39P INHIBITION OF THE CYTOCHROME P4501A, P4502B AND P4503A SUBFAMILIES IN THE LIVER OF RATS TREATED WITH FLEROXACIN

M.R. Martínez-Larrañaga, M.J. Díaz, M.A. Martínez, M.L. Fernandez-Cruz, M.T. Frejo, M. Martínez, Tafur, M. & A. Anadón. Department of Toxicology, Faculty of Veterinary Medicine, Universidad Complutense, 28040 Madrid, Spain.

Fleroxacin is a new member of the class of fluoroquinolones. The drug has good activity (i.e. minimum inhibitory concentrations at less than 2 mg/L against 90% of strains) against a wide range of Gram-positive and Gram-negative bacteria. The drug is metabolised to form antimicrobially active N-demethyl-fleroxacin and inactive N-oxide-fleroxacin.

Limited data are available on drug-drug interactions involving fleroxacin. The most important reported drug-drug interaction is a decrease in systemic availability of fleroxacin after ingestion of aluminium- or magnesium-containing antacids. Because the new fluoroquinolones are considered to be a general inhibitors of cytochrome P450 enzymes (Edwards *et al.*, 1988; Anadón *et al.*, 1990), the purpose of this study was to determine whether fleroxacin, when administered *in vivo* to adult male Wistar rats, selectively inhibits hepatic microsomal cytochrome P450 (CYP) enzymes.

Rats weighing initially 200 g were deprived of food for 6 h before the single daily oral administrations of fleroxacin at the dose of 50 mg/kg for 6 days. Fleroxacin was administered by gavage. Control rats received 0.5 ml of saline solution. Fleroxacin-treated and control animals were killed 24 h after the last administration and the livers were removed. The livers were individually homogenized and microsomal pellets were prepared for drug metabolising enzyme determinations (Martínez-Larrañaga *et al.*, 1996).

Treatment with fleroxacin resulted in a significant decrease in the hepatic NADPH-dependent reduction of cytochrome *c* (20%) and the *N*-demethylation of erythromycin(CYP3A1) (20%), and no statistically significant change in total cytochrome P450 and cytochrome b_5 contents. In addition, treatment with fleroxacin caused a substantial decrease in the *O*-demethylation of methoxyresorufin (CYP1A2)(18%) and *O*-depentylation of pentoxyresorufin (CYP2B1)(33%). In contrast, treatment with fleroxacin had no significant effect on the *O*-deethylation of ethoxyresorufin (CYP1A1).

Our results demonstrated that fleroxacin inhibited some members of CYP1A, CYP2B and CYP3A subfamilies. Thus, taken collectivelly the above findings are consistent with the suggestion that these changes may contribute to an interaction between fleroxacin and the metabolism of other drugs administered concomitantly.

This work was supported by CICYT, Project SAF96-0044, Spain.

Anadón, A., Martínez-Larrañaga, M.R., Fernandez, M.C. *et al.* (1990). *Antimicrob. Agents Chemother.* **34**(11), 2148-2151.
Edwards, D.J., Bowles, S.K., Svenson, C.K. *et al.* (1988). *Clin. Pharmacokinet.* **15**, 194-203.
Martínez-Larrañaga, M.R., Anadón, A., Diaz, M.J. *et al.*(1996). *Toxicol. Appl. Pharmacol.* **141**, 185-194.

40P EXPRESSION OF P450 ENZYMES IN PRIMARY RAT VENTRAL MESENCEPHALIC CULTURE

Gilbert E., Edwards R.*, Schulz-Utermoehl T.*, Boobis A.*, Rose S. & Jenner P.
Neurodegenerative Disease Research Centre, Pharmacology Group, King's College London, LONDON UK.
*Clinical Pharmacology, Imperial College School of Medicine, LONDON UK.

In the adult rat brain only CYP2C13/6, CYP2D2, CYP2D5, and CYP2E1 are found in the substantia nigra (Riedl *et al.* in press, Watts *et al.* in press). Cultured foetal ventral mesencepalic cells are commonly used to study the effects of compounds on nigral dopaminergic neurones, but the expression of P450 enzymes is unclear. For this reason we have now investigated the expression of P450 enzymes in cultured foetal rat ventral mesencephalic cells.

Pregnant Sprague-Dawley rats at day E14 (where E1 is the first day following overnight mating) were killed by stunning followed by decapitation. Foetuses were removed and ventral mesencephalon dissected out under sterile conditions. The tissue was pooled, plated at a density of 1×10^6 cells/cm^2 on coverslips and grown without change of media (37°C, 5% CO_2, 100% humidity) under conditions based on the method of Barker & Johnson (1995). At day 6 the cells were fixed in 4% paraformaldehyde and immunocytochemistry performed using antibodies against tyrosine hydroxylase (TH, 1:200), glutamic acid decarboxylase (GAD, 1:200), and glial fibrillary acidic protein (GFAP, 1:200), as phenotypic markers, and highly specific anti-peptide antibodies against CYP2B1/2, CYP2C11, CYP2C12, CYP2C13/6, CYP2D5, CYP2E1, and NADPH-P450-oxidoreductase (all at 1:200, Edwards *et al.* 1995). Immunoreactivity was visualised using Vectastain ABC peroxidase kit (Vector Labs) with 3,3'-diaminobenzidine and hydrogen peroxide. Immunopositive cells were manually counted using a blinded protocol on randomly selected regions from each slide and expressed as a percentage of the total number cells present (which were counter-stained using cresyl violet).

The average numbers of TH-, GAD-, and GFAP-immunopositive cells (3%, 48%, and 4% respectively) were in agreement with previously published values for these cultures (Colton *et al.*, 1995, Goto *et al.*, 1997). Large numbers of cells were highly immunoreactive for all the P450 enzymes studied (Table 1) as was as for NADPH-P450-oxidoreductase immunoreactivity (74%), but the type of cells involved remains to be elucidated.

Table 1: Percentage of cells showing positive immunoreactivity.

P450 enzyme	Mean % positive cells* (min, max)
CYP2B1/2	69 (60, 74)
CYP2C11	47 (44, 50)
CYP2C12	68 (60, 71)
CYP2C13/6	66 (61, 71)
CYP2D5	51 (48, 53)
CYP2E1	71 (65, 80)

*Number of immunopositive cells as a percentage of total number of cresyl violet stained cells from 3 - 8 separate cultures.

The high levels of expression suggests that the P450 enzymes are not restricted to one type of cell However, the pattern of expression is different to that found in the adult rat substantia nigra. This raises the question as to whether the expression observed is due to induction from the stress of the culturing process, or whether P450 enzymes in neuronal and/or glial cells are developmentally regulated.

E.Gilbert is supported by a Wellcome Trust Studentship.

Barker & Johnson, 1995. *Neuronal Cell Culture* IRL Press.
Colton C., Pagan F., Snell J. *et al.*, 1995. *Exp. Neurol.* **132** 54-61.
Edwards R., Singleton A., Murray B. *et al.*, 1995. *Bichem. Pharmacol.* **49** 39-47.
Goto K., Hochizuki H., Halton T. *et al.*, 1997. *Brain Res.* **754** 260-268.
Riedl A., Brown C., Rose S. *et al.*. *Br. J. Pharmacol.* (in press, Harrogate meeting).
Watts P., Riedl A., Douek D. *et al. Neurosci.* (in press).

41P AN ASSESSMENT OF THE POTENTIAL OF ROPINIROLE, A DOPAMINE RECEPTOR AGONIST, TO INHIBIT VARIOUS HUMAN CYTOCHROME P450 ENZYMES

J. C. Bloomer, S. E. Clarke and R. J. Chenery (introduced by C. M. Kaye), Department of Drug Metabolism and Pharmacokinetics, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts, AL6 9AR.

The dopamine agonist, ropinirole, has recently been shown, at clinically relevant concentrations, to be a substrate for CYP1A2 (Bloomer *et al*, 1997). Although this compound has some structural features consistent with substrate models for CYP2D6, there was no metabolism by this enzyme. A recent report has indicated that various dopamine receptor agonists, including ropinirole, have potential to inhibit CYP2D6 (Wynalda and Wienkers, 1997). In the present study the potential of ropinirole to inhibit various human P450 enzymes, including CYP2D6 and CYP1A2, was investigated.

Ethoxyresorufin O-dealkylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), tolbutamide hydroxylase (CYP2C9), S-mephenytoin 4-hydroxylase (CYP2C19), bufuralol 1'-hydroxylase (CYP2D6), chlorzoxazone 6-hydroxylase (CYP2E1), cyclosporine oxidase (CYP3A), testosterone 6β-hydroxylase (CYP3A) and lauric acid ω-hydroxylase (CYP4A) were the enzyme activities used to investigate the inhibitory potential of ropinirole, the methods for which have been reported previously (Bloomer *et al*, 1994). The substrates were incubated with human liver microsomes (n=3) and an NADPH regenerating system, in the absence and presence of ropinirole (1-250 μM). The percentage inhibition of each enzyme activity with 250 μM ropinirole was determined and, for bufuralol 1'-hydroxylase, IC_{50} values were calculated.

With 250 μM ropinirole over 80% inhibition of bufuralol 1'-hydroxylase was observed. There was no or minimal (<20%) inhibition of the other enzyme activities investigated, including ethoxyresorufin O-dealkylase. The IC_{50} for the inhibition of bufuralol 1'-hydroxylase was 9.3 μM ± 3.0 (mean ± SD, n=3). Such an IC_{50} value is consistent with the approximate 60% CYP2D6 inhibition with 10 μM ropinirole reported by Wynalda and Wienkers (1997). The IC_{50} reported by these authors (0.54 μM) is an order of magnitude more than would be expected from their inhibition data, suggesting an inconsistency.

Maximal plasma concentrations of ropinirole are on average 0.1 μM and are unlikely to exceed 0.3 μM after administration to man (data on file at SB Pharmaceuticals). An IC_{50} value of 9.3 μM represents a ropinirole concentration much in excess of typical plasma levels, therefore limiting the likelihood of a drug interaction involving ropinirole and CYP2D6.

Bloomer, J.C., Baldwin S.J., Smith G.J. *et al* (1994) British Journal of Clinical Pharmacology, 38, 557-566.
Bloomer, J.C., Clarke S.E. & Chenery R.J. (1997) Drug Metabolism and Disposition, 25 (7), 840-844.
Wynalda, M.C. & Wienkers, L.C. (1997) Drug Metabolism and Disposition, 25 (10), 1211-1214.

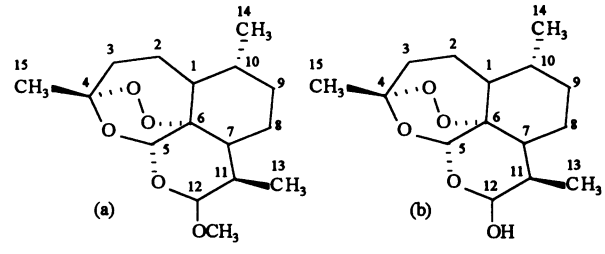
42P THE BILIARY METABOLITES OF β-ARTEMETHER, AN ENDOPEROXIDE ANTIMALARIAL

John O. Bell, Geoffrey Edwards, James L. Maggs, Laurence P. Bishop, Paul M. O'Neill, Stephen A. Ward, Peter A. Winstanley and B. Kevin Park, Department of Pharmacology & Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3GE.

β-Artemether (AEM; fig 1a) is an O-methyl derivative of the endoperoxide antimalarial, artemisinin (De Vries & Dien, 1996). AEM is regarded as a pro-drug of dihydroartemisinin (DHA; fig 1b) in humans. DHA undergoes rapid glucuronylation and biliary excretion in rats (Maggs *et al.*, 1997). The glucuronide is inactive. We have now characterised the biliary metabolites of AEM.

[13-^{14}C]- AEM (35 μmol kg^{-1} in DMSO) was given i.v. to pentobarbitone-anaesthetized and jugular vein cannulated male Wistar rats (210 to 250g) and CD1 mice (42 to 49g). Between 0-5 h, 35.2 ± 3.0 % and 15.4 ± 1.0 % (mean ± S.D, n=6) of the radiolabelled dose was recovered in the bile of rats and mice respectively. The terminal recovery in urine was 1.7 ± 0.7 % dose ml^{-1} (rat) and 13.8 ± 6.0 % ml^{-1} (mouse) and in plasma 0.01 ± 0.01% ml^{-1} (rat) and 6.0 ± 2.1 % ml^{-1} (mouse). Biliary metabolites were analysed by reversed-phase radiometric HPLC (5μm particle size; C-8 Phenomenex Ultracarb® column) using a gradient of acetonitrile (20-35 % v:v, 15min; 35-70 % v.v, 10min): 0.1M ammonium acetate and characterised by LC-MS. Recovery of the total radiolabelled dose was 96.3 ± 8.5 % (rat) and 89.8 ± 7.6 % (mouse). The major metabolites in rat bile (0-3 h; 32.3 ± 2.6 % of radiolabelled dose) were the glucuronides of 9-OH-DHA (R_t 15 min; 37.0 ± 6.5 % of eluted ^{14}C radioactivity; n=5) and DHA (R_t 31 min; 22.0 ± 2.5 %). Three unassigned monohydroxy glucuronides (R_t 13 min; 5.2 ± 1.3 %; R_t 22 min, 15.3 ± 2.0 %; R_t 25 min, 4.5 ± 1.1 %) and a dihydroxy glucuronide (R_t 9 min, 6.1 ± 1.6 %) were also found. Mice formed glucuronides of DHA (48.4 ± 11.3 %), 9-OH-DHA (20.7 ± 13.1 %) and two hydroxy glucuronides (R_t 22 min; 10.8 ± 1.9 %; R_t 25 min; 4.6 ± 3.2 %) but also the -OCH_2OH glucuronide (R_t 33 min; 11.7 ± 5.8 %). Hydroxylation is therefore a major route of AEM metabolism in rat and mouse. 9-OH AEM is known to have antimalarial activity *in vitro* (Lee & Hufford, 1991).

Figure1. Structure of (a) artemether and (b) dihydroartemisinin.



De Vries P.J. & Dien T.K. (1996) *Drugs*, 52, 818-836.
Lee I.-S. & Hufford C.D. (1991) *Pharmacol. Ther.*, 48, 345-355.
Maggs J.L., Madden S., Bishop L.P. *et al.*, (1997) *Drug Metab. Dispos.*, 25, 1200-1204.

43P EVIDENCE AGAINST CLOMETHIAZOLE PREVENTING MDMA-INDUCED NEURODEGENERATION BY FREE RADICAL SCAVENGING

M.I. Colado, E. O'Shea, B. Esteban, R. Granados & *A.R. Green. Dept. Farmacol., Fac. Med., Univ. Complutense, Madrid 28040, Spain & *Astra Arcus, Bakewell Rd., Loughborough LE11 5RH.

Administration of 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') to rats produces degeneration of 5-HT neurones in the brain, and this degeneration is attenuated by injection of clomethiazole (CMZ) (Colado *et al.*, 1993). The neuroprotection is produced partly by prevention of MDMA-induced hyperthermia and partly by an unrelated mechanism (Colado *et al.*, 1997a). We have now investigated whether this second mechanism involves a free radical scavenging action, since increased free radical formation is involved in MDMA-induced damage (Colado *et al.*, 1997a).

Male DA rats (170-200g) were implanted with a microdialysis probe in the hippocampus. The next day probes were perfused with artificial csf containing salicylic acid (0.5 mM) and dialysate collected for measurement 2,3-dihydroxybenzoic acid (2,3-DHBA), raised levels of which indicate increased free radical formation (for details see Colado *et al*, 1997a). Animals given CMZ (50 mg kg^{-1}i.p) 5min prior and 55min post MDMA (15 mg kg^{-1} i.p.) were placed in a cage with a homeo-thermic blanket (see Colado *et al.*, 1997b) to ensure that their body temperature was elevated to that of rats given only MDMA.

Both MDMA and MDMA+CMZ treated rats showed a similar hyperthermic response (data not shown). The concentration of 2,3-DHBA in the dialysate rose rapidly after MDMA (15 mg kg^{-1} i.p.) and was significantly increased for over 6.5h (Figure 1). No attenuation of the enhanced 2,3-DHBA formation was observed in the MDMA treated rats also injected with CMZ (Figure 1).

These data demonstrate that CMZ does not alter MDMA-induced increases in free radical formation. Since CMZ is neuroprotective even when animals are hyperthermic (Colado *et al.* 1997b), we conclude that its protective mechanism does not involve free radical scavenging.

Colado, M.I. *et al.* (1993) *Br. J. Pharmacol.* **108**, 583-589.
Colado, M.I. *et al.* (1997a) *Br. J. Pharmacol.* **121**, 889-900.
Colado, M.I. *et al.* (1997b) *Br. J. Pharmacol.* **122**, 304P.

