action on dendritic as opposed to axonal morphology. NO can cause an increase in MAP2 immunoreactivity and this effect may be mediated by cGMP.Recent evidence suggests that the ability of NGF to induce differentiation in PC12 cells may be mediated by NO (Peunova & Enikolopov,1995). It will be of interest to see if the observed NGF-induced increase in MAP2 immunoreactivity is also mediated by NO.

We thank the Wellcome Trust,MRC and Merck,Sharp & Dohme for support.

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453P EFFECTS OF PAEONOL ON THE ACTION POTENTIAL AND IONIC CURRENTS OF GUINEA-PIG VENTRICULAR MYOCYTES

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Paeonol is a principal constituent of Mudanpi, a traditional Chinese medicine prepared from the root bark of *Paeonia suffruticosa.* Previous studies have shown extracts of Mudanpi to have a significant protective effect in dogs against acute myocardial ischaemia (Ma *et al,* 1984). This study investigated the effects of paeonol on the action potential (AP) and ionic currents of guinea-pig ventricular myocytes, isolated as previously described (Bates and Gurney, 1993). The cells were continuously perfused at room temperature with a solution of composition (in mM): NaCl, 112; KCl, 5.4; $MgCl_2$, 1.0; $CaCl_2$, 1.0; NaH_2PO_4, 1.0; HEPES, 5.0; Glucose, 10; $NaHCO_3$, 24. The whole-cell, patch-clamp technique was used to record the AP and ionic currents. To record Na current (I_{Na}), the external Na concentration was reduced to 20mM by substituting TEACl for NaCl. The pipette solution usually contained (in mM): KCl, 140; $MgCl_2$, 1; EGTA, 5; HEPES, 10; ATP, 2; with CsCl replacing KCl for measurement of I_{Na}.

The main effect of paeonol on the AP was to depress the upstroke velocity (APV) and duration (APD), seen at concentrations from 80 to 640μM with IC_{50} of 254μM and 288μM, respectively. At 320μM, paeonol induced inhibition of APV by 21 ± 4% (mean±sem; P<0.01, n=9) and of APD by 26 ± 4% (P<0.001, n=9). The effect of paeonol on APV was associated with blockade of voltage gated I_{Na}. Inhibition of I_{Na} was voltage-dependent with IC_{50}=63μM at -110mV and 18μM at -90mV. Inhibition was not use-dependent, indicating that paeonol does not block open Na channels. Paeonol (160μM) shifted the voltage-dependence of inactivation towards more hyperpolarized potentials, suggesting that it preferentially bound to inactivated Na channels. Paeonol (320μM) had no effect on Ca current, indicating that APD shortening was not due to blockade of Ca channels. In the presence of glibenclamide (3μM), APD shortening by paeonol was preserved, suggesting that it is also not due to opening of ATP-sensitive K channels. Lignocaine also shortens APD, associated with block of a slowly inactivating I_{Na} (Wasserstrom & Salata, 1988). In the presence of 10μM lignocaine, paeonol (160μM) failed to decrease further AP amplitude or duration, implying that paeonol may share a common mechanism with lignocaine. Comparing the effects of the two agents on I_{Na} blockade showed that paeonol had a slower onset (~ 4min) and produced a smaller maximal block of I_{Na} (57 ± 7%, n=4) than lignocaine, which fully blocked I_{Na} in 2min. In conclusion, paeonol acts as a selective Na channel blocker and could be useful clinically as an antiarrythmic agent.

Y-L. Ma is a K C Wong scholar. We are grateful to Dr. S.E. Bates for help and discussion.

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454P ANALYSIS OF THE EFFECTS ON VASOCONSTRICTOR AGONISM OF ENDOTHELIUM REMOVAL, L-NAME AND ELEVATION OF TONE IN THE RAT ISOLATED COMMON CAROTID ARTERY

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In this increasingly used experimental model of a large conducting artery we have analysed factors which, in other vessels, influence pharmacological analysis of vasoconstrictors. Most striking were a very substantial influence of constitutive nitric oxide production and the absence of any evidence for responses mediated via the α_2-adrenoceptor sub-types, often uncovered in vessels by raising the tone or inhibiting nitric oxide production, e.g. (Templeton et al., 1989; Maclean et al., 1993).