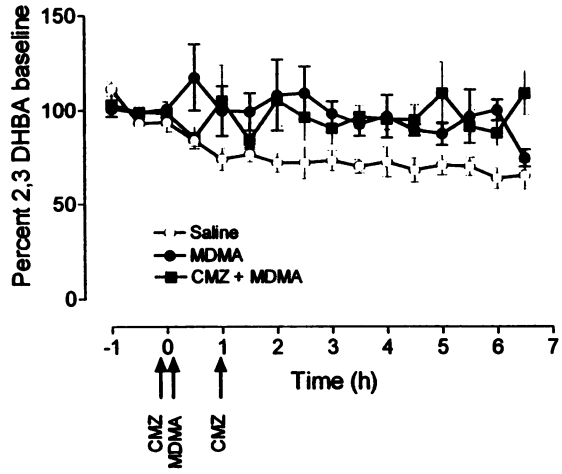


Figure 1. Percentage changes in 2,3-DHBA in the dialysate from a hippocampal probe implanted in rats injected with MDMA (15mg kg^{-1}) or MDMA plus clomethiazole (50 mg kg^{-1} x 2) injected at arrows. In MDMA-treated rats the 2,3-DHBA conc. was elevated above that seen in saline injected animals [$F(1,13=17.32, p<0.001$]. This elevation was not altered significantly by clomethiazole treatment. Mean baseline values were: saline, 6.0 ± 0.6; MDMA/CMZ, 5.4± 0.6; MDMA, 4.9 ± 0.6 (pmol mg^{-1}, n=7-8)

M.I.C. thanks CICYT (SAF 1560/95) and Astra Arcus for support.

44P ROLE OF BODY TEMPERATURE IN THE PROTECTIVE ACTION OF HALOPERIDOL AGAINST MDMA-INDUCED NEURODEGENERATION

E. O'Shea, B. Esteban, R. Granados, *A.R. Green & M.I. Colado. Dpto. Farmacologia., F. Medicina, Univ. Complutense, Madrid 28040, Spain & *Dept. Pharmaceut. Sci., De Montfort Univ., Leicester LE1 9BH.

Previous studies have demonstrated that haloperidol (HAL) protects against the neurotoxicity induced by 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') in rat brain (e.g. Hewitt & Green, 1994). HAL does not produce hypothermia, but does block the MDMA-induced rise in temperature. Although some drugs are neuroprotective through a mechanism which is independent of changes in temperature (Colado *et al.*, 1997a,b; Malberg *et al.*, 1996), a decrease in the hyperthermic response seems to be the determinant factor in the protection exerted by other compounds (Farfel & Seiden, 1995). We have now examined whether HAL protects against MDMA-induced neurodegeneration of 5-HT nerve terminals in rats kept at normal and high ambient temperatures.

Male Dark Agouti rats (175-200g) were injected with HAL (2 mg kg^{-1}, i.p.) or vehicle 5 min before and 55 min after MDMA (15 mg kg^{-1}, i.p.). HAL was suspended in peanut oil at 2 mg ml^{-1}. Another group of rats treated with HAL and MDMA were placed in a cage covered with an homeothermic blanket to keep their body temperature similar to that of rats given MDMA alone. Rectal temperature was measured over 6 h following MDMA administration. Seven days later the rats were killed and 5-HT reuptake sites quantified in the cortex by [^{3}H]-paroxetine binding, a measure of neuronal damage.

MDMA administration resulted in a long-lasting hyperthermia (1.5°C above the saline group) which was totally abolished by HAL co-administration. HAL also prevented the loss in cortical [^{3}H]-paroxetine binding seen 7 days after MDMA administration (Figure 1). When this experiment was repeated maintaining rats at high ambient temperature, the blocking effects of HAL on both hyperthermia and neurodegeneration induced by MDMA were absent (Figure 1). These results agree with those found with methamphetamine (Bowyer *et al.*, 1994) supporting the view that the neuroprotective effect of HAL might be due solely to its ability to abolish MDMA-induced hyperthermia.

Bowyer, J.F. *et al.* (1994) J. Pharmacol. Exp. Ther. 268, 1571-1580.
Colado, M.I. *et al.* (1997a) Br. J. Pharmacol. 121, 889-900.
Colado, M.I. *et al.* (1997b) Br. J. Pharmacol. 122, 304P.
Farfel, G.M. & Seiden, L.S. (1995) J. Pharmacol. Exp.Ther. 272, 860-867.
Hewitt, K.E. & Green, A.R. (1994)Neuropharmacology 33, 1589-1595.
Malberg, J.E. *et al.* (1996) J. Pharmacol. Exp. Ther. 278, 258-267.

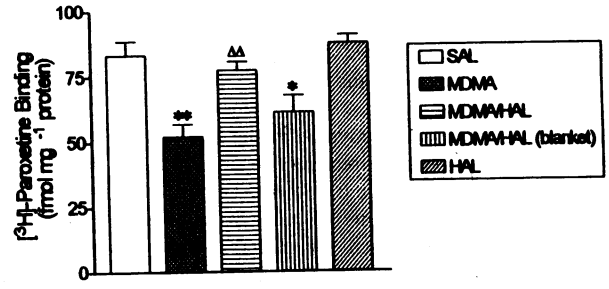


Fig. 1. Effect of haloperidol (HAL, 2 mg kg^{-1}, i.p.) given 5 min before and 55 min after MDMA (15 mg kg^{-1}, i.p.) on cortical [^{3}H]-paroxetine binding 7d later. Rats given MDMA and HAL were kept at normal and high (blanket) ambient temperature. Results shown as mean ± s.e. mean, n = 6-10. Different from saline-treated: *$P<$ 0.05, **$P<$ 0.01. Different from MDMA-treated: $^{\Delta\Delta}P<$ 0.01 (Newman-Keuls test).

MIC thanks CICYT (SAF 1560/95) and Astra Arcus for support.

45P IDENTIFICATION OF CLOMETHIAZOLE METABOLITES IN THE GERBIL

[1]A.R.Green, [2]T.K.Murray, A. Misra & [3]A.J. Cross, [1]Astra Arcus, Bakewell Rd, Loughborough LE11 5RH, [2]Astra Arcus, S-151 85, Södertälje, Sweden & [3]Astra Arcus USA, 755 Jefferson Road, Rochester NY 14623,USA.

There is substantial evidence that clomethiazole (CMZ) is neuroprotective in both global and focal models of acute ischaemic stroke (see Green & Cross, 1994). Several of these neuroprotective studies were performed in gerbils, an animal in which CMZ has a short plasma half life (Cross *et al.*, 1995), raising the possibility that active metabolites may be responsible for, or assist in, the protection afforded by CMZ administration. Major metabolites reported to occur in man include (company code in brackets): 5-(l-hydroxy-2-chloroethyl)-4-methylthiazole (NLA-715), 5-(l-hydroxyethyl)-4-methylthiazole (NLA-272) and 5-acetyl-4-methylthiazole (NLA-511) (see Dollery, 1991).We have now investigated whether these metabolites occur in gerbils.

Male Mongolian gerbils (60-80g) were injected with CMZ (600 μmol kg^{-1} i.p.). The concentration of CMZ, NLA-715, NLA-272 and NLA-511 measured in plasma and brain between 5 - 240 min after administration, using an adaptation of the h.p.l.c. method of Kim & Khanna (1983) for CMZ. Following a single injection of CMZ (600 μmol kg^{-1}) there was a rapid increase in the plasma concentration of CMZ, which was maximum at 5 min and declined rapidly thereafter (Figure 1) with a plasma half life of 25min. The brain drug concentration paralleled both the rise and decline, although the peak cerebral concentration was approx. 40% higher (229 ± 56 nmol g^{-1} n=6). Significant concentrations of NLA-715 were present in plasma and brain for at least 2 h post injection with small but measurable levels of NLA-272 and NLA-511 (Figure 1).

The data confirm the short half life of CMZ previously reported (Cross *et al.*, 1995) and the fact that brain concentrations of the compound are higher than blood following i.p. administration. They also demonstrate that NLA-715 is found in significant amount after a neuroprotective dose of CMZ.

Cross, A.J. *et al.* (1995). *Br. J. Pharmacol,* **114**, 1625-1630.
Dollery, C.(1991). In: *Therapeutic drugs pp C184-C188,* London, Churchill-Livingstone.
Kim, C. & Khanna, J.M. (1983). *J. Liq. Chromatogr.* **6**, 907-916.
Green, A.R. & Cross, A.J. (1994). *Prog. Neurobiol,* **44**, 463-484.

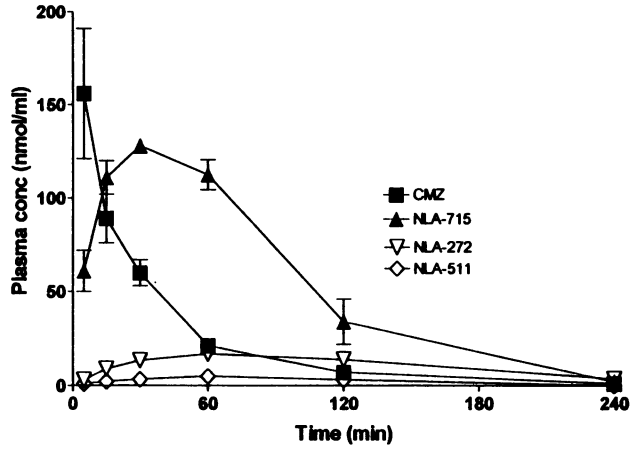


Figure 1: Plasma concentration of CMZ and various metabolites following a dose of 600μmolkg^{-1} clomethiazole (i.p.)Results shown as mean ± s.e.mean (n=6).

46P THE ACTION OF CLOMETHIAZOLE METABOLITES IN THE GERBIL MODEL OF GLOBAL ISCHAEMIA

[1]A.J. Cross, J. A. Jones, [2]T. K. Murray & [3]A. R. Green, [1]Astra Arcus USA, 755 Jefferson Road, Rochester NY 14623, USA, Astra Arcus, S-151 85 Södertälje, Sweden and [3]Astra Arcus, Bakewell Road, Loughborough LE11 5RH.

In another communication to this meeting (Green *et al.*, 1998) we reported that the neuroprotective compound clomethiazole (see Green & Cross, 1994; 1997) had a short half life and formed one major metabolite (NLA-715) with lower concentrations of 2 others (NLA-272, NLA-511) raising the possibility that the neuroprotective action of clomethiazole might be due, at least in part, to the effect of active metabolites. We therefore examined the effect of these metabolites in the gerbil model of global ischaemia.

Male Mongolian gerbils (60-80g) were subjected to transient forebrain ischaemia by a 5 min bilateral carotid artery occlusion (see Cross *et al.*, 1991; 1995). Clomethiazole or its metabolites were injected i.p. 60 min later in 2 separate experiments. Four days after the ischaemic insult, degeneration of the CA1 region was measured in 20μm coronal sections of the hippocampus stained with Cresyl Violet as detailed by Cross *et al.* (1995).

In both experiments clomethiazole (600 μmol kg^{-1}) provided substantial protection from ischaemia-induced hippocampal degeneration (Table 1). In contrast, no statistically significant protection was seen following administration of any of the metabolites (Table 1) when they were given at the same dose as clomethiazole (600 μmol kg^{-1}).

The clomethiazole metabolites studied are all found in high concentration in the brain following their intraperitoneal administration, indicating good brain penetration (Green *et al.*, unpublished observations). These data suggest strongly therefore that the metabolites examined are not involved in the neuroprotective action of clomethiazole in the gerbil model of global ischaemia.

Cross, A.J. *et al.* (1991). *Br. J. Pharmacol,* **104**, 406-411.
Cross, A.J. *et al.* (1995). *Br. J. Pharmacol,* **114**, 1625-1630.
Green, A.R. & Cross, A.J. (1994). *Prog. Neurobiol,* **44**, 463-484.
Green, A.R. & Cross, A.J. (1997) *Eur. Neuropsychopharmacol.* **7**, Suppl. 2, S81
Green, A.R. *et al.* (1998) Poster, this meeting.

Table 1: Effect of clomethiazole and various metabolites (all injected at a dose of 600μmol kg^{-1} i.p.) on hippocampal degeneration following bilateral carotid artery occlusion in the gerbil.

Treatment	n	Hippocampal degeneration (%)
Saline	12	64 ± 8
Clomethiazole	12	27 ± 7 *
NLA-715	8	62 ± 6
Saline	10	70 ± 4
Clomethiazole	10	9 ± 5 +
NLA-272	10	58 ± 9
NLA-511	10	50 ± 10

Results shown as mean ± s.e. mean. Analysis of variance demon-strated a significant effect of treatment with post-hoc tests demonstrating a significant difference between controls and clomethiazole. * ($t = 3.48$, $p<0.01$), + ($t = 9.53$, $p<0.01$)

47P COMPARISON OF THE TIME COURSE OF THE TREMOROGENIC EFFECT OF TACRINE, DONEPEZIL AND NXX-066 IN RATS

[1]T.K. Murray, M.F. Snape, [2]A.J. Cross & [3]A.R. Green. [1]Astra Arcus, S-151 85, Södertälje, Sweden, [2]Astra Arcus USA, Rochester, NY 14623, USA & [3]Astra Arcus, Loughborough, LE11 5RH

The acetylcholinesterase (AChE) inhibitors tacrine and donepezil (E-2020) are now in clinical use for the treatment of Alzheimer's Disease. We have compared the acute cholinergic pharmacology of these reversible inhibitors with the slowly reversible non-competitive inhibitor NXX-066 [(3aS-cis)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo(2,3b)indol-5-(1,2,3,4-tetra-hydroquinolinyl) carbamate ester], measuring both tremor and salivation following intraperitoneal and oral administration.

Male Lister Hooded rats weighing 250-400g were used. Various doses of the AChE inhibitors were either injected i.p. or given orally by gastric lavage. Tremor and lacrimation were measured as described by Hunter *et al.* (1989).

After construction of a dose response curve for each drug following its i.p. or oral administration (data not shown), a near maximal tremorigenic dose was chosen for examination of the duration of action of each compound. Administration i.p. resulted in a rapid increase in tremor following tacrine (84 μmol kg^{-1}), donepezil (18 μmol kg^{-1}) and NXX-066 (53 μmol kg^{-1}) with significant tremor still apparent 8 h after tacrine and NXX-066 (Figure 1). A substantial salivation response was also seen after tacrine, while only a small response was seen after NXX-066 and there was no observable salivation in the donepezil-treated animals.

After oral administration all 3 drugs (tacrine: 256 μmol kg^{-1}, donepezil: 24 μmol kg^{-1} and NXX-066: 53 μmol kg^{-1}) again demonstrated a dose dependent increase in tremor. The onset was slow after NXX-066 administration, although the drug had a long duration of action (Figure 1). Tremor was also clearly observable 6 h after tacrine but marginal 4 h after donepezil (Figure 1). Salivation was again marked after tacrine, while both other drugs induced little salivatory response.

These data suggest that tacrine shows poor selectivity for centrally- (tremor) versus peripherally- (salivation) mediated effects, in contrast to both NXX-066 and donepezil. The tremorogenic effect of donepezil is relatively short in comparison to the other compounds even when given orally. It is also clear that substantially higher doses of tacrine are required to induce a similar degree of tremor when it is given orally rather than i.p., in contrast to the other 2 compounds.

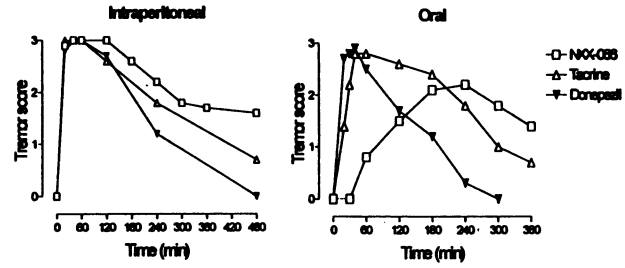


Figure 1. Effect of NXX-066, tacrine and donepezil on tremor when given either intraperitoneally or orally. Doses given in text., Results show mean score in a group of 6 animals per time point.

Hunter, A.J., Murray, T.K., Jones, J.A., Cross, A.J. & Green, A.R. (1989). *Br. J. Pharmacol.* **98**, 79-86.

48P THE POTENCY OF TACRINE, DONEPEZIL, RIVASTIGMINE AND METRIFONATE IN INDUCING TREMOR AND SALIVATION IN RATS

S. Dronfield, K. Egan, C. A. Marsden & *A. R. Green. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH & *Astra Arcus, Bakewell Rd., Loughborough LE11 5RH

Three acetylcholinesterase (AChE) inhibitors have now been registered in one or more countries for the treatment of mild to moderate Alzheimer's Disease (tacrine, rivastigmine [ENA 713] and donepezil [E-2020]), with an application filed for a fourth (metrifonate). However, there appear to have been no animal studies published comparing the acute cholinergic pharmac-ology of these compounds. We have, therefore, examined the potency of all four compounds in inducing overt cholinergic responses following their oral administration to rats, as well as investigating their duration of action in this species.

Male Hooded Lister rats (180-260 g) were administered by gastric lavage various doses of the AChE inhibitors or drug vehicle (n=6/group). Using the methods for behavioural observation described by Hunter *et al.* (1989), tremor (score 0 - 3), lachrimation (score 0 - 3), salivation (weight in mg) and rectal temperature were measured over the next 6 h by an observer blind to the treatment condition.

All four compounds dose-dependently induced tremor (Figure 1) and hypothermia. Tacrine also induced marked salivation, while the other compounds at the doses used had little or no effect (Figure 1). The lacrimation response showed a similar dose-response profile to salivation.

Following a sub-maximal tremorogenic dose (rivastigmine: 6.25 μmol kg^{-1}, donepezil: 20 μmol kg^{-1}, tacrine: 150 μmol kg^{-1}, metrifonate: 388 μmol kg^{-1}) both tacrine and donepezil treated rats still displayed a significant tremor response (score of approx 1.5) 6 h after administration. In contrast, both rivastigmine and metrifonate treated rats showed little tremor by 2 h (score 0.3 - 0.5) and no response at 3 h.

We conclude that tacrine, in contrast to the other compounds, shows poor selectivity for centrally- (tremor) versus peripherally- mediated effects (salivation and lacrimation). In addition, metrifonate displays both a low potency and a brief duration of action.

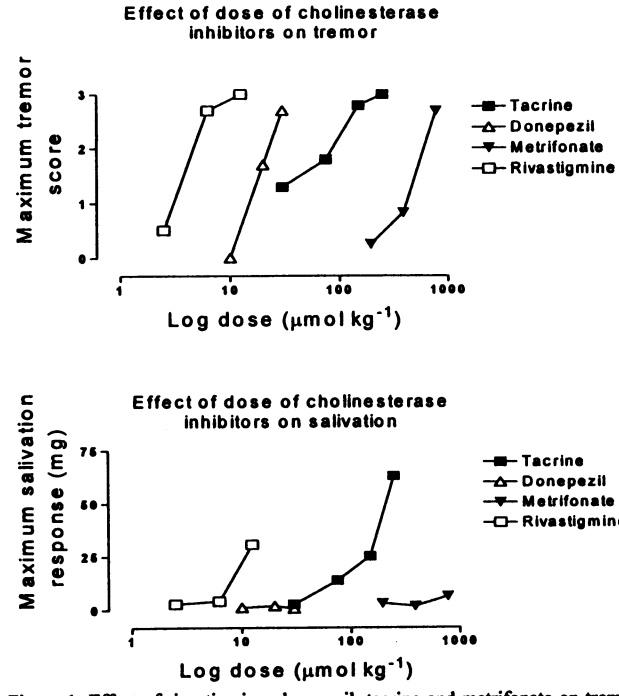


Figure 1. Effect of rivastigmine, donepezil, tacrine and metrifonate on tremor and salivation in rats. Results shown as mean value of 6 rats per dose.

Hunter, A.J., Murray, T.K., Jones, J.A., Cross, A.J. & Green, A.R. (1989). *Br. J. Pharmacol.* **98**, 79-86.

49P 4-AMINOPYRIDINE HAS SIGNIFICANT ANTICHOLINESTERASE ACTIVITY AT MICROMOLAR CONCENTRATIONS *IN VITRO*

I. Khan, J.W. Smythe and A.T. Evans Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, West Yorkshire. BD& 1DP.

4-Aminopyridine is a weak, nonspecific potassium channel blocker (Bouchard and Fedida, 1995). It has been found particularly useful in animal behavioural studies due to its ability to cross the blood brain barrier (e.g. Poorheidari *et al,* 1996). We have been interested in developing novel cysteine protease inhibitors from the reversible anticholinesterase, tacrine (tetrahydroaminoacridine) (Qayum *et al,* 1996), and have therefore been examining a number of aminoacridine, aminopyridine and aminoquinoline structures to attempt to differentiate anticolinesterase and antiprotease structural requirements. In view of the widespread use of millimolar concentrations of 4-aminopyridine in pharmacological studies, we were therefore surprised to identify a relatively potent anticholinesterase activity of this compound in the micromolar range.

Cholinesterase activity was measured using a simple colourimetric assay and a commercially available enzyme preparation derived from electric eel (Sigma). The assay mixture contained 0.5 mM bromophenol blue and 50 mM acetylcholine chloride at pH 8.0. 4-aminopyridine was dissolved in a minimum quantity of methanol and further diluted in distilled water to appropriate stock concentrations. 10µl of drug solution (or vehicle control) was added to 180µl of assay mix, and the reaction started by addition of 10µl of enzyme solution (1 unit). Acetylcholine hydrolysis was measured as an increase in absorbance at 450 nm. Assay incubations were carried out in triplicate, and on three separate occasions. Control incubations showed a mean rate of hydrolysis equivalent to the production of 0.9 (±0.3) µmoles of acetic acid per minute (n = 3), as determined by interpolation of absorbance values on a standard curve. Drug activity was determined following a five minute incubation at 35 °C. Enzyme activity was linear over this period, and addition of the vehicle did not alter enzyme activity. Drug addition did not significantly alter the absorbance of the assay solution compared to vehicle controls.

4-amino pyridine was capable of complete inhibition of enzyme activity at a concentration of 50µM (apparent IC_{50} 5µM)($p < 0.05$ Anova and post hoc Bonferroni t-test) (n = 3). In the same assay system, tacrine and its planar analogue, 9-aminoacridine, were more potent (maximum inhibition of 100 % at 0.5µM for both)(apparent IC_{50} 0.08 µM for 9-aminoacridine; 0.05 for tacrine) ($p<0.05$) (n = 3).

The results suggest that the concentrations of 4-aminopyridine currently employed in pharmacological studies would exert a major anticholinesterase action, which may underlie the ability of this drug to enhance cholinergic transmission. We would advise caution in the interpretation of data gained from studies with this compound.

Bouchard, R., and Fedida, D. (1995) *J. Pharmacol Exp. Ther.*, **275**, 864 - 868.
Poorheidari, G., Stanhope, K.J. and Pratt, J.A. (1995) *Br. J. Pharmacol.* **116**, 366P.
Qayum, S., Shafique, M., Kerslake, S. *et al* (1997) *Br. J. Pharmacol.* **120**, 273P

50P DEMONSTRATION OF NEUROPROTECTION BY THE NOVEL ANTICONVULSANT, BTS 72 664, AFTER PERMANENT FOCAL CEREBRAL ISCHAEMIA IN RATS

S.L. Smith, K.F. Martin[1] & D.J. Heal[1], School of Biological Sciences, University of Manchester, Manchester M13 9PT, and [1]Knoll Pharmaceuticals Research and Development, Nottingham NG1 1GF.

In spite of the fact that cerebral ischaemia is a major cause of morbidity and mortality, drug therapy for this condition remains inadequate and prevention is still the most effective treatment (De Deyn *et al.*, 1997). This study was undertaken to assess the neuroprotective efficacy of the novel anticonvulsant drug, BTS 72 664 ((*R*)-7-[1-(4-chlorophenoxy)ethyl]-1,2,4-triazolo[1,5-α]pyrimidine), in an experimental model of cerebral ischaemia. This was permanent middle cerebral artery occlusion (MCAo) in rats. The resulting damage was assessed histolopathologically by measurement of lesion volumes.

Animals were anaesthetised with halothane and subjected to MCAo by electrocoagulation, below the rhinal fissure. Body temperature was maintained at 37.0±0.5°C throughout the surgical procedure by use of a thermostatically-controlled heated operating blanket. Fifteen minutes after coagulation and severence of the MCA, the animals were dosed with 50mg/kg of drug (n=12) or equivalent volume of vehicle (n=11) by oral gavage. The animals were dosed thereafter at 12 hourly intervals for a total of 36h. After 48h, animals were perfused trans-cardially with paraformaldehyde in 0.2M sodium phosphate buffer. Brains were further fixed in the skulls for at least 24 hours at 4°C. The brains were sectioned (30μm thickness) using a vibratome and stained with luxol fast blue/cresyl fast violet. Extent of ischaemic damage was assessed in 10 coronal planes at 500μm intervals from +2.2mm to -2.3mm relative to Bregma. Lesion sizes were assessed by an operator unaware of treatment procedures. Slides were placed onto a slide illuminator (Carl Zeiss; 17.5x magnification) and areas of damage were delineated onto stereotaxic maps. Lesion areas were measured using a SeeScan image analyser and lesion volumes calculated using computational analysis. Statistical analysis of data was by 2-way ANOVA. All data are mean ± s.e. mean.

After MCAo, damage was present in the parietal, insular, frontal and forelimb cortical regions. Secondary damage due to oedema formation in the cortex and corpus callosum was also found in the outer region of the caudate-putamen. Treament of animals with BTS 72664 resulted in a 31% reduction of lesion volume when compared with vehicle-treated animals. Lesion volumes were 82.6±6.5 mm^3 for vehicle-treated rats and 56.9±6.1 mm^3 for drug-treated animals ($P<0.005$) when measured 48h post-insult.

This histopathological study, together with the data provided by Smith *et al.* (1998b) on functional improvement after treatment with this drug, has provided evidence for neuroprotection by the novel anticonvulsant drug, BTS 72 664, in a model of permanent focal ischaemia. These results are of relevance to the clinical situation as the animals were treated post-insult. The peak brain levels of BTS 72664 have been shown to be 30 min after oral gavage (unpublished observations, Knoll Pharmaceuticals). Therefore, the time for the drug to reach maximum effectiveness in this study was approximately 45 min post-MCAo. In conclusion, BTS 72 664 maybe of value as a neuroprotective agent for the treatment of patients suffering progressing brain damage due to occlusion of a major cerebral artery.

De Deyn, P.P., De Reuck, J., Deberdt, W. *et al.* (1997) *Stroke*, 28, 2347-2352.
Smith, S.L., Martin, K.F. & Heal, D.J. (1998b) This meeting.

51P IMPROVEMENT OF FUNCTIONAL DEFICIT AFTER PERMANENT FOCAL ISCHAEMIA IN RATS PROVIDED BY THE NOVEL ANTICONVULSANT, BTS 72 664

S.L. Smith, K.F. Martin[1] & D.J. Heal[1], School of Biological Sciences, University of Manchester, Manchester M13 9PT and [1]Knoll Pharmaceuticals Research and Development, Nottingham NG1 1GF.

This novel study assessed the post-insult neuroprotective efficacy of the anticonvulsant, BTS 72 664 ((*R*)-7-[1-(4-chlorophenoxy)ethyl]-1,2,4-triazolo[1,5-α]pyrimidine), after middle cerebral artery occlusion (MCAo) in rats. Most techniques of assessement of brain damage in models of stroke employ histological methods. In patients, functional and neurological outcome measures are used. There is increasing evidence that neurologic deficits and lesion size may not correlate (eg Ginsberg and Busto, 1989). Therefore, it is important to measure functional deficit as well as lesion volumes. After MCAo, there is damage in the cortical area controlling forelimb function (Smith *et al.* 1998a), therefore forelimb dysfunction on the side contralateral (CL) to the ischaemic damage can be predicted. The forelimb-fault test developed by Hernandez and Schallert (1988) for functional assessment of cortical damage was modified for this study.

Rats were trained to cross an elevated grid (3cm openings) to escape from a bright light into a dark box. Forelimb slips through the grid were counted as errors. Ipsilateral (IL) errors were subtracted from those of the CL forelimb. Using halothane anaesthesia, the MCA was electrocoagulated proximal to the rhinal fissure. Body temperature was maintained at 37.0±0.5°C by use of a thermostatically-controlled heated operating blanket. The animals were tested on days -5, -2 and 3, 6, 8 and 10 post-surgery. The test was established in a preliminary experiment using untreated control, sham-operated and MCAo groups. BTS 72 664 (n=11; 50 mg/kg by oral gavage, every 12h for 36h) or vehicle (n=10) were given 15 min after MCAo. Effect of treatments on IL and CL forelimb errors were analysed using ANCOVA and forelimb symmetry pre-intervention by 1-way ANOVA. All data are mean ± s.e. mean.

In the validation experiment, sham-operated rats did not differ from untreated controls in the number of CL errors taken. All animals took an equal number of total steps across the grid. MCAo rats made an increased number of CL errors compared with the sham-operated group on days 3, 6 and 8, but not day 10, indicating a recovery of function in this test by day 10. In the rats treated with BTS 72 664 or vehicle, forelimb errors were found to be symmetrical when measured pre-intervention (days -5 and -2; IL 1.5±0.17, CL 1.0±0.33; n.s.). Neither vehicle nor drug treatment caused any change in errors executed with the IL forelimb after MCAo (Days 3, 6 and 8; vehicle 5.17±0.46, BTS 72 664 5.53±0.32; n.s.). Treatment with BTS 72 664 caused a reduction of contralateral forelimb errors on day 8 (vehicle 1.19±0.42, BTS 72 664 0.03±0.3; $P<0.05$). Administration of this drug also produced a decrease of contralateral errors when days 3, 6 and 8 were combined (vehicle 3.1±0.64, BTS 72 664 0.99±0.54; $P<0.05$).

These findings indicate that BTS 72 664 reduced the functional deficit in this test overall and also accelerated the recovery of animals back to normal function by day 8. The time for the drug to reach maximum effectiveness in this study was approximately 45 min post-insult (Smith *et al.* 1998a). Together with the histopathological reduction of damage described by Smith *et al.* (1998a), these results indicate that BTS 72 664 may have therapeutic value as a neuroprotective agent for the treatment of cerebral ischaemia.

Ginsberg, M.D. & Busto, R. (1989) *Stroke*, 20, 1627-1642.
Hernandez, T.D. & Schallert, T. (1988) *Exp. Neurol.* 102, 318-324.
Smith, S.L., Martin, K.F. & Heal, D.J. (1998a) This meeting.

52P TOLERANCE TO THE CLOZAPINE DISCRIMINATIVE STIMULUS

Z.S Burgess & A.J. Goudie, Dept. of Psychology, Liverpool University, P.O. Box 147, Liverpool, L69 7ZA.

Clozapine represents a major advance in the treatment of schizophrenia, for which it is administered chronically over many years. It is therefore important to develop models of its chronic effects and to study associated neuroadaptations. The aim of this study was to investigate in vivo the development of tolerance to the clozapine discriminative stimulus.

Female Wistar rats (n = 11; 350-450g), maintained at 21°C, were trained in daily 15 min operant sessions to discriminate clozapine (5 mg/kg, i.p.) dissolved in 0.1N HCl diluted with distilled water and NaOH to a pH of 5 and administered 30 minutes before sessions in a quantal two lever Fixed Ratio 30 food rewarded operant drug discrimination assay (Goudie & Leathley, 1993). Once the discrimination was established (the group level of accuracy of lever selection was at least 85% correct/day throughout the study) generalisation tests were introduced, separated by at least two baseline days. Clozapine initially induced dose-related generalisation between 0.3125 to 5 mg/kg (the ED_{50} by log/linear least squares regression analysis was 0.84 mg/kg, r^2 for the regression line = 0.94). Chronic clozapine (i.p., 10 mg/kg, b.i.d., 0900 and 1600 h) was administered for 10 days during which discrimination training was suspended. On days 11-13 the generalization curve was recomputed. Clozapine again induced dose-related generalisation, but the dose/effect curve was shifted significantly ($p < 0.05$ by probit analysis [SPSS for Windows Release 6.1]) in parallel 3.5 fold to the right (ED_{50} = 2.98 mg/kg, $r^2 = 0.57$).

Subsequently, the rats were left for 16 further days, and the generalization curve computed for a third time. The tolerance acquired as a result of chronic clozapine was spontaneously completely lost. Clozapine yet again induced dose-related generalization, but the dose/effect curve was almost identical to that initially computed (ED_{50} = 0.96 mg/kg, $r^2 = 0.97$), as there was a significant, parallel shift to the *left* in the generalization curve ($p < 0.05$ by probit analysis) relative to that computed after chronic clozapine treatment.

Thus we observed spontaneously reversible tolerance, as evidenced by parallel shifts to the right and then back to the left in the dose-response curves. Since the tolerance observed was spontaneously lost, it was most likely pharmacodynamic in nature. The mechanism(s) involved in this tolerance is as yet unknown. The study of clozapine tolerance in the drug discrimination assay may provide a useful behavioural model system to study clinically relevant neuroadaptations occurring during chronic clozapine treatment

Goudie, A.J. & Leathley, M.J. (1993) In *Behavioural Neuroscience.* ed. A Sahgal, pp 145-167. Oxford University Press.

53P CROSS-TOLERANCE TO THE CLOZAPINE DISCRIMINATIVE STIMULUS INDUCED BY THE NOVEL PUTATIVE ATYPICAL ANTIPSYCHOTIC JL13

Z.S Burgess & A.J. Goudie, Dept. of Psychology, Liverpool University, P.O. Box 147, Liverpool, L69 7ZA.

The clozapine congener JL13 (5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3,-b][1,5]benzoxazepine) is a putative atypical antipsychotic which generalises to clozapine in drug discrimination assays (Bruhwyler *et al.*, 1997). The aim of this study was to investigate the ability of JL13 to induce cross-tolerance to the clozapine discriminative stimulus, prior studies having shown that chronic clozapine induces tolerance to its own stimulus properties (Burgess & Goudie, 1998).

Female Wistar rats (n = 10, 350-450g), maintained at 21^{0}C, were trained in daily 15 min operant sessions to discriminate clozapine (5 mg/kg, i.p.) dissolved in 0.1N HCl diluted with distilled water and NaOH to a pH of 5 and administered 30 min before sessions in a quantal two lever Fixed Ratio 30 food rewarded operant drug discrimination assay (Goudie & Leathley, 1993). Once the discrimination was established (the group level of accuracy of lever selection was at least 85% correct/day throughout the study) generalisation tests were introduced, separated by at least two baseline days. Clozapine initially induced the expected dose-related generalisation between 0.3125 to 5 mg/kg (the ED_{50} by log/linear least squares regression analysis was 0.84 mg/kg, r^2 for the regression line = 0.94). Chronic JL13 (i.p., 20 mg/kg, b.i.d., 0900 and 1600 h) was then administered for 10 days during which discrimination training was suspended. On days 11-13 the generalisation curve was recomputed. Clozapine again induced dose-related generalisation, but the dose/effect curve was shifted significantly ($p < 0.05$ by probit analysis [SPSS for Windows Release 6.1]) in parallel 3.3 fold to the right (ED_{50} = 2.8 mg/kg, r^2 = 0.92). Subsequently, the rats were simply left for 16 further days, and the generalisation curve computed for a third time. The tolerance acquired as a result of chronic JL13 was spontaneously completely lost. Clozapine yet again induced dose-related generalisation, but the dose/effect curve was similar to that initially computed (ED_{50} = 0.54 mg/kg, r^2 = 0.89), as there was a significant, parallel shift to the *left* in the generalisation curve ($p < 0.05$ by probit analysis) relative to that computed after chronic clozapine treatment.