Male Wistar rats were killed by overdose with pentobarbitone sodium, after which the left and right carotid arteries were removed. Two rings 3-4mm in length were cut from each vessel and mounted for tension recording under 3g tension in Krebs' solution at 37°C, bubbled with 95% O_2/5% CO_2, and allowed to equilibrate for one hour. When appropriate, the endothelium was removed by gently rolling the tissue around a thin wire. Tissues were then exposed to 1μM noradrenaline (NA) then 1μM acetylcholine to test the integrity of the endothelium. The contraction to NA 1μM was used as a standard response to compare the size of subsequent responses.

In unrubbed preparations, relative to noradrenaline (NA), phenylephrine (PE) was a full agonist and UK-14304 was a partial agonist (mean max. NA 1.1g, PE 1.0g, UK 0.4g; n=12) (see Nagadeh et al, 1994). Addition of L-NAME (100μM), to test the effect of blocking NO synthesis, produced a substantial contraction (58±10% of NA 1μM standard, n=10). Subsequently, sensitivity to agonists increased and the largest response obtained to UK-14304 became greater (1.45g) than the "maxima" to the "full" agonists NA and PE (which were not significantly increased). This enhanced response to UK-14304 was resistant to rauwolscine but highly sensitive to prazosin ($pA_2 = 9.2$) confirming its α_1-adrenoceptor agonism (Nagadeh et al, 1994). The potentiation by L-NAME could be reversed by addition of L-arginine (300μM). Other means of raising the tone per se, including 5HT, KCl and angiotensin II produced the anticipated small potentiations but these were significantly smaller than for L-NAME.

In rubbed preparations: relaxation to Ach was trivial; the sensitivities and maxima to the agonists lay between unrubbed controls and unrubbed + L-NAME. Addition of L-NAME to rubbed preparations produced a significantly smaller contraction than in unrubbed (20±6% of NA 1μM) and a smaller, though still present, shift in agonist maxima and sensitivities.

Conclusions: i. constitutive NO activity has substantial inhibitory influence on vasoconstrictor responses, ii. potentiation caused by inhibition of NO synthesis is a more powerful influence than our other attempts at synergism, iii. the lesser effect of rubbing c.f. L-NAME may or may not indicate a source of NO other than the endothelium but does indicate that, in maximising vasoconstrictor effects, rubbing is less effective. In pharmacological analysis of vasoconstriction in large blood vessels, L-NAME or similar should be considered as an adjunct.

This laboratory is a member of the EC Biomed project "EureCa" BMHI-CP94-1375.

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455P EFFECT OF DEXAMETHASONE AND BREFELDIN A ON ACTIVITY AND SUBCELLULAR LOCALIZATION OF INDUCIBLE NITRIC OXIDE SYNTHASE

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Administration of lipopolysaccharide (LPS) to J774.2 macrophages induces nitric oxide synthase (iNOS) activity 50% of which is in the particulate fraction (Hecker et al., 1992). iNOS is present in vesicles of primary macrophages and this could account for the presence of iNOS in the membrane fraction (Vodovotz et al., 1995). The aim of this study was to define the subcellular localisation of iNOS and to investigate the effects on iNOS activity and distribution in J774.2 macrophages of dexamethasone (DEX), and brefeldin A (BFA) which causes disruption of the Golgi apparatus and hence inhibits nascent protein transport (Misumi et al., 1986).

J774.2 macrophages were cultured to 95% confluence in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1%penicillin-streptomycin and 2mM L-glutamine. Cells were treated with LPS (1μg/ml) and NOS activity was measured by the detection of nitrite (μM) in the medium using the Griess reaction (Gross et al., 1991). Cells were fixed in 4% paraformaldehyde for 5 min and labelled for iNOS using a primary antibody dilution of 1:5000 (Bryant et al., 1995). The cells were visualised using both light and confocal laser microscopy. The effect of a 1h pre-exposure to dexamethasone (0.1μM) on iNOS activity and protein expression in response to LPS was measured. BFA (0.1-100μM) was added for either 1h or 6h after the cells had been exposed to LPS for 14h and its effect on nitrite release measured.