Thus, like clozapine (Burgess & Goudie, 1998), chronic JL13 induced spontaneously reversible tolerance. The mechanism by which this cross-tolerance developed is unknown, although given its spontaneous reversibility the tolerance observed is assumed to be pharmacodynamic in nature. These data show clearly that clozapine and JL13 have very similar stimulus properties in the drug discrimination assay, since JL 13 does not simply generalise to clozapine (Bruhwyler *et al.* 1997); it also induces cross-tolerance to the clozapine stimulus.

Bruhwyler, J., Liegeois, J-F., Bergman, J *et al* (1997) Pharmacological Research, 36, 255-264.
Burgess, Z.S. & Goudie, A.J. (1998) This meeting.
Goudie, A.J. & Leathley, M.J. (1993) In *Behavioural Neuroscience.* ed. A Sahgal, pp 145-167. Oxford University Press

---

54P OLANZAPINE GENERALISATION TO THE CLOZAPINE DISCRIMINATIVE STIMULUS IS DETERMINED BY CLOZAPINE TRAINING DOSE

A.J. Goudie & A. Taylor, Dept. of Psychology, Liverpool University, P.O. Box 147, Liverpool, L69 7ZA.

Clozapine is discriminated by rats and clozapine-like atypical neuroleptics generalise to it (Goudie & Taylor, 1998). In this study we assessed the extent to which the dose used to train a clozapine discrimination determines the extent to which olanzapine, a clozapine congener with clinically validated atypical neuroleptic actions, generalises to clozapine.

Two groups of female Wistar rats (ns = 20; 190-265g), maintained at 21^{0}C, were trained in daily 15 min operant sessions to discriminate clozapine at *either* 5 *or* 2 mg/kg (i.p.). Clozapine was dissolved in 0.1N HCl, diluted with distilled water and NaOH to a pH of 5, and administered 30 min before sessions in a quantal two lever Fixed Ratio 30 food rewarded operant drug discrimination assay (Goudie & Leathley, 1993). The discrimination was established more rapidly in rats trained on 5 as opposed to 2 mg/kg. After 70 training sessions the former group showed 100% correct responses, whilst the latter showed only 85% correct. Subsequently, generalisation tests were introduced with clozapine and olanzapine. In both training groups clozapine induced dose-related generalisation. The ED_{50}s, by log/linear least squares regression analyses, were 2.1 mg/kg (r^2 for the regression line=0.9) in the 5 mg/kg group and 0.8 mg/kg (r^2=0.9) in the 2 mg/kg group. Thus the generalisation curve was shifted 2.6 fold to the *left* in the 2 mg/kg group due to its *acquired* greater sensitivity to clozapine. Clozapine did not suppress operant responding in generalization tests in either training group.

In generalisation tests with olanzapine in both training groups olanzapine induced dose-related generalisation, although considerably more generalization was seen in the 2 mg/kg training group (maximum=75%, ED_{50} = 0.6 mg/kg, r^2 = 0.9) than in the 5 mg/kg training group (maximum =38%). In contrast to clozapine, olanzapine only generalised at doses which suppressed operant responding. The response rate dose/effect curves differed significantly [$F(1,37)=7.4$, $p=0.01$], since there was a parallel shift to the *right* in the 5 mg/kg compared to the 2 mg/kg training group. The ED_{50}s for the 5 and 2 mg/kg training groups were 2.08 and 1.16 mg/kg respectively. Thus less response suppression was seen at any specific dose in the 5 mg/kg group, due presumably to the development of greater cross-tolerance to drug-induced rate-suppression between clozapine and olanzapine in the rats trained on the higher dose of clozapine.

These data show that:- i) Olanzapine generalises to clozapine, although more generalization was seen in rats trained on a low dose of clozapine; ii) Olanzapine differs from clozapine as it only generalises at rate suppressant doses; and iii) Olanzapine and clozapine show cross-tolerance in terms of their rate suppressant actions. Collectively, the results demonstrate both similarities and differences between clozapine and olanzapine.

Goudie, A.J. & Leathley, M.J. (1993) In *Behavioural Neuroscience.* ed. A Sahgal, pp 145-167. Oxford University Press.
Goudie, A.J. & Taylor, A. (1998) *Psychopharmacology.* In Press.

55P LACK OF EVIDENCE FOR THE ROLE OF D4 RECEPTORS IN THE BEHAVIOURAL EFFECTS OF CLOZAPINE IN RATS

J.A. Smith & A.J. Goudie, Dept. of Psychology, Liverpool University, P.O. Box 147, Liverpool, L69 7ZA.

Clozapine is a major advance in the treatment of schizophrenia, although its mechanism of action is elusive. Recently, there has been speculation about a role for D4 antagonism in clozapine's actions, due mainly to its relative selectivity at D4 receptors (Van Tol *et al.* 1991), but also as a result of suggestions that D4 receptors may be elevated in post mortem brain tissue of schizophrenics (Seeman *et al.* 1993). The aim of this study was to investigate the role of D4 receptors in the discriminative stimulus (cue) properties of clozapine using the highly selective D4 antagonist (Patel *et al.* 1996) L-745,870 (3-{[4-(4-chlorophenyl) piperazin-1-yl)] methyl} -1H-pyrrolo [2,3b] pyridine).

Female Wistar rats (n = 14; 280-360g), maintained at 21^{0}C, were trained in daily 15 min sessions to discriminate clozapine (5 mg/kg, i.p.) dissolved in 0.1N HCL, diluted with distilled water, buffered with NaOH to a pH of 5 and administered 30 min before sessions in a quantal two lever Fixed Ratio 30 food rewarded operant drug discrimination assay (Goudie & Leathley, 1993). Once the discrimination was established (the group level of accuracy of lever selection was at least 85% correct/day throughout the study), the ability of clozapine (i.p. 30 min pre-session) to induce the clozapine cue was studied at various doses, and then generalisation tests with L-745,870 (1, 3.3 and 10 mg/kg, i.p. administered 30 min before sessions), were introduced, separated by at least 2 baseline days.

As expected, clozapine produced dose-related drug lever selection, [ED_{50} = 1.41 mg/kg]. However, L-745,870 did not generalise to clozapine, the maximal generalisation being 14% at the highest dose (10 mg/kg). This was the only dose at which responding was suppressed by a small, but significant amount - 28%, [Repeated measures ANOVA followed by Dunnett's tests ($F(3,39) = 7.37, p = 0.0005$)].

The response suppression induced by L-745,870 at 10 mg/kg shows that, despite the lack of generalisation, L-745,870 was behaviourally active. The effects seen with L-745,870 contrast with the generalisation seen with clozapine. Thus D4 antagonism *alone* does not mimic clozapine. However, D4 antagonism with concurrent effects at other receptor(s) *may* mimic clozapine. The finding that D4 antagonism alone does not mimic the clozapine cue parallels recent findings that L-745,870 is *not* itself an effective antipsychotic (Kramer *et al.* 1997)

Goudie, A.J. & Leathley, M.J. (1993) In *Behavioural Neuroscience.* ed. A Sahgal, pp 145-167. Oxford University Press.

Kramer, M.S., Last, B., Getson, A. *et al.* (1997) *Arch Gen Psychiatry* 65, 567-572.

Patel, S., Patel, S.H., Marwood, R. *et al.* (1996) Brit J Pharmacol 119 (suppl), 29P.

Seeman, P., Guan, H.-C. & Van Tol, H.M. (1993) *Nature* 365, 441-445.

Van Tol, H.H.M., Bunzow, J.R., Guan, H.-C. *et al.* (1991) *Nature* 350, 610-614.

56P DEVELOPMENT OF A RADIOLIGAND BINDING ASSAY TO SELECTIVELY LABEL 5-HT$_7$ RECEPTORS IN RAT BRAIN

R. L. Stowe and N. M. Barnes.
Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT UK.

The 5-HT$_7$ receptor has been cloned from rat, mouse, guinea pig and human tissue. 5-HT$_7$ receptor mRNA is localised in cortical and limbic brain regions which together with a high affinity for many antipsychotic agents suggests a role for the receptor in control of affective behaviour (e.g. Ruat et al; 1993). Competition assays using [^{3}H]5-CT ([^{3}H]5-carboxamidotryptamine) to label the 5-HT$_7$ receptor in rat brain have demonstrated shallow curves when methiothepin competes for the labelled receptor (Boyland et al; 1996). Similarly, we have reported shallow competition curves with a variety of compounds in rat whole brain and hypothalamus (Stowe and Barnes 1996 and 1998). This study used [^{3}H]5-CT to further characterise 5-HT$_7$ receptor binding in rat brain tissue.

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

In homogenates of rat whole brain a range of structurally diverse compounds competed for (±)-pindolol (10µM)/WAY100635 (100nM)-insensitive [^{3}H]5-CT binding in a monophasic manner with Hill coefficients around unity (see Table 1) and at concentrations consistent with their affinities for the recombinant 5-HT$_7$ receptor (5-CT > 5-HT > Methiothepin > Pimozide > 8-OHDPAT; Table 1). In homogenates of rat hypothalamus, these compounds also competed with unity Hill coefficients and displayed a similar affinity profile.

Table 1. Affinities and Hill coefficients for a range of compounds competing for (±)-pindolol (10µM)/WAY100635 (100nM)-insensitive [^{3}H]5-CT binding to homogenates of rat whole brain. Data represents mean ± S.E.M.

Compound	pIC_{50}	Hill Coefficient	'n'
5-CT	9.17 ± 0.03	1.00 ± 0.03	3
5-HT	8.34 ± 0.26	1.07 ± 0.02	3
Methiothepin	7.92 ± 0.13	0.96 ± 0.09	3
Pimozide	7.84 ± 0.07	1.04 ± 0.07	3
8-OHDPAT	6.65 ± 0.05	1.13 ± 0.09	3

These results indicate that under the present binding conditions [^{3}H]5-CT appears to label one binding site in rat whole brain, the pharmacology of which resembles the 5-HT$_7$ receptor. These assay conditions are currently being utilised to study the distribution of 5-HT$_7$ receptors in rat brain using receptor autoradiography.

R.L. Stowe is recipient of a Wellcome Trust Prize Studentship.

Boyland, P.S., Eastwood, S., Ellis, C. et al. (1996) Br. J. Pharmacol. 117, 132P.

Ruat, M., Traiffort, E., Leurs, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90, 8547-8551.

Stowe , R.L. and Barnes, N. M. (1996) Br. J. Pharmacol. 119, 366P.

Stowe, R.L. and Barnes, N. M. (1998) Br. J. Pharmacol. (in press).

57P NO EVIDENCE FOR A DIFFERENCE IN CENTRAL 5-HT$_3$ RECEPTOR EXPRESSION BETWEEN GAERS AND AN OUTBRED CONTROL STRAIN OF RATS

S. Fletcher, D.A. Richards & N.M. Barnes. Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham, B15 2TT U.K.

The genetic absence epilepsy rat from Strasbourg (GAERS) has been validated as an experimental model of human absence epilepsy on the basis of neurophysiological, behavioural, pharmacological and genetic studies (Marescaux *et al.*, 1992). There is evidence that excess GABA-mediated inhibition may underlie the genesis of the spike-and-wave discharges which characterise absence seizures (e.g. Liu *et al.*, 1991). As 5-HT$_3$ receptors are thought to be involved in modulating the release of GABA (e.g. Ropert & Guy 1991), and are expressed by GABAergic interneurons (Morales & Bloom 1997), the present study investigated whether there was any difference in the expression of 5-HT$_3$ receptors between GAERS and an outbred control strain of rats, using the technique of *in vitro* autoradiography.

Following removal, rat brains (female Wistar, 12 week old, <250 g) were immediately frozen at -80°C. Frozen brain regions were cut using a cryostat, thaw mounted on to gelatin-coated glass slides, and stored at -80°C until use. Thawed (4°C) slide-mounted sections were pre-incubated in Tris/Krebs buffer (mM; Tris, 50; NaCl, 118.0; KCl, 4.75; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11.0; pH 7.4) for 30 min at 4°C, before being incubated in Tris/Krebs buffer (4°C) containing 0.4 nM [^{3}H]-(S)-zacopride in the absence (total binding) or presence of 1.0 μM granisetron (non-specific binding) for 60 min. The sections were washed twice for 5 min in ice-cold Tris/Krebs buffer, and rinsed for 1 sec in ice-cold distilled water. The sections were then rapidly dried in a stream of cold dry air before being exposed to tritium-sensitive film (Hyperfilm-[^{3}H], Amersham) along with tritium standards (Amersham) for 14 weeks. Developed autoradiographs were analysed and quantified (with reference to the tritium standards), using an image analysis system (MCID, Imaging Research Inc.). Total and non-specific binding (fmol mg^{-1} wet weight tissue equivalent) was determined for each area from 63-108 sections per rat.

Highest levels of specific [^{3}H]-(S)-zacopride binding were detected in the cerebral cortex of both the GAERS and the control rats (3.34±0.54 and 2.88±0.67 fmol mg^{-1} for the GAERS and the controls respectively, mean±SEM, n=3), with the binding being largely associated with the outer layers of the cortex. High levels of binding were also detected in the hippocampus (2.91±0.40 and 2.43±1.01 fmol mg^{-1} for the GAERS and the controls respectively, mean±SEM, n=3). Lower levels of binding were detected in some other brain regions (e.g. cerebellum, thalamus), although total binding was not consistently above the level of non-specific binding in these regions. There was no significant difference in 5-HT$_3$ receptor levels between the GAERS and the control rats in any of the brain regions examined ($p < 0.05$, 2-tailed unpaired t-test).

In conclusion, the present studies indicate that there is no significant difference in central 5-HT$_3$ receptor expression between GAERS and a control strain of rats.

S. Fletcher is a recipient of an A.J. Clark Studentship from the British Pharmacological Society.

Marescaux, C., Vergnes, M. & Depaulis, A. (1992). *J. Neural Transm.* [Suppl] 35, 37-69.
Liu, Z., Vergnes, M., Depaulis, A. & Marescaux, C. (1991). *Brain Res.* 545, 1-7.
Ropert, N. & Guy, N. (1991). *J. Physiol.* 441, 121-136.
Morales, M. & Bloom, F.E. (1997). *J. Neurosci.* 17, 3157-3167.

58P MECHANISMS FOR THE ENHANCED ACETYLCHOLINE RELEASE INDUCED BY 5-HT$_3$ RECEPTOR ANTAGONISTS IN RAT ENTORHINAL CORTEX SLICES

M. Díez-Ariza, MJ. Ramírez, B. Lasheras and J. Del Río, Department of Pharmacology, School of Medicine, University of Navarra, Pamplona, Spain.

It has been shown that 5-HT$_3$ receptors mediate the inhibitory effect of 5-HT on acetylcholine (ACh) release in the entorhinal cortex (Barnes *et al.*, 1989). The 5-HT$_3$ receptor antagonist ondansetron produced a concentration-dependent enhancement in K$^+$-evoked [^{3}H]-ACh efflux and this effect was potentiated by the GABA$_A$ receptor antagonist bicuculline (Ramírez *et al.*, 1996). Since 5-HT$_3$ receptors are probably located on GABAergic neurons in this cortical area (Morales *et al.*, 1996), we suggested that 5-HT$_3$ receptor antagonists block the excitatory influence on GABA neurons which inhibit cholinergic systems. It has also been described that ondansetron has a unique ability to block voltage-gated potassium channels in a human neuroblastoma cell line, a property not shared by other 5-HT$_3$ receptor ligands (Toral *et al.*, 1995). We have investigated whether the same potentiation of ACh release was found by using other 5-HT$_3$ or GABA$_A$ receptor antagonists and also the effect of non-selective K$^+$-channel blockers and their interactions with some of these drugs.
The entorhinal cortex from male Wistar rats was cross-chopped into 350 μm slices, and superfused with gassed Krebs-Ringer buffer (KRB). The slices were labelled by incubation with [3H]Choline and superfused at 0.45 ml/min. After equilibration, release was evoked by 20 mM KCl at 12 min (S1) and 45 min (S2). Fractions were collected at 3-min intervals and [3H]ACh release was estimated from the tritium overflow. S1 y S2 were calculated as K$^+$-stimulated tritium increase on basal efflux and the results were expressed as S2/S1 ratio. All test drugs were added 15 min before S2. Data are means ± S.E.M. (n = 6 - 9).
The 5-HT$_3$ receptor antagonists ondansetron, granisetron and MDL 72222 (0.01-1 μM) produced a concentration-dependent increase in K$^+$-evoked [3H]ACh efflux. (S2/S1 ratio was 0.87±0.05, 1.19±0.09, 1.28±0.22, 1.25±0.03 for control, ondansetron, granisetron and MDL 72222, 0.1μM each, respectively). Bicuculline and flumazenil, two antagonists at different sites of the GABA$_A$ receptor, were studied in combination with 5-HT$_3$ antagonists. Bicuculline, 0.1 μM, and flumazenil, 10μM, markedly potentiated the releasing effect of ondansetron (S2/S1 raised from 1.19±0.09 to 2.35±0.28 and 2.20±0.36 respectively), but not of MDL 72222 or granisetron. Non-selective potassium channel blockers (KCB) such as tetraethylammonium (TEA) and barium were also studied for their intrinsic effect on ACh efflux and also in combination with bicuculline. Whereas both KCB increased ACh release (S2/S1 ratios were 0.87±0.05, 1.83±0.12 and 1.14±0.22 for control, TEA, 1mM, and barium, 10 μM, respectively), K$^+$-evoked ACh release was not further enhanced in the presence of bicuculline.
The results indicate that the unique interaction of ondansetron with GABA systems cannot be ascribed to a blockade of potassium channels, as these drugs, which are known to facilitate the release of numerous neurotransmitters, are unable to modify the effect of a typical GABA$_A$ antagonist. It is also doubtful that all of the 5-HT$_3$ antagonists enhance ACh release through a reduction in the inhibitory influence of GABA systems on cholinergic neurons. Yet, it is still conceivable that some 5-HT$_3$ antagonists may not act in a depolarized in vitro preparation because of a different site of action at the ligand-gated ionic channel or because of other unknown factor. In vivo ACh release studies will be probably useful to address this issue.

Supported by EEC, BIO4 CT96-0752.

Barnes, JM., Barnes, NM., Costall, B. *et al.* (1989) Nature, 338, 762-763.
Morales, M., Battenberg, E., de Lecea, L. *et al.* (1996) Mol. Brain Res. 36, 251-260.
Ramírez, MJ., Lasheras, B., Cenarruzabeitia, E. *et al.* (1996) Brain Res. 712, 274-280.
Toral, J., Hu, W., Critchett, D. *et al.* (1995) J. Pharm. Pharmacol. 47, 618-622.

59P EFFECT OF GLUTATHIONE DEPLETION ON MDMA-INDUCED SEROTONERGIC NEUROTOXICITY IN NEONATE RATS

N. Aguirre, I. Hervías, J. Del Río & B. Lasheras, Department of Pharmacology, School of Medicine, University of Navarra, Pamplona, Spain.

Administration of 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") to adult rats causes a long lasting loss of brain 5-hydroxytryptamine (5-HT) concentration and increases the formation of hydroxyl free radicals and lipid peroxidation (Sprague and Nichols, 1995), suggesting that MDMA-induced 5-HTergic neurotoxicity is related to oxidative damage. In spite of this, the administration of MDMA to immature rats neither causes long-term reductions in brain 5-HT concentrations or 5-HT transporter density (Broening *et al.*, 1994), nor increases lipid peroxidation probably due to the high free radical scavenging activity present in the tissues of young animals (Colado *et al.*, 1997). As glutathione (GSH) is a key component of the antioxidant system, we have investigated whether depletion of GSH in rat pups, by administration of L-buthionine-(S,R)-sulfoximine (BSO), potentiates the toxicity induced by MDMA.

Fourteen-day-old male Wistar rats received saline or BSO (3 mmol kg^{-1}, i.p., b.i.d. for 2 consecutive days). One hour later rats were killed and GSH levels were measured in the frontal cortex and in the hippocampus by spectrophotometry. Four other groups of rats received saline or BSO (see above) 1 h before saline or MDMA (20 mg kg^{-1}, i.p.). These rats were killed at postnatal day 23 (PND23), the frontal cortices and the hippocampi were dissected free to determine 5-HT concentration, by HPLC with electrochemical detection, as well as the density of [^{3}H]paroxetine-labelled 5-HT uptake sites.

BSO treatment reduced significantly ($P < 0.001$) GSH levels in the frontal cortex (75%) and in the hippocampus (70%). However, it did not produce by itself any change in 5-HT content or in 5-HT transporter density in any of the brain regions examined. As expected, MDMA did not either cause long lasting serotonergic deficits when compared to control rats. However, when MDMA was administered to the rat pups previously treated with BSO, 5-HT content and 5-HT tranporter density were significantly decreased in the frontal cortex and in the hippocampus ($P < 0.05$; Table 1).

Table 1. Effect of combined administration of BSO and MDMA on 5-HT content and 5-HT transporter density at PND 23

Region	Treatment	5-HT (pg mg^{-1} wet tissue)	Transporter (fmol mg^{-1} prot)
F. Cortex	Control	350±14	321±16
	MDMA	328±12	299±17
	BSO	338±13	291±18
	BSO+MDMA	241±10*	230±15*
Hippocampus	Control	388±14	214±12
	MDMA	351±10	208±11
	BSO	398±15	217±13
	BSO+MDMA	288±16*	160±14*

Values are means ± S.E.M. n = 6. * $P < 0.05$, vs. control group (ANOVA+Tukey test).

These data show that MDMA administration to immature rats does not produce damage to the neonate and suggest that free radical formation is involved in MDMA-induced neurotoxicity.

Supported by EEC, BIO4 CT96-0752.

Broening, H.W., Bacon, L. & Slikker, W. (1994) J Pharmacol Exp Ther **271**, 285-293.
Colado, M.I., O'Shea, E., Granados, R. *et al.*, (1997) Br J Pharmacol **121**, 827-833.
Sprague, J.E. & Nichols, D.E. (1995) J Pharmacol Exp Ther **273**, 667-673.

60P BROMOCRIPTINE ACTS AS A PARTIAL AGONIST AT THE HUMAN 5-HT_{2A} RECEPTOR

A.L. Mitchell, S.L. Phipps, D.G. Grahame-Smith and J.M. Elliott. Department of Clinical Pharmacology, University of Oxford, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE, U.K.

The dopamine D2 receptor agonist bromocriptine is used in the treatment of Parkinson's disease. This compound also displays moderate affinity for the rat 5-HT_{2A} receptor (Ki = 83nM; Choudhary et al., 1995), although its efficacy at this receptor subtype is unknown. Here we report that bromocriptine acts as a partial agonist at human 5-HT_{2A} receptors stably expressed in the neuroblastoma cell-line SH-SY5Y, and that chronic exposure to bromocriptine down-regulates the 5-HT_{2A} receptor.

SH-SY5Y cells transfected with and stably expressing the human 5-HT_{2A} receptor (Newton et al., 1996) were grown in 24 well plates and labelled with [^{3}H]inositol (1μCi/ml) for 48 hours in inositol-free Dulbecco's Modified Eagle's Medium (DMEM) and 5% dialysed foetal calf serum (dFCS) at 37°C. Following two washes in inositol free DMEM, cells were incubated for 30 minutes with 10 mM LiCl then bromocriptine (10 nM - 10 μM) was added for 15 minutes at 37°C. Maximal response was induced by 10 μM 5-HT. Reactions were terminated with ice cold methanol, and [^{3}H]inositol phosphates extracted using Dowex AG1-X8 anion exchange columns. For binding studies, cells were grown to confluency in DMEM with 5% dFCS, then transferred into either pure DMEM (control) or DMEM plus bromocriptine for 24 hours. Cells were harvested and membranes prepared by homogenisation in 5 mM Tris/EDTA (pH 7.4), washed three times and resuspended in incubation buffer (50 mM Tris, 5 mM $MgCl_2$, 1 mM EGTA and 1 mg/ml ascorbic acid, pH 7.4) before incubation with [^{3}H]ketanserin (0.2-6 nM) for 60 minutes at 37°C. Reactions were terminated by rapid filtration through Whatman GF/C filters. Non-specific binding was determined using 10μM mianserin.

The application of bromocriptine to SH-SY5Y/5-HT_{2A} cells resulted in a dose-dependent increase in PI hydrolysis. The potency of bromocriptine to stimulate PI hydrolysis (pEC_{50} = 7.07 ± 0.07; n=3) agreed closely with its previously reported affinity for the 5-HT_{2A} receptor. Efficacy of bromocriptine represented approximately 55 ± 6% of the maximal response induced by 5-HT. In the presence of the 5-HT_{2A} receptor antagonist ketanserin (10 nM), the dose-response curve was shifted in a dextral manner with no reduction in Emax, indicating pK_b (ketanserin) = 8.83. Prolonged exposure of cells to 100 nM bromocriptine for 24 hours did not significantly alter [^{3}H]ketanserin binding capacity or affinity of cell membranes. However, exposure of cells to 2 μM bromocriptine induced a significant decrease in [^{3}H]ketanserin binding capacity (Bmax = 70 ± 6 % of control, n=3, $p<0.05$), without apparent effect on binding affinity.

This study demonstrates that the dopamine D2 receptor agonist bromocriptine also acts as an effective partial agonist at the human 5-HT_{2A} receptor. Consistent with previous studies which have demonstrated agonist down-regulation of the 5-HT_{2A} receptor, prolonged exposure to bromocriptine induced a reduction in receptor number in these cells. Since there is a close correlation between efficacy at the 5-HT_{2A} receptor and hallucinogenic potency (Glennon, 1990), this study may have implications for the hallucinatory side effects seen with bromocriptine and other antiparkinsonian drugs.

Choudhary, M.S., Sachs, N., Uluer, A., Glennon, R.A., et al., (1995) Mol. Pharmacol. 47, 450-457.
Glennon, R.A. (1990) Neuropsychopharmacol. 3, 509-517.
Newton, R.A., Phipps, S.L., Flanigan, T.P., Newberry, N.R. et al. (1996) J. Neurochem. 67, 2521-2531.

61P COMPARISON OF [^{3}H]IFENPRODIL BINDING IN NATIVE AND STABLY EXPRESSED RECOMBINANT NMDA RECEPTORS

P. Richards, P.H. Hutson and S. Grimwood, Merck, Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

Ifenprodil is an N-methyl-D-aspartate (NMDA) receptor antagonist proposed to modulate the NMDA receptor through a polyamine binding site (Carter *et al.*, 1990) and has recently been shown to be selective for NMDA receptors containing the NR2B subunit (Williams *et al.*, 1993). However, ifenprodil also has high affinity for a number of other receptors which has limited its use as a specific NMDA receptor radioligand. In the present study we have determined the assay conditions required to investigate NMDA receptor-specific [^{3}H]ifenprodil binding and characterised binding to both native tissue and NR1a/NR2B receptors stably expressed in L(tk-) cells.

NMDA receptor-specific [^{3}H]ifenprodil binding was achieved using 50 mM Tris-acetate buffer (pH 7.0) containing 100 µM R(+)-3-(3-hydroxyphenyl)-N-propylpiperidine hydrochloride ((+)PPP), 1 µM GBR 12909 and 1 µM GBR 12935. 100 µg rat cortex / hippocampus membranes or cells stably transfected with human NR1a/NR2B receptors (Grimwood *et al.*, 1996) were incubated with [^{3}H]ifenprodil for 2h @ 4 ºC over a concentration range of 0.1 - 100 nM for saturation experiments or at 5 nM for inhibition curves. The incubation was terminated by filtration through GF/B filters using either a 24-cell Brandel harvester or a 96-well Tomtec harvester. Non-specific binding was determined in the presence of 10 µM (±)-(1S*,2S*)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol ((±)CP 101,606).

Binding parameters for NMDA receptor-specific [^{3}H]ifenprodil binding to rat brain membranes (n=3) and stably expressed NR1a/NR2B receptors (n=4) revealed B_{max} values of 2.45 ± 0.41 and 1.83 ± 0.56 pmol/mg protein (arithmetic mean ± s.e.mean), respectively, K_d values of 24.8 (21.2, 28.9) nM and 33.5 (22.5, 49.8) nM (geometric mean (-s.e.mean, +s.e.mean), respectively and Hill coefficients of 1.04 ± 0.04 and 1.03 ± 0.12, respectively. Under the assay conditions described above, [^{3}H]Ifenprodil did not bind to untransfected L(tk-) cells, stably transfected human NR1a assemblies, stably transfected recombinant human NR1a/NR2A receptors or HEK 293 cells transiently transfected with the human NR2B subunit (data not shown). The NR2B-selective antagonists ifenprodil, (±)CP 101,606 and (±)-(R*,S*)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidine propanol ((±)Ro-25-6981) inhibited [^{3}H]ifenprodil binding to rat brain membranes with K_i values (nM) of 32.6 (30.4, 34.9) (n=5), 16.2 (14.1, 18.7) (n=8) and 11.1 (9.30, 13.3) (n=5), respectively and [^{3}H]ifenprodil binding to human NR1a/NR2B receptors with K_i values (nM) of 166 (139, 198) (n=8), 11.1 (9.53, 12.9) (n=8) and 11.8 (9.61, 14.6) (n=9), respectively, consistent with previously reported affinity values for these compounds (Trube *et al.*, 1996; Menniti *et al.*, 1997).

These results demonstrate that, in the absence of commercially available radioligands specific for NR2B-containing NMDA receptors, with careful consideration of the assay conditions, [^{3}H]ifenprodil can be used to investigate these receptors.

Carter C.J., Lloyd K.G., Zivkoric B., *et al.* (1990) *J. Pharmacol. Exp. Ther.* 253, 475.
Grimwood S., Le Bourdellès B., Atack J.R., *et al.* (1996) *J. Neurochem.* 66, 2239-2247.
Menniti F., Chenard B., Collins M., *et al.* (1997) *Eur. J. Pharmacol.* 331, 117-126.
Trube G., Ehrhard P., Malherbe P., *et al.* (1996) *Soc. Neurosci. Abstr.* 22, 693.4.
Williams K., Russell S.L., Shen Y.M., *et al* (1993) *Neuron* 10, 267.

62P TRANSIENT INDUCTION OF INDUCIBLE NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY IN NMDA RECEPTOR-MEDIATED NEUROTOXICITY IN THE RAT CAUDATE PUTAMEN

Iravani M.M., Babwah R., Rose S. and Jenner P. *Neurodegenerative Diseases Research Centre*, Pharmacology Group, King's College London, Manresa Road, London SW3 6LX.

Inducible nitric oxide synthase (iNOS) is expressed in a number of brain pathologies including Parkinson's disease (Hunot et al. 1996), and nitric oxide (NO) has been implicated in the mechanisms of cell death (Bolanos et al, 1997). Additionally, stimulation of N-methyl-D-aspartate (NMDA) receptors may play a role in neuronal cell death (Turski et al, 1991). Whether iNOS is induced during NMDA-mediated cell death is not known. For this reason, we investigated the role of iNOS in NMDA-mediated neurodegeneration. In this study we have looked at the presence of iNOS-immunoreactivity (-ir) in the caudate putamen (CPu) in relation to NMDA-induced neurotoxicity.

Male Wistar rats (180-200g) were anaesthetised with Sagatal (60 mg.kg^{-1}, i.p.). NMDA (15 µg in 1 µl, RBI) was injected unilaterally into the left CPu (A, 0.48 mm; L, 2.5 mm; V, 5 mm from bregma) at a rate of 1 µl.min^{-1} using standard stereotaxic techniques. Control animals (n=5) were received vehicle (phosphate buffered saline, PBS). NMDA-treated rats were divided into 3 groups. Groups 1 (n=3) and 2 (n=2) were treated with NMDA alone. Group 3 (n=3) were pre-treated with the iNOS inhibitor, aminoguanidine (25 mg.kg^{-1} AG; i.p.). After 16 hours, groups 1 and 3 were perfused-fixed with 4% paraformaldehyde under sagatal anaethesia, brains removed and contiguous sections (30µm) cut through the striatum. NMDA group 2 were similarly treated following 7 days recovery. Sections were processed for cresyl violet (Nissl), NADPH-diaphorase histochemistry or iNOS (polyclonal; 1:2000 dil.) and GFAP (monoclonal; 1:200 dil.) immunohistochemistry. Area of lesion was assessed by area of faint Nissl staining using quantitative image analysis.

Injection of vehicle into the CPu produced no obvious signs of neurodegeneration as assessed by Nissl histochemistry. No iNOS-ir was seen, however, some weak GFAP-ir was present around the needle tracts. Sixteen hours following injection of NMDA (group 1), large lesions were seen in the CPu, accompanied by very intense expression of iNOS- and GFAP-ir. Interestingly, the intensity of NADPH-diaphorase staining was reduced. This may have been due to a loss of nNOS positive cells as reported previously (Mackenzie et al., 1997). Seven days following injection of NMDA (group 2) large lesions accompanied with the appearance of numerous cell debris were apparent in the CPu. There was no evidence of iNOS-ir although GFAP-ir was only moderately reduced. Following pretreatment with AG, (group 3), sixteen hours after NMDA administration, there was a marked reduction (57 ± 11%) in the size of lesion compared to NMDA group 1 ($p<0.05$, paired t-test).

This study demonstrates that iNOS-ir is increased in the rat striatum following the focal injection of NMDA, but that this induction is not maintained. Furthermore, inhibition of iNOS by AG results in a reduction of NMDA-mediated neurotoxicity suggesting a role for iNOS in the process of neuronal cell death. In conclusion, these data suggest that the NMDA-mediated neurotoxicity is at least partly mediated through iNOS-mediated NO release.

Bolanos JP, Almeida A, Stewart V et al (1997) *J Neurochem* **68**: 2227-2240
Hunot S, Bossiere F, Faucheux B et al.(1996) *Neuroscience* **72**, 355-363.
Mackenzie GM, Jackson MJ, Jenner P & Marsden CD (1997) *Synapse* **26**, 301-316.
Turski L, Bressler K, Rettig K-J et al (1991) *Nature* **349**: 414-418.