Nitrite release was detected from the cells after exposure to LPS for 24h. Nitrite release was first detected at a low level by 4h, the accumulation reaching a maximum at 24h (n=3; see table 1). Activity was suppressed by 32±3.1% after pre-treatment with DEX (n=3). Treatment of the cells for either 1h or 6h with BFA, at concentrations that suppresses iNOS (Paul et al., 1995), had no effect on nitrite accumulation (n=3)

Time (h)	0	2	4	6	8	15	24
Nitrite (μM)	0	0	0.85 ±0.2	12.1 ±0.9	20.2 ±0.4	46.7 ±7.5	77.3 ±3.4

Table 1: LPS -induced time course of nitrite accumulation

Treatment of cells with LPS induced positive immunolabelling of some, but not all, J774.2 macrophages (n=8). Untreated cells or cells where primary antiserum was replaced with normal rabbit serum did not label. iNOS protein was detectable in cells at 4h and was maximal at 15 and 24h. Visualisation of the immunolabelling by confocal laser microscopy showed strong subcellular localisation in the perinuclear region and some association with the plasma membrane (n=6). This localisation was most pronounced at 24h (n=3). DEX reduced the amount of protein detectable in the macrophages, although no clear difference in the subcellular distribution of iNOS was apparent (n=3).

We conclude that LPS induces iNOS protein in J774.2 macrophages which is expressed maximally by 15h. The subcellular localisation of the iNOS is confined to the perinuclear and the plasma membrane region. The perinuclear localisation could be associated with vesicles in the Golgi apparatus, although an inhibitor of Golgi protein transport had no effect on nitrite release from these cells.

This work was supported by the Wellcome Trust.

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456P ROLE OF NO AND FREE RADICALS AS MEDIATORS OF LEUKOCYTE-INDUCED CONTRACTIONS OF RABBIT AORTIC RINGS

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Leukocytes have been implicated in the development of vascular injury, including atherosclerosis and restenosis (Halliwell 1989). Leukocytes have the ability to release a variety of substances, some of which cause vascular contraction and others vascular relaxation and which can interact with mediators released from the endothelium (Salvemini *et al* 1989). The aim of this study is to investigate the changes in vascular tone when unstimulated rabbit leukocytes are added to rings of rabbit thoracic aorta *in vitro*, and the role of free radicals and nitric oxide.

Blood was extracted from the pulmonary artery of pentobarbitone anaesthetised male New Zealand White rabbits into syringes containing sodium citrate (3.8% w/v). Leukocytes were isolated by dextran sedimentation, hypotonic lysis and ficoll-hypaque density gradient centrifugation. Thoracic aortic rings were mounted on parallel steel wires in Krebs solution containing indomethacin (10^{-6}M) at 37°C. Following contraction with 5-hydroxytryptamine (10^{-7}M), leukocytes were added to the bath. L-nitroarginine methyl ester (L-NAME 10^{-3}M) was added to the rings 30 minutes prior to precontraction or to the leukocytes 30 minutes prior to addition to the organ bath. Scavenging drugs were added 1 minute prior to addition of leukocytes. Free radical generation from zymosan stimulated leukocytes was measured in the presence or absence of scavenging drugs in rabbit whole blood by luminol-enhanced chemiluminescence.

Addition of leukocytes to aortic rings caused a concentration dependent contraction which was abolished when the ring was incubated with L-NAME (Table 1). The contraction was enhanced following incubation of the leukocytes with L-NAME. Addition of L-NAME to both leukocytes and the ring produced similar results to the addition of L-NAME to the ring alone. Sodium azide, an inhibitor of myeloperoxidase (10^{-5}M, 10^{-4}M and 10^{-3}M) inhibited zymosan-induced chemiluminescence in rabbit whole blood (93±4, 98±1, 100± 0.2% inhibition respectively). Superoxide dismutase (SOD 100U/ml) or catalase (3000U/ml) also inhibited chemiluminescence (74±6, 51± 5% inhibition respectively). The combination of SOD and catalase produced similar inhibition to SOD alone (79±6% inhibition). Mannitol (0.1M), a hydroxyl radical scavenger was the least effective of the scavengers studied (39±7% inhibition). SOD (100U/ml) did not modify the contraction induced in rabbit aortic rings by $6x10^5$ leukocytes.

Table 1 Rabbit aortic ring (% change in 5-HT-induced tone) n=4-6.

	Leukocytes per 5 ml bath			
	$3x10^4$	10^5	$6x10^5$	10^6
control	-10±4	+5±7	+32±15	+42±13
L-NAME				
cells	+2±2	+21±6	+43±9	+50±9
ring	-15±5	-16±5	-5±5*	-4±5*
cells + ring	-7±4	-13±4	-17±6*	-21±4*

Values are expressed as mean±s.e.m. *p<0.05 compared with control.

It is concluded that endothelial derived nitric oxide contributes in some way to the contraction seen when leukocytes are added to aortic rings. Free radical generation by activated rabbit leukocytes appears to involve myeloperoxidase products and superoxide although superoxide does not appear to be involved in the contractile response to leukocytes.

S.K was supported by an MRC research studentship.

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457P DIFFERENTIAL CD_{14} STAINING AND INDUCTION OF HYPOREACTIVITY BY LPS IN RAT ARTERIES

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Ex vivo response to noradrenaline (NA) was investigated in conductance and resistance arteries of Wistar rat after intraperitoneal administration of lipopolysaccharide (LPS). Expression of CD_{14}, the receptor for the LPS-(LPS) binding protein complex was visualized by confocal microscopy using CD_{14} antibodies. Male Wistar rats (12-14 week old) were treated with an intraperitoneal bolus injection of either *Escherichia coli* LPS (30 mg/kg, LD_{50}: 32.76 mg/kg, 055:B5, Difco) in 0.1 ml saline/100 g body wt or in saline (0.9% NaCl). After 4 h the rats were killed, thoracic aorta, distal femoral arteries and branch II or III of mesenteric arteries (internal diameter: 150-200 µm) were removed and were mounted either in an organ bath or a myograph. The vessels were maintained in organ chambers filled with physiological solution (PSS) of the following composition in mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 1.25 (aorta) or 2.5 (femoral and mesenteric arteries), glucose 5.5 continuously kept at 37°C and gassed with a mixture of 95% O_2, 5% CO_2 (pH 7.4). All the experiments were performed on vessels with intact endothelium. Contractile responses to maximally active concentration of NA (1 µM for the aorta and 10 µM for the small femoral and mesenteric arteries) were tested at 4 h after treatment of the rat for 4 h with either LPS or saline solution. This period corresponds to a lag time of 8 h after the pretreatment of the rat with either LPS or saline solution. Relaxations of the vessels with exogenous L-arginine (1 mM) and reversion of these relaxations by the inhibitor of nitric oxide (NO) synthase, N^{ω}-nitro-L- arginine-methyl-ester (L-NAME, 300 µM) were assessed on vessels pre-contracted with NA at 8 h. Tissue distribution of CD_{14} staining of different vessels were performed using monoclonal murine anti-human CD_{14} biotin conjugated antibodies, that were revealed with monoclonal murine anti-biotin FITC conjugated. CD_{14} staining was performed after a lag time of 8 h after the pretreatment with either LPS or saline solution. CD_{14} staining was detected using confocal microscopy which enables accurate localization of antibodies. In accordance with previous studies (Julou-Schaeffer et al., 1990; Schneider et al., 1992; 1994), no changes in contractile responses to NA and no relaxations to L-arginine were observed on different types of vessels from control rats. Hyporeactivity to NA was observed in the aorta after LPS-treatment. Unlike the aorta, no depression of responses to NA was observed in small femoral and mesenteric arteries. Nevertheless, addition of L-arginine induced relaxation in the small femoral and mesenteric arteries as in the aorta in LPS but not in control rats. L-NAME (300 µM) reversed the relaxant effect of L-arginine in any vascular tissue tested. CD_{14} staining was observed on endothelial and smooth muscle layers in all of the vascular preparations tested. LPS-treatment did not induce any change in CD_{14} staining. In the aorta, CD_{14} labelling was observed in the endothelial layer and in at least three layers of smooth muscle cells. The small femoral and mesenteric arteries contained intense and uniform labelling of CD_{14} on the endothelial layer but only a weak and diffused labelling in the smooth muscle layer. The results indicate that LPS induces regional differences of vascular hyporeactivity. The latter involves the activation of L-arginine-NO pathway. The differential CD_{14} labelling together with the regional differences of LPS-induced hyporeactivity to NA within the arteries strongly suggest that CD_{14} expression in blood vessels plays a role in the development of vascular hyporeactivity during endotoxemia.