63P NITRIC OXIDE SYNTHASE INHIBITOR L-NAME ENHANCES PHENCYCLIDINE-INDUCED BEHAVIOURS AND NEURAL ACTIVATION IN RAT BRAIN

M. Bujas Bobanovic[1], S.M. Dursun[1,2] & H.A. Robertson[1], Depts of [1]Pharmacology and [2]Psychiatry, Dalhousie University, Halifax, NS, Canada, B3H 4H7

Nitric oxide (NO) is an intracellular messenger in the CNS and plays a role in the pathophysiology of schizophrenia (Das *et al.*, 1996). Phencyclidine (PCP) has been used as pharmacological model of schizophrenia (Steinpreis, 1996). The aim of this study is to characterize the functional interaction between PCP and the NO system. The effect of L-NAME, a non-selective NO synthase inhibitor, on PCP-induced behaviors and immediate early gene expression was determined.

Adult male Sprague-Dawley rats (250-300 g; N=4 per group) received i.p. injection of saline or L-NAME (5, 10, 25 mg/kg) alone or 15 min before PCP (5 mg/kg). Behavioral syndromes were evaluated using direct observation. Neural activation was mapped using c-fos immunocytochemistry. At 1 h following PCP injection rats were anesthetized and perfused transcardially with saline, followed by fixative. Brains were removed and postfixed for 48 hrs. Fos immunostaining was visualized in sagittal sections (50 μm) using the standard avidin-biotin technique.

After PCP alone behavioral responses occurred within 1-2 min. The predominant behavior changes were sniffing, licking, grooming and rearing, while hyperlocomotion and head weaving were moderate and seen only occasionally. Neither ataxia nor motor coordination deficits were observed. L-NAME alone failed to produce behavioral changes compared with saline treated animals. However, pretreatment with L-NAME resulted in a enhancement of the PCP-induced behaviors. The predominant behavioral changes were intense hyperlocomotion, rapid head weaving, turning, backpedalling and ataxia.
There was a low level of c-fos nuclear staining in the control animals throughout the prefrontal, occipital and piriform cortex, in inferior colliculus, and dorsal parts of caudate putamen. Occasional fos-positive cells were detected in the frontal and entorhinal cortex, cerebellar granule cell layer and in the hippocampus. PCP increased the number of fos-positive neurons in the same brain regions, with the exception of hippocampus and inferior colliculus. In addition, PCP induced a low-level fos protein expression in superior colliculus, presubiculum and ventromedial parts of caudate putamen, while moderate expression was observed in several thalamic nuclei. L-NAME, 5 mg/kg, failed to influence saline-induced c-fos expression. L-NAME 10 and 25 mg/kg increased the number of saline-induced fos-positive neurons in cerebellar granule cell layer and occipital cortex. Following L-NAME 25 mg/kg, c-fos was also induced in thalamic nuclei. Pretreatment with L-NAME resulted in a significant increase of the PCP-induced fos expression, while the distribution pattern remained almost the same. The most prominent and very dense immunostaining was observed in the occipital cortex, presubiculum and cerebellar granule cell layer, followed by prefrontal, frontal, piriform and entorhinal cortex. Moderate staining was detected in superior and inferior colliculi, thalamic and subthalamic nuclei, and to a lesser degree also in ventral and dorsal parts of caudate putamen.

Our results demonstrate that the NOS inhibitor, L-NAME, enhances both PCP-induced behaviors and neural activation, as determined by c-fos expression, suggesting an important role for nitric oxide in the action mechanism of PCP.

Das, I. *et al.* (1996) *Neurosci. Lett.* 215, 209-211.
Steinpreis, R.E. (1996) *Behav. Brain Res.* 74, 45-55.

64P IMPLICATION OF GLUTAMATE IN THE EXPRESSION OF A CALCIUM-INDEPENDENT NO SYNTHASE AFTER OXYGEN AND GLUCOSE DEPRIVATION IN RAT FOREBRAIN SLICES

A. Cárdenas, J. De Alba, M.A. Moro, J.C. Leza, P. Lorenzo & I. Lizasoain. Dept. Farmacología, Facultad de Medicina, UCM, Madrid, Spain.

Inducible NO synthase (iNOS) is a high-output inducible isoform of NOS which might contribute to the tissue damage after cerebral ischaemia (for rev., see Iadecola *et al.*, 1997). We have recently demonstrated that a calcium-independent NOS activity, which corresponds to iNOS as shown by the detection of both iNOS mRNA and protein, is expressed in different CNS cell types including neurones when using rat forebrain slices exposed to oxygen and glucose deprivation (OGD) (Moro *et al.*, 1998). The mechanisms of iNOS expression after cerebral ischaemia remain elusive. The activation of the transcription factor nuclear factor κB (NF-κB) has been recognized as an essential requirement for the expression of this gene (Xie *et al.*, 1994). Stimulation of glutamate receptors can activate NF-κB in neurones (Guerrini *et al*, 1995; Kaltschmidt *et al.*, 1995). We therefore decided to investigate whether glutamate might also be involved in the mechanisms by which OGD leads to the expression of iNOS in rat forebrain slices.

Rat forebrain slices were prepared as described (Moro *et al.* 1998). NOS activity was determined according to Salter *et al.* (1991). Glutamate concentration in slice incubation solutions was measured by HPLC (Lindroth & Mopper, 1979).

OGD for 20 min caused the appearance of a calcium-independent NOS activity (3.2 ± 0.2 pmol/min.mg prot, n=4, $p<0.05$). Calcium-independent NOS activity in control slices was not detected. In the presence of the NMDA receptor antagonist dizocilpine (100 nM), the expression of the calcium-independent NOS activity caused by OGD was blocked (0.1 ± 0.5 pmol/min.mg prot, n=4, $p<0.05$). In this context, OGD for 20 min caused the release of glutamate to the bathing solution (12.7 ± 0.2 vs. 0.5 ± 0.1 μM glutamate in OGD- and control slices, respectively, n= 4-8, $p<0.05$). In addition, incubation of control slices with glutamate (100 μM) for 20 min also caused the expression of a calcium-independent NOS activity (3.4 ± 0.7 pmol/min.mg prot, n=4, $p<0.05$). Finally, the inhibitor of the activation of NF-κB pyrrolidine dithiocarbamate (PDTC; 100 μM) inhibited the induction of the calcium-independent NOS activity found in slices exposed to glutamate (100 μM; 0.4 ± 0.2 pmol/min.mg prot, n=4, $p<0.05$). These data suggest that activation of NMDA receptors by glutamate released after OGD is involved in the expression of a calcium-independent NOS activity in rat forebrain slices *via* activation of the transcription factor NF-κB.

Iadecola, C. (1997). *Trends Neurosci.* 20, 132-139.
Moro, M.A., De Alba, J., Leza, J.C. *et al.* (1998). *Eur. J. Neurosci.* 10 (in press).
Salter, M., Knowles, R.G. & Moncada, S. (1991) *FEBS Lett.* 291, 145-149.
Lindroth, P. & Mopper, K. (1979). *Anal. Chem.* 51, 1667-1674.
Guerrini, L., Blasi, F. & Denis-Donini, S. (1995). *Proc. Natl. Acad. Sci. U.S.A.* 92, 9077-9081.
Kaltschmidt, C., Kaltschmidt, B. & Baeuerle, P.A. (1995). *Proc. Natl. Acad. Sci. U.S.A.* 92, 9618-9622.

65P PROTECTIVE EFFECTS OF CYCLIC GMP ON THE NEURONAL DEATH INDUCED BY SIN-1 IN THE PRESENCE OF SUPEROXIDE DISMUTASE

I. Lizasoain, P. Fernández-Tomé[1], J.C. Leza, P. Lorenzo & M.A. Moro. Dept. Farmacología, Facultad de Medicina, UCM and [1]Inst. Farmacología-Toxicología, CSIC, Madrid, Spain.

The nitrovasodilator 3-morpholinosydnonimine (SIN-1) slowly decomposes to release both nitric oxide (NO) and superoxide (O_2^-) (Feelisch *et al.*, 1989). The effect of superoxide dismutase (SOD) against cell damage induced by the simultaneous generation of NO and O_2^- is controversial (Lipton *et al.*, 1993; Gergel *et al.*, 1995). On the other hand, NO increases cGMP concentrations *via* activation of soluble guanylate cyclase (SGC) and, interestingly, cGMP may have neuroprotective actions (Garthwaite and Garthwaite, 1988). In this context, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) has been shown to be a specific and potent inhibitor of SGC (Garthwaite *et al.*, 1995; Moro *et al.*, 1996). In the current report we decided to study the effects of SOD and ODQ on SIN-1-induced cell death in 1-week-old rat cortical neurones in primary culture, in order to clarify the role of the different species involved in this process.

Primary cultures of cortical cells were performed as described (Koh & Choi, 1987). Cell viability was monitored colorimetrically by MTT assay (Hansen *et al.*, 1989).

SIN-1 caused a concentration-dependent neuronal death (LC_{50}= 2.5 ± 0.5 mM, n=3-12, $p<0.05$), which was potentiated by SOD (100 U/ml; 52.0 ± 5.3 and 17.0 ± 9.0% decrease in cell viability of 0.5 and 2 mM SIN-1-treated cells, respectively, n=3-12, $p<0.05$). The effect of SOD (100 U/ml) on SIN-1- induced cell death was abolished by catalase (100 U/ml, n=3-12, $p<0.05$), suggesting that it is mediated by H_2O_2. SIN-1 (0.5 mM)/SOD (100 U/ml)-induced cell death was enhanced in the presence of HbO_2 (50 μM, 67.1 ± 1.4% decrease in cell viability of SIN-1/SOD-treated cells, n=3, $p<0.05$) and of ODQ (0.01-1 μM, EC_{50}= 0.073 ± 0.004 μM, n=3-6), consistent with the idea that NO-induced cGMP formation has a protective effect in these conditions. The cGMP mimetic, 8-bromo-cGMP reversed the potentiating effect induced by ODQ on SIN-1/SOD-induced neuronal death (n=3, $p<0.05$). Taken together, these data suggest that H_2O_2 is the species responsible of the potentiation by SOD of SIN-1-induced cell death and that cGMP elevations confer cytoprotection against this H_2O_2-mediated component of cell death.

Feelisch, M., Ostrowski, J. & Noack, E. (1989). *J. Cardiovasc. Pharmacol.* 14 (Suppl. 11), S13-S22.

Gergel, D., Mišík, V., Ondriaš, K. *et al.* (1995). *J. Biol. Chem.* 270, 20922-20929.

Garthwaite, G. & Garthwaite, J. (1988). *Neuroscience* 26, 321-326

Garthwaite, J., Southam, E., Boulton C.L. *et al.* (1995). *Mol. Pharmacol.* 48, 184-188.

Hansen, M.B., Nielsen S.E. & Berg K. (1989). *J. Immunol. Meth.* 119, 203-210.

Koh, J. Y. & Choi, D. W. (1987). *J. Neurosci. Meth.* 20, 83-90.

Lipton, S.A., Choi, Y.-B., Pan, Z.-H. *et al.* (1993) *Nature* 364, 626-632.

Moro, M.A., Russell, R.J., Cellek, S. *et al.* (1996). *Proc. Natl. Acad. Sci. USA.* 93, 1480-1485.

66P DOWN-REGULATION OF CALCIUM-DEPENDENT NO SYNTHASE BY CALCIUM-INDEPENDENT NO SYNTHASE AFTER OXYGEN AND GLUCOSE DEPRIVATION IN RAT FOREBRAIN SLICES

J. De Alba, A. Cárdenas, M.A. Moro, J.C. Leza, P. Lorenzo & I. Lizasoain. Dept. Farmacología, Facultad de Medicina, UCM, Madrid, Spain.

There is increasing evidence that nitric oxide (NO) may play complex roles in the pathophysiology of cerebral ischaemia. However, the precise role that NO plays in the mechanisms of ischaemic brain damage has been a source of controversy, since this agent might be either beneficial or detrimental to the ischaemic brain (for rev., see Iadecola 1997). We have recently shown that a calcium-independent NOS activity, which corresponds to inducible NOS (iNOS) as shown by the detection of both iNOS mRNA and protein, is expressed after oxygen and glucose deprivation (OGD) in rat forebrain slices (Moro *et al.*, 1998). We have now used this system to study the effect of OGD on the calcium-dependent NOS isoform.

Male Sprague-Dawley rats were killed by decapitation, the forebrain slices were prepared as described (Moro *et al.*, 1998). NOS activity was measured by monitoring the conversion of L- [U-^{14}C]arginine into [U-^{14}C]citrulline (Salter *et al.*, 1991).

OGD for 20 min caused a decrease in the calcium-dependent NOS activity at 180 min after the end of the OGD period (1.6 ± 0.3 vs 8.6 ± 1.2 pmol/min. mg prot in OGD and control slices, respectively, n=16, $p<0.05$). This decrease was parallel to the increase in the calcium-independent NOS activity in rat forebrain slices exposed to OGD (0 vs 3.2 ± 0.2 pmol/min. mg prot in control and OGD slices, respectively, n=16, $p<0.05$). The induction of the calcium-independent NOS activity was inhibited after addition of the induction inhibitor dexamethasone (1 μM; 0.1 ± 0.1 pmol/min. mg prot, n=12, $p<0.05$) and the protein synthesis inhibitor cycloheximide (10 μM; 0 pmol/min.mg prot, n=12, $p<0.05$). Concomitantly, these compounds produced a recovery in the calcium-dependent NOS activity after OGD in rat forebrain slices (3.0 ± 0.3 and 4.3 ± 0.3 pmol/min. mg prot respectively, n=16, $p<0.05$). Oxyhaemoglobin (10μM) also caused a recovery in the calcium-dependent NOS activity after OGD (5.8 ± 0.6 pmol/min. mg prot, n=6, $p<0.05$) suggesting that NO is responsible for the down-regulation of calcium-dependent NOS. Finally, the exposure to the NO donor DETA-NONOate (1 mM) during 200min caused a decrease in the calcium-dependent NOS activity present in control rat forebrain slices (8.6 ± 1.2 vs 2.5 ± 0.5 pmol/min. mg prot in control and DETA-NONOate treated slices, respectively, n=16, $p<0.05$). These data suggest that calcium-independent NOS expression down-regulates calcium-dependent NOS activity in rat brain slices exposed to OGD. These studies suggest important and complex interactions between NOS isoforms which may help to gain further insight into the physiological and pathophysiological events that occur during and after cerebral ischaemia.

Iadecola, C. (1997). *Trends Neurosci.* 20, 132-139.

Moro, M.A., De Alba, J., Leza, J.C. *et al.* (1998). *Eur. J. Neurosci.* 10 (in press).

Salter, M., Knowles, R.G. & Moncada, S. (1991) *FEBS Lett.* 291, 145-149.

67P BASAL DOPAMINE RELEASE AND THE RESPONSE TO ACUTE HALOPERIDOL IN THE CORE AND SHELL SUB-REGIONS OF RAT NUCLEUS ACCUMBENS

R.J. Moran, W.R. Bell[1] & D.J. King. Department of Therapeutics and Pharmacology and [1]School of Psychology, The Queen's University of Belfast, Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL

Acute, systemic administration of the dopamine D_2 antagonist haloperidol increases DA release in the nucleus accumbens, as measured by *in vivo* microdialysis. However, reports tend not to distinguish between the putative motor and limbic subregions of the nucleus accumbens, namely the core and the shell. The use of disparate methodologies to study dopaminergic transmission in the nucleus accumbens core and shell has produced conflicting results. In the present study we used microdialysis in an attempt to clarify some of these apparent inconsistencies.

Male, Sprague-Dawley rats (200 - 300 g) were implanted with concentric microdialysis probes in either the core or shell of the nucleus accumbens. Animals were randomly divided into four groups (n = 6) on the basis of probe location or treatment. Dialysate samples were collected from awake rats every twenty minutes. Dopamine and metabolites were measured by high-performance liquid chromatography with electrochemical detection. Results were expressed as a percentage of the concentration in three baseline samples, collected before administration of haloperidol (0.1 mg kg^{-1}; i.p.) or saline (1.0 ml kg^{-1}; i.p.). Basal dialysate dopamine concentration was higher in the core region of the nucleus accumbens than in the shell (5.03 ± 0.78 nM in the core and 2.10 ± 0.33 nM in the shell; $P<0.01$). Dialysate concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were slightly higher in the core region. Values for core and shell, respectively, were: DOPAC, 0.58 ± 0.03 µM and 0.47 ± 0.03 µM ($P=0.01$); HVA, 0.26 ± 0.02 µM and 0.20 ± 0.02 µM ($P<0.05$). Haloperidol increased dopamine, DOPAC and HVA dialysate concentrations in the core to a maximum of 202 ± 37%, 214 ± 31% and 228 ± 32% of baseline ($P<0.001$), respectively. In the shell, maximal respective increases in dopamine, DOPAC and HVA concentrations were 298 ± 39%, 247 ± 12% and 270 ± 17% ($P<0.001$). There was a trend towards a greater dopamine increase following haloperidol in the shell compared with the core ($P<0.1$). Saline had no effect on any of the compounds measured. These results, obtained using microdialysis, differentiated between the core and shell regions of the nucleus accumbens on the basis of basal dopamine and metabolite concentrations, but not in terms of response to acute haloperidol. The present findings show much agreement with recent microdialysis studies (Kalivas & Duffy, 1995; King et al, 1997) but differ, in some respects, from previous reports, which used homogenized tissue and voltammetry (Deutch & Cameron, 1992; Marcus et al, 1996). Methodological differences may explain any disparity.

Deutch, A.Y. & Cameron, D.S. (1992). *Neuroscience*, **46**, 49-56.
Kalivas, P.W. & Duffy, P. (1995). *Brain Res.*, **675**, 325-328.
King, D., Zigmond, M.J. & Finlay, J.M. (1997). *Neuroscience*, **77**, 141-153.
Marcus, M.M., Nomikos, G.G. & Svensson, T.H. (1996). *Eur. Neuropsychopharmacol.*, **6**, 29-38.

68P TREATMENT WITH I_2-IMIDAZOLINE LIGANDS ATTENUATES THE DEVELOPMENT OF TOLERANCE TO MORPHINE-INDUCED ANTINOCICEPTION IN RATS

M.A. Boronat, G. Olmos & J.A. García-Sevilla. Laboratory of Neuropharmacology, Associate Unit of the Institute Cajal/CSIC, Department of Biology, University of the Balearic Islands, E-07071 Palma de Mallorca, Spain.

Chronic exposure to opiate agonists leads to drug tolerance, which is characterized by a decrease in antinociception efficay. Agmatine, an endogenous ligand for imidazoline receptors (IR), has been shown to attenuate tolerance to morphine-induced antinociception (Kolesnikov *et al.*, 1996), suggesting a role of IR (I_1- and/or I_2-types) in the modulation of opioid tolerance. The aim of this study was to assess if idazoxan, an α_2-adrenoceptor antagonist that also interacts with IR, and other more selective IR-ligands, could also modulate opiate tolerance in rats.

Antinociceptive responses to opiate drugs were measured by the tail-flick (TF) test (D'Amour & Smith, 1941) in male Sprague-Dawley rats (250-300 g). The TF latency (TFL) was defined as the time from the onset of radiant heat to tail withdrawal (baseline TFLs: 3-4 s). TFLs were determined 30 min after the last drug injection. TFLs are expressed as mean ± s.e. mean. One-way ANOVA followed by Scheffé's test was used for statistical comparisons.

The acute administration of morphine (10 mg kg^{-1}, i.p., n= 4) or pentazocine (10 mg kg^{-1}, i.p., n= 4) resulted in cut-off times for TFLs (9-12 s). As expected, the initial antinociceptive response to the opiates was lost after chronic (13 days) treatment (tolerance). When idazoxan (10 mg kg^{-1}, i.p., n= 4) was given chronically 30 min before the opiates it almost completely prevented morphine tolerance (TFL increased by 143±10% at day 13, $P<0.01$) and markedly attenuated tolerance to pentazocine (TFL increased by 71±5% at day 13, $P<0.05$). Idazoxan alone did not modify TFLs. The concurrent chronic administration (10 mg kg^{-1}, i.p., 13 days) of 2-BFI (2-(2-benzofuranyl)-2-imidazoline), LSL 60101 (2-(2-benzofuranyl)-2-imidazol) and LSL 61122 (2-styryl-2-imidazoline), selective I_2-imidazoline receptor ligands, and morphine (10 mg kg^{-1}, i.p., n= 4 for each treatment), also prevented (2-BFI) or attenuated morphine tolerance (TFLs increased by 64±6% to 172±10% at day 13, $P<0.01$). These I_2-ligands alone did not modify TFLs. In contrast, the concurrent chronic (13 days) treatment of RX 821002 (2-methoxy idazoxan, 10 mg kg^{-1}, i.p.) and RS 15385-197 (3-methoxy-12-methanesulfonyl-isoquino-naphthyridine, 1 mg kg^{-1}, i.p.), selective α_2-adrenoceptor antagonists, and morphine (10 mg kg^{-1}, i.p., n= 4 for each treatment) did not attenuate morphine tolerance. Similarly, the concurrent chronic treatment of moxonidine (1 mg kg^{-1}, i.p.), a mixed I_1-imidazoline receptor and α_2-adrenoceptor agonist, and morphine (10 mg kg^{-1}, i.p., n= 4) did not alter the development of tolerance to the opiate.

Together, the results indicate that chronic treatment with I_2-imidazoline ligands attenuates the development of tolerance to opiate drugs. This finding offers the I_2-ligands as promising therapeutic co-adjuvants in the management of chronic pain with opiate drugs.

This study was supported by DGICYT Grant PB94-0002 Mod C

D'Amour, F.E. & Smith, D.L. (1941) J. Pharmacol. Exp. Ther. 72, 74-79.
Kolesnikov, Y., Jain, S. & Pasternak, G.W. (1996) Eur. J. Pharmacol. 296, 17-22.

69P PRE-TREATMENT WITH THE NON-NMDA ANTAGONIST DNQX ATTENUATES ECS-INDUCED GENE EXPRESSION FOR BDNF IN RAT BRAIN

T.S.C. Zetterström, R. McQuade and Q. Pei,
Oxford University - SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE

Electroconvulsive shock (ECS) administration to rats has been shown to cause alterations in the expression of a number of genes including potassium channel subunits and brain derived neurotrophic factor (BDNF) (Pei et al., 1997; Zetterström et al., 1997). The mechanism behind the effect of ECS on BDNF gene expression is not clear. However, BDNF mRNA abundance in hippocampal neurones has been shown to be enhanced by ECS in a similar way to that observed following the administration of kainate, suggesting that excitatory glutaminergic neurotransmission mediates the elevation of BDNF gene expression seen after ECS. The aim of the present was to investigate the effect of pretreatment with the AMPA/kainate glutamate antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) on ECS induced elevation of BDNF gene expression in rat brain.

Male Sprague-Dawley rats (250-270g) were used in all experiments. Rats were anaesthetised with halothane and received a single ECS (150V, 50Hz, 1s, via ear clip electrodes) and control rats were anaesthetised with halothane and had electrodes placed but no current delivered. In the DNQX experiments, rats were pretreated 30 min before the ECS administration with either vehicle or DNQX (2 nmol/µl, dissolved in 1 N NaOH, pH adjusted to 7.4 with 0.2 N HCl) in a volume of 10 µl into the right ventricle over a period of 5 min using a microinfuson pump. Three hours after the ECS or sham administration, rats were anaesthetised with sodium pentobarbitone, transcardially perfused and their brains were removed. Hippocampal sections (12 µm) were mounted on slides and pretreated for ISHH using a standard procedure (Pei et al., 1997). After pretreatment, an oligonucleotide complementary to bases 642-686 of the rat BDNF cDNA was 3'-tail labelled with [^{35}S]-dATP and added to the brain sections. The relative abundance of BDNF mRNA was determined by densitometric quantification of autoradiograms in dentate gyrus and piriform cortex. Levels of BDNF mRNA are expressed as nCi/g tissue and values are the means±s.e.mean of n=4-5 rats/group. Statistical analyses were performed using one way ANOVA and Dunnetts t-test.

Rats which were pretreated with DNQX 30 min before ECS administration still showed tonic-clonic seizure activity following the ECS administration, although the seizures were generally shorter lasting compared to the vehicle treated rats.The basal expression of BDNF mRNA in dentate gyrus was: 114±31, at 3 h after the administration of a single ECS this was increased to 1282±124. There was also an increase in mRNA abundance following a single ECS in piriform cortex, basal expression was here: 101±28 and a single ECS increased the levels to 332±45. Pretreatment with DNQX resulted in an almost complete inhibition of ECS induced enhancement of BDNF gene expression in the dentate gyrus (143±16, $p<0.001$ compared to ECS treated vehicle injected rats). However the ECS induced action was only partially inhibited in piriform cortex by DNQX (206±27, $p<0.002$, compared to controls).
In summary, we have shown that the increase in BDNF mRNA expression seen in the dentate gyrus following a single ECS is likely to be mediated by glutaminergic AMPA/kainate receptors, while additional mechanisms are likely to contribute to the ECS induced elevation of BDNF mRNA in piriform cortex.

Pei, Q. et al (1997) *Neuroscience.* **78**, 343-350.
Zetterström, T et al (1997) *Br. J. Pharmacol.* in press, Harrogate BPS

70P THE DOSE-DEPENDENT EFFECTS OF PHENCYCLIDINE ON BEHAVIOUR AND IMMEDIATE EARLY GENES IN RATS

M. Bujas Bobanovic[1], S.M. Dursun[1,2] & H.A. Robertson[1], Depts of [1]Pharmacology and [2]Psychiatry, Dalhousie University, Halifax, NS, Canada, B3H 4H7

In humans, phencyclidine (PCP) is known to produce a syndrome of behavioral effects which have many characteristics in common with schizophrenia (Steinpreis, 1996). Therefore, we have investigated the effects of PCP on immediate early genes (IEGs) in rat brain to obtain a clue for the neuromolecular basis of schizophrenia.

Adult male Sprague-Dawley rats (250-300 g; N=4 per group) received i.p. injection of saline or PCP (2.5; 5; 7.5; 10; 25 and 50 mg/kg). Behavioral syndromes were evaluated using direct observation. The effect of PCP on IEGs was determined using c-fos, c-jun and NGFI-A immunocytochemistry. At 1 h following PCP injection rats were anesthetized and perfused transcardially with saline, followed by fixative. Brains were removed and postfixed for 48 h. Immunostaining was visualized in sagittal sections (50 µm) using the standard avidin-biotin technique. C-jun and NGFI-A immunoreactivity was examined also at 3, 6, 12 and 24 h following PCP injection.

Behavioral responses occurred within 1-2 min of PCP administration. PCP 2.5 and 5 mg/kg had similar behavioral effects such as sniffing, licking, grooming and rearing. After PCP 7.5 and 10 mg/kg rats became hyperactive with head weaving, turning, backpedalling and ataxia. At higher doses of PCP, 25 and 50 mg/kg rats initially laid stationary and exhibited slight head weaving and tremor, followed by crawling locomotion.

Saline administration induced a low-level fos protein expression in the prefrontal, occipital and piriform cortical regions, inferior colliculus and dorsal parts of caudate putamen. Following PCP 2.5-50 mg/kg, moderate fos staining was observed in prefrontal, retrosplenial and piriform cortices, thalamic and subthalamic nuclei and in ventromedial parts of caudate putamen. Rare fos positive nuclei were detected in the molecular and Purkinje cell layer and white matter of the cerebellum. Patchy fos immunoreactivity was observed in the granule cell layer throughout the cerebellar cortex and this expression was dose-dependent. Moreover, PCP dose-dependently induced c-fos expression in entorhinal, frontal and occipital cortices. The most prominent enhancement was observed in deep cortical layers. Following 7.5 and 10 mg/kg, moderate staining was detected in superior colliculus, presubiculum and in ventrolateral parts of caudate putamen. At higher doses of PCP, 25 and 50 mg/kg, the most prominent and very dense immunostaining was observed in the neurons of the occipital cortex, presubiculum, cerebellar granule cell layer and ventrolateral parts of caudate putamen, followed by superior colliculus, frontal and entorhinal cortical regions. In addition, PCP 50 mg/kg induced dense fos protein expression in cerebellar nuclei. In contrast to the c-fos, PCP did not affect saline-induced expression of c-jun or NGFI-A, neither at 1 nor at 3, 6, 12 and 24 h after PCP injection.

The behavioral syndrome induced by PCP is consistent with the previous reports (Steinpreis, 1996). This study also indicates that PCP induces c-fos, but not c-jun or NGFI-A gene expression in rat brain. These results might suggest that c-fos plays an important role in the behavioral and/or psychotomimetic effects of PCP.

Steinpreis, R.E. (1996) *Behav. Brain Res.* 74, 45-55.

71P THYROXINE ENHANCES COGNITIVE PERFORMANCE IN RATS CHALLENGED WITH SCOPOLAMINE

J. W. Smith, B. Costall and J.W. Smythe
Neuropharmacology Research Group, Dept. of Pharmacology, Univ. of Bradford, Bradford BD7 1DP

Loss of central cholinergic neurotransmission induced experimentally, or accompanying pathological conditions such as Alzheimer's disease (AD), produces profound cognitive impairments (Fibiger, 1991). Recent reports suggest that thyroid hormones augment cholinergic function in the hippocampus as revealed by immunohistochemical changes in cholinergic innervation patterns (Schwegler, 1995) In a previous report, we showed preliminary data suggesting that a high dose of a thyroid hormone, administered acutely, enhanced cognitive performance in a water maze task (Shirley *et al.*, in press). In the present study we have investigated whether or not thyroxine (T4; tetra-iodothyronine), one of the principal thyroid hormones, would ameliorate cognitive deficits produced by scopolamine in a water maze task.

Adult male, Lister hooded rats (350-500 g) were injected with vehicle (VEH; physiological saline+40 mg/kg ethanol), or scopolamine (SCOP; 1 mg/kg IP). Forty minutes later each animal was injected with either 0, 2.5 or 5.0 mg/kg T4, given IP, and tested 40 min later in a water maze (n=10/group). Rats were given 4 trials of training to locate the platform, with each trial limited to a 60 sec maximum. Four days later, rats were tested for their retention of the task. Data (latencies to locate the platform, aggregates of the 4 trials) were assessed by ANOVA (applying a Greenhouse-Gieser correction) and *post hoc* testing was performed using Bonferroni corrected t-tests.

ANOVA revealed a significant interaction between T4 treatment and day of testing $F(2,59)=7.83$, $P<.01$. As shown in Fig. 1, T4 at the high dose significantly improved day 4 retention performance of the task compared to the 0 mg/kg group regardless of pretreatment with VEH or SCOP. Further analysis revealed a significant main effect of SCOP treatment $F(1,59)=11.1$, $P<.001$. As illustrated in Fig. 1, rats treated with SCOP displayed higher latencies, an effect that was absent on day 4. There was no effect of any drug on swim speed.

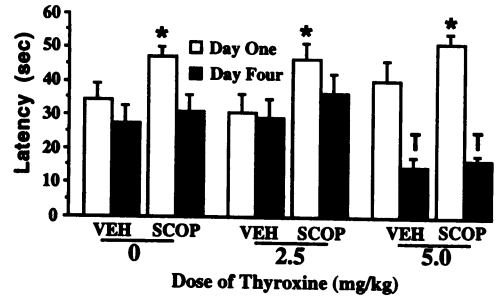


Figure 1. Effects of VEH or T4 on latencies to locate a hidden platform in a water maze task following pretreatment with VEH or SCOP. Means ± sem are shown.
*significantly different from VEH,0 mg/kg group on day 1
Tsignificantly different from VEH,0 mg/kg group on day 4

In conclusion, acute thyroid hormone administration improves cognitive performance in rats, possibly by elevating cholinergic function in the brain. Low level T4 treatment might be usefully employed to augment cognitive performance in some pathological conditions such as AD.

Fibiger, H.C. (1991) *Trends Neurosci.* 14, 220-223.
Schwegler, H. (1995) *Neurosci. Lett.* 198, 197-200.
Shirley, A. *et al. Br. J. Pharmacol.* (in press).

72P EFFECTS OF ANTIDEPRESSANT TREATMENTS ON mRNA EXPRESSION OF G PROTEIN-COUPLED POTASSIUM CHANNEL (GIRK) SUBUNITS IN THE RAT BRAIN

Q. Pei, L. Lewis, K. Ainsworth, and T. S. C. Zetterström,
Oxford University - SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE

Previous studies have shown that various chronic antidepressant treatments such as electroconvulsive shocks (ECS) or long term lithium diet alter behavioural responses mediated by monoamine receptors. Interestingly, some of these effects are mimicked by K^+-channel blocking agents, suggesting that chronic antidepressant treatments may change K^+-channel function (Wang and Grahame-Smith, 1992). We have recently shown that repeated ECS results in an upregulation of the voltage dependent K^+ channel subunit Kv 4.2 in rat hippocampus (Pei et al., 1997). Another family of potassium channels comprises the G-protein coupled inward rectifier K^+-channels (GIRKs) which play important roles in mediating synaptic functions of neurotransmitters in the brain (Liao et al., 1996). The aim of the present study was to investigate the effects of several chronic antidepressant treatments, chronic ECS, fluoxetine, tranylcypromine (TCP) or desipramine (DMI), lithium diet on gene epression of GIRK1 and GIRK2 subunits, in the rat brain using in situ hybridization histochemistry (ISHH).

Male Sprague-Dawley rats were used in all experiments. In the ECS group, rats received a shock (150v, 50 Hz, 1 second, via ear clips under halothane anaesthesia) every other day for 10 days (a total of 5 shocks). Sham rats received halothane and identical handling without current delivered. For antidepressant drug treatments, rats received either fluoxetine (5 or 10 mg/kg, i.p.), TCP (2.5 mg/kg, i.p.) or DMI (10 mg/kg, i.p.) twice daily for 14 days. Control rats received twice daily saline injection (1 ml/kg). For chronic lithium experiments, rats were fed with pellets containing 0.1% Li_2CO_3 for 21 days. Rats were then killed 24 h after the last administration and brains were removed and processed for ISHH using ^{35}S-dATP labelled antisense GIRK1 and GIRK2 oligonucleotides. The relative mRNA abundance of GIRK1 and GIRK2 was determined by densitometric quantification of autoradiograms. Values (mean±SEM) are expressed as a percentage of respective control groups. Statistical significance was tested using ANOVA and Dunnett's t-test.

Chronic ECS increased mRNA abundance of GIRK1 and GIRK2 to 123 ± 3% and 155 ± 5.9% (% of sham, n=10 rats/group, $p<0.01$) respectively in dentate gyrus. No significant changes in either GIRK1 or GIRK2 were detected in other brain regions measured such as CA1 and CA3 of the hippocampus or parietal cortex. Gene expression for both GIRK1 (data not shown) and GIRK2 was unaltered in dentate gyrus by chronic administration with fluoxetine, 5 (data not shown) or 10 mg/kg (90 ± 4.2%, n=6), TCP, 2.5 mg/kg (89 ± 3.7%, n=5) and DMI, 10 mg/kg (95 ± 5.6%, n=6). Chronic lithium diet also failed to alter GIRK1 and GIRK2 (102±4%, n=5) mRNA abundance in the rat dentate gyrus, or any other parts of the rat brain.

In summary, we have shown that chronic ECS induces a significant upregulation in mRNA abundance of both GIRK1 and GIRK2 in the dentate gyrus, but not in any other regions of the rat brain. The effects on GIRK mRNA abundance in the dentate gyrus was unique for ECS, since other antidepressant treatment including chronic administration of fluoxetine, TCP and DMI and lithium diet, did not affect GIRK1 and GIRK2 mRNA expression.

Wang, H.& Grahame-Smith, D.G.(1992) Neuropharmacol. 31, 413-419.
Pei, Q. et al., (1997) Neuroscience, 78, 343-350.
Liao et al., (1996) J. Neuroscience, 15, 7132-7150.

73P ELEVATED SENSITIVITY TO THE INCREASE IN SEROTONIN TURNOVER AFTER α_2-ADRENOCEPTOR BLOCKADE IN MICE WITH THREEFOLD OVEREXPRESSION OF α_{2C}-ADRENOCEPTOR SUBTYPE

E. MacDonald[1], J. Sallinen[2], T. Viitamaa[3], A. Haapalinna[3], M. Scheinin[2], R.E. Link[4] & B.K. Kobilka[4], [1]Dept. Pharmacol. & Toxicol., Univ. Kuopio, PL 1627, Kuopio, [2]Dept. Pharmacol., Univ. Turku, Kiinamyllynk. 10, Turku, [3]Orion Corporation - Orion Pharma, PL 425, Turku, Finland, [4] Dept. Mol. Cell. Physiol. Stanford Univ., CA 94305, U.S.A.

Of the three known α_2-adrenoceptor (α_2-AR) subtypes, the α_{2C}-AR has remained an enigma. It is present in the brain though not the periphery but mice with genetic manipulation of this receptor i.e. knock-outs or mice with receptor over-expression (OE) do not show any gross developmental, behavioural or neurochemical differences from their wild type (WT) controls. Equally, the genetically manipulated mice do not differ from controls when challenged with a subtype non-selective α_2-agonist (Sallinen *et al.* 1997). We now describe the effects of challenge with a subtype non-selective α_2-antagonist, atipamezole (ATI) (Haapalinna *et al.* 1997) in OE and WT mice, concentrating on the changes in serotonergic neurochemistry.

We used female, 8 month old mice, weight 29 ± 2 g (S.D.), the strain being derived from FVB/N mice. The OE mice (n = 49) have 3-fold over-expression of α_{2C}-AR in the brain (Sallinen *et al.* 1997) compared to the WT controls (n=52). They were injected with ATI (s.c.) or saline and 1 h later killed by decapitation. Since preliminary experiments failed to reveal any strain dependent regional differences in neurochemistry, the brains were not dissected into subregions. Whole brain concentrations of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC and electrochemical detection. Statistical analysis was ANOVA (strain; strain + dose) with Dunnett's test (doses of ATI vs saline; $P < 0.05$ = significant).

There were no strain-related differences between untreated OE mice and their WT controls. After ATI treatment, 2 factor ANOVA (strain; dose) failed to reveal any significant differences for 5-HT. For 5-HIAA, there was a significant dose-related difference between groups, but for the 5-HIAA/5-HT ratio (an indirect measure of 5-HT turnover) both dose and species showed significant differences and there was a trend for an interaction of dose with strain ($P = 0.08$). Table 1 shows the values for 5-HT and the 5-HIAA/5-HT ratio.

Table 1: Concentrations (± s.e. mean) of 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio in mouse brain 1h after ATI injection

ATI	5-HT (nmol/g)		5-HIAA (nmol/g)		5-HIAA/5-HT	
(μg/kg)	WT	OE	WT	OE	WT	OE
Saline	4.4±0.1	4.6±0.2	2.9±0.1	2.7±0.1	0.67±.04	0.60±.03
125	4.6±0.3	4.6±0.2	2.7±0.1	2.9±0.1	0.60±.05	0.64±.03
250	4.6±0.2	4.2±0.3	3.0±0.1	3.2±0.1*	0.67±.04	0.79±.05*
500	4.6±0.2	4.0±0.2	3.2±0.1	3.2±0.1*	0.70±.03	0.81±.03*

Each group consisted of 12 - 16 mice. * = significantly different ($P<0.01$) from mice treated with saline (Dunnett's test)

In conclusion, mice with 3-fold over-expression of the α_{2C}-AR do not differ from their WT controls in terms of brain levels of 5-HT or 5-HIAA or in unstimulated 5-HT turnover as assessed by the ratio of 5-HIAA/5-HT. However, these mice were more sensitive to the effects of an α_2-antagonist (ATI) to increase the turnover of 5-HT. Thus, the ability of α_2-antagonists to increase 5-HT release in brain is in part mediated through removal of α_{2C}-AR inhibitory tone on serotonergic neurones.

Haapalinna, A., Viitamaa, T., MacDonald, E. *et al.* (1997) *Naunyn-Schmeideberg's Arch. Pharmacol.* 356, 570-582.
Sallinen, J., Link, R.E., Haapalinna, A. *et al.* (1997) *Mol. Pharmacol.* 51, 36-46.

74P MODULATION OF ARACHIDONIC ACID RELEASE AND CELL GROWTH BY PHOSPHOLIPASE A_2 INHIBITORS IN A549 CELLS

Q Choudhury, Diane T Mckay, RJ Flower & JD Croxtall
Dept. Biochemical Pharmacology, The William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ.

Prostaglandins (PGs) are growth factors in many cell lines (Otto et al., 1982; Karmali et al., 1979) including the A549 cell line (Croxtall & Flower, 1992). They are formed by the action of cyclooxygenase on arachidonic acid (AA) liberated from phospholipid pools by rate-limiting phospholipase A_2 (PLA_2) enzymes including the calcium-dependent low mol. wt. (14 kDa) secretory PLA_2 ($sPLA_2$), the calcium-dependent high mw (85 kDa) cytosolic PLA_2 ($cPLA_2$), and the recently described calcium-independent high mw (80 kDa) cytosolic PLA_2 ($iPLA_2$). In this study we investigated the ability of PLA_2 inhibitors to control AA release and thus A549 cell proliferation.

A549 cells were maintained in cell culture medium DMEM/F12 supplemented with 10% FCS, 1% PS in a humidified atmosphere of 95% O_2/5% CO_2 at 37°C. For AA release (AAR) experiments, the cells were seeded onto 12-place multiwell plates at a density of 3×10^5 cells per ml per well in medium and labelled with 9.25 kBq of ^{3}H-AA per well. Medium containing stimulators, inhibitors or vehicle was added to the cells and the released ^{3}H-AA was measured using a scintillation counter. For cell proliferation (CP) experiments, cells were seeded at a density of 5×10^4 cells per ml per well in culture medium containing stimulators, inhibitors or vehicle and cell numbers were determined after 72 h using a Coulter counter. All experiments were performed in triplicate, data are reported as mean (%) ± s.e. mean.

EGF (10nM)/Thapsigargin (50nM) and IL-1ß (1ng/ml) stimulated AAR by 1019.3±1.8 and 176.4±10.9% above control levels respectively, whereas EGF (10nM) and IL-1ß (1ng/ml) stimulated CP by 76.2±2 and 42.1±1.5% above control levels respectively. The $sPLA_2$ inhibitor (ONO-RS-82) (20μM) had no effect on AAR stimulated by EGF/Tg or IL-1ß (<5%) and was also ineffective at 1 μM in preventing basal (<1%), EGF (<4%) or IL-1ß (10%) stimulated CP. The $cPLA_2$ inhibitor arachidonyl trifluoromethyl ketone ($AACOCF_3$) at 0.1 μM and above inhibited EGF/Tg and IL-1ß stimulated AAR (max inhibitions of 33.7±1.5 and 63.4±3%) but had no effect (<4%) on basal CP but inhibited EGF and IL-1ß dependent CP in a dose dependent manner with a max inhibitions of 36.6±0.8 and 75±0.5% with IC_{50}s seen at 80nM and 3μM respectively. The $iPLA_2$ inhibitor, haloenol lactone suicide substrate (HELSS) at 0.5μM and above inhibited EGF/Tg and IL-1ß stimulated AAR but to a lesser extent than $cPLA_2$ inhibitor (25.7±4 and 46±9%). It also inhibited basal, EGF and IL-1ß induced CP (22.8±1.5, 20.7±0.6 and 37.3±0.5%) in a dose depedent manner with IC_{50}s seen at 750nM, 160nM and 180nM respectively.

Previously, we have demonstrated that A549 cells express $cPLA_2$ but not $sPLA_2$ (Tokumoto et al., 1993) and this is congruent with the inactivity of ONO-RS-82 and the effect of $AACOCF_3$ in this system. Interestingly, data obtained with HELSS implies that the recently described $iPLA_2$ also seems to be active in these cells although we cannot totally rule out a non-specific effect of HELSS on $cPLA_2$. Although $iPLA_2$ has not been formally identified in A549 cells, it has been found in most cells and tissues examined (Balsinde & Dennis, 1997) and may be important in membrane remodelling. Like the NSAIDS and the glucocorticoids, both of which suppress PGs generations, PLA_2 inhibitors also reduce CP presumably by restricting the synthesis of growth-promoting eicosanoids.

Otto, AM *et al.*, (1982). *PNAS*, USA. **79**:4992-4996.
Karmali, RA *et al.*, (1979) *Pharm. Res. Comm.* **11**:69-75.
Croxtall, JC & Flower, RJ. (1992)*PNAS*, USA **89**:3571-3575
Tokumoto, H. *et al.*, (1993) *BBA.* **1164**: 236-242.
Balsinde, J. & Dennis, EA. (1977)*JBC.* **272**:26, 16069-16072
This work was supported by The Wellcome Trust.

75P DO SESQUITERPENE LACTONES ACT AS ANTI-INFLAMMATORY AGENTS THROUGH A PROTEIN SYNTHESIS-MEDIATED MECHANISM?

J.L. Ríos, R.M. Giner, M.C. Recio, S. Máñez, M.L. Uriburu* & J.R. de la Fuente*. Department de Farmacologia, Facultat de Farmàcia, Universitat de València, Spain; *Consejo de Investigación, Universidad Nacional de Salta, Argentina.

Sesquiterpene lactones constitute a phytochemical group with relevant pharmacological properties including impairment of some mediators of inflammation (Hwang *et al.*, 1996). In this study we examined the anti-inflammatory activity of seven sesquiterpene lactones isolated from extracts of *Parthenium* species (De la Fuente *et al.*, 1997) and searched for their mode of action.

Adult female Swiss mice (25-30 g; n=6) were used. Ear thickness was measured with a micrometer before and 4 h after the application of 2.5 µg/ear of tetradecanoylphorbol acetate (TPA). The sesquiterpene lactones and indomethacin (0.5 mg/ear), dissolved in acetone were topically applied together with TPA. In the ethyl phenylpropiolate (EPP, 1 mg/ear)-test drugs were applied 16 h before challenge, using dexamethasone (0.05 mg/ear) as a standard. Ear thickness was measured 1 h after EPP. Blockage of the activity against serotonin (5-HT) induced vascular permeability in mice, by a glucocorticoid (GC) antagonist (progesterone) and m-RNA (actinomycin D) or protein synthesis (cycloheximide) inhibitors was performed (Recio *et al.*, 1995). Edema was determined by plethysmometry. Test compounds were applied *s.c.* into the dorsal area at 50 mg/kg. Percentages of edema reduction are expressed as the mean with S.E.M. Dunnet's *t*-test for unpaired data is used for statistical evaluation (** $p<0.01$).

All sesquiterpene lactones were active in the TPA-test with inhibitions ranging 61-79%. In the EPP-test, a model in which corticoid drugs are effective, confertdiolide was the most active compound with 63% edema reduction (46.0 ± 11.0**µm *vs* control, 124.3 ± 18.7 µm). It was then assayed on the blockage by the anti-GC and the m-RNA or protein synthesis inhibitors in order to explore the possible interaction with the steps involved in the mechanism of action of steroids.

Table 1. Effects of confertdiolide on 5-HT induced edema

	ΔV±SEM (ml x 10^{-2})	%I
Control (5-HT)	10.3 ± 0.7	
Confertdiolide	4.0 ± 0.4**	61
Confertdiolide + Progesterone	3.4 ± 0.5**	67
Confertdiolide + Actinomycin D	8.5 ± 0.5	17
Confertdiolide + Cycloheximide	8.5 ± 0.9	17

It is concluded that the pseudoguaianolide structure is a simple skeleton endowed with anti-inflammatory activity, independently of the substitution with the common hydroxyl or acetyl radicals and their stereochemistry. Confertdiolide exerts its effect through a mechanism related to the m-RNA synthesis and function, without interacting with GC receptor.

This work was supported by Spanish MCE (grant PM95-0150).

Hwang, D., Fischer, N.H., Jang, B.C. *et al.* (1996) *Biochem. Biophys. Res. Commun.* 226: 810-818.
De la Fuente, J.R., Novara, L. Alarcón S.R. *et al.* (1997) *Phytochemistry* 45: 1185-1188.
Recio, M.C., Giner R.M., Máñez, S. *et al.* (1995) *Planta Med.* 61: 9-12.

76P PAROXETINE DOWN-REGULATES CORTICOSTEROID RECEPTORS IN RAT BRAIN AND THYMUS

M.Maurya, A.E. Theodorou & [1]R.W Horton. Faculty of Science and Technology, NESCOT, Surrey, KT17 3DS and [1]Department of Pharmacology & Clinical Pharmacology, St. George's Hospital Medical School, London, SW17 0RE.

Some antidepressant drugs, when administered chronically, increase the density of corticosteroid receptors (CR) in the brain of adrenalectomised and non-adrenalectomised rats (Przegalinsky & Budziszweka, 1993 ; Reul *et al*, 1993,1994 ; Theodorou *et al*, 1997). In the present study we have measured CR in cortex and thymus from rats with intact adrenals following long-term treatment with paroxetine, a selective serotonin reuptake inhibitor (SSRI).

Paroxetine (5mg kg, p.o. once daily) or distilled water vehicle was administered to male Sprague-Dawley rats (~200g at the start of the study) for up to 28 days. Rats were killed 24 hours after 1, 7, 14 and 28 days of drug administration. Dissected tissues were frozen on dry ice and stored at –70°C until assayed. CR binding assays were carried out by incubating 100µL of the cytosolic fraction prepared from each of the tissues with ^{3}H-dexamethasone (concentrations ranging from 0.3-20nM) for 20-24 hours at 0-4°C. Non-specific binding was determined using 5µM hydrocortisone.

Bound and free ^{3}H-dexamethasone were separated by filtration under vacuum (Rosser *et al*, 1995). Binding parameters were determined by non-linear regression analysis to a one-site binding model. Statistical analysis was carried out using two-way ANOVA followed by Student's t-test.

A significant reduction (up to 35%) in the density of CR in paroxetine-treated rats, relative to controls, was observed in the cortex and thymus at days 14 and 28, respectively. There were no differences in Kd values between the groups in either tissue. For example, in cortex from control and paroxetine treated rats at day 14, Kd values (mean ± sem, n=7, in nM) were 2.0 ± 0.7 and 2.3 ± 0.6, respectively.

These results suggest that the concentration of CR in the brain is modified in a similar way to that of peripheral tissues. The exact mechanism by which this occurs is uncertain but drug-induced changes in corticosterone may be involved. In addition, unlike tricyclic antidepressants and MAO-inhibitors, which increase CR following chronic administration, paroxetine appears to be producing the opposite effect.

Przegalinski E. & Budzisweka B.(1993) *Neurosci.Lett.*161,215-218
Reul J.M.H.M *et al* (1993) *Endocrinology* 133, (No.1) 312-320
Reul J.M.H.M *et al* (1994) *Neuroendocrinology* 60, 509-519
Rosser D.J *et al* (1995) *Br.J.Pharmacol.* 116, Proc.Suppl.(Oct):101P
Theodorou A.E. *et al* (1997) *Br. J. Pharmacol.*123, Proc.Suppl., 236P
We would like to thank NESCOT for supporting this work, and Smithkline Beecham for providing us with paroxetine.

Table 1. Effect of repeated administration of paroxetine on ^{3}H-dexamethasone binding (Bmax values) in rat cortex and thymus.

Duration of drug administration (days)	1		7		14		28	
	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine
Cortex	101 ± 17	115 ± 13	93 ± 11	104 ± 9	114 ± 13	74 ± 3 *	101 ± 9	92 ± 7
Thymus	417 ± 30	433 ± 34	ND	ND	ND	ND	336 ± 22	256 ± 22*

Bmax (fmol/mg protein)values are mean ± sem (n=6-8). * $p<0.05$ compared to respective controls. ND = not determined.

77P DEXAMETHASONE AMELIORATES CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN RATS

A. Lecci, S. Meini, M. Tramontana, S. Giuliani, M. Criscuoli & C.A. Maggi. Pharmacology Department, Menarini Ricerche, Florence, Italy.

Kinin B_1 receptor-mediated responses are induced after tissue injury or inflammation and steroidal antiinflammatory drugs like dexamethasone (DEX) can prevent this phenomenon (Marceau, 1995). We have investigated the motor effects of i.v. administration of the selective