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458P CHARACTERISATION OF PHOSPHATIDIC ACID PHOSPHOHYDROLASE-2 IN AIRWAY SMOOTH MUSCLE

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Phosphatidic acid (PA) has been shown to have a number of intracellular functions. A potential role for PA in mitogenesis has also been proposed (Khan *et al.*,1994;Fukami *et al.*,1992). In guinea-pig airway smooth muscle cells (GPASM) PA can be derived from phosphatidylcholine (PC) hydrolysis via phospholipase C (PLC) and phospholipase D (PLD) in response to a variety of agonists, including Bradykinin and PDGF. In bradykinin stimulated GPASM cells PLD-derived PA appears to be upstream of cAMP formation which, in these cells, is anti-mitogenic (Stevens *et al* ., 1994). Thus, regulation of the PA concentration within these cells may be critical to their proliferative potential. Phosphatidic acid phosphohydrolase (PAP), which dephosphorylates PA to generate diacylglycerol (DAG), may therefore have a pivotal role in regulating cell function. Distinct PAP isoforms have been described in a number of cell types (Jamal *et al.*, 1991; Jamdar & Cao 1994). We have previously demonstrated the presence of a PAP-2 type activity in membrane fractions of GPASM cells (Tolan & Pyne 1995). The present work investigates the species specificity of PAP-2 type activity in membrane fractions of GPASM cells and examines the regulation of this activity.

Primary cultures of GPASM cells were prepared from a guinea-pig tracheal smooth muscle strip. Cells were grown to confluency in DMEM supplemented with 10% FCS/10% DHS and utilised for experiments between 16-21 days after initial preparation. Membranes were obtained by centrifugation (48 000 x g for 20min). PAP activity was measured by assaying the release of [^{32}P]Pi from [^{32}P]-PA. The Assay method was validated by thin layer chromatography analysis of reaction products. Assays were performed at 30°C for 5min using 150μM PA (~6500dpm/nmol). Triton X -100 was included in the assay at a fixed ratio of PA : Triton X -100 (1:10).

A PAP activity has been detected in membrane fractions of GPASM cells. This activity has been found to be Mg^{2+} independent and insensitive to inhibition by NEM, suggesting that it is a PAP -2 type activity. Sphingosine inhibited this activity in a concentration dependent manner. PAP activity exhibited no preference for PA species containing long or short acyl chains (dioleoylPA C18:1,18:1 100% activity, dioctanoylPA C8:0:8:0 84.5±29% activity). However PAP activity was least active against PA species containing stearoyl/arachidonyl acyl chains (C18:0,20:4 35.4±7.1% activity). Furthermore, pre-incubation of the membranes with a non-hydrolysable analogue of GTP (GppNHp) had no effect on PAP activity. All values represent mean±sd for three independent experiments.

These results suggest that the PAP-2 type activity in GPASM cells is more active against PA species derived from phospholipids other than the phosphoinositols which are rich in stearoyl/arachidonyl acyl chains. Furthermore, there appears to be no direct regulation of membrane PAP activity by G-proteins.

This work was funded by The Wellcome Trust.

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459P COMPUTER-ASSISTED COURSEWARE IN DRUG METABOLISM

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The study of drug metabolism is an integral part of Pharmacology courses but often suffers from being heavily chemically-orientated with many drug structures and chemical reactions to master. A lecture-based course in drug metabolism that keeps the students' attention is somewhat difficult to devise as it can involve showing many different reaction sequences and mechanisms of reaction without the ability for animation. On this basis, we have designed and implemented a computer-based courseware package in drug metabolism using animated reaction sequences.

The courseware is designed as an introduction to drug metabolism for science, dental, medical and pharmacy students. No prior knowledge of drug metabolism is assumed although a grounding in chemistry and chemical reactions is useful to make best use of the courseware. The aim of the courseware is to give students an understanding of the basic principles of drug metabolism including routes of metabolism. The courseware runs on a PC with 386 processor running at a minimum of 25MHz with VGA 16-colour graphics capability. Windows 3.1 is also required. The courseware comes as a self-loading archive file.

The courseware covers where drug metabolism occurs, why it is important and the major routes of phase 1 and phase 2 drug metabolism (including the enzymes and cofactors involved). The synthesis of cofactors involved in phase 2 metabolism is also covered as is the cytochrome P450 catalytic cycle including the electron transport chain, the nomenclature of cytochromes P450 and the further metabolism of glutathione conjugates. There are short quizzes after each section and a longer integrative quiz, based on aspirin metabolism, at the end of the courseware. The quizzes can be by-passed if the student is quickly revising. The direct route through the courseware can be completed in about 45 minutes but can be extended to 9 hours if all information is used. The courseware can be seen as complementary to the textbook, "Introduction to Drug Metabolism" (Gibson and Skett, 1995).

The courseware has been evaluated and checked in a number of Departments over the past year and is now available in its final version.

This courseware has been developed by the Pharma-CAL-ogy Consortium under TLTP funding from the HEFCE.

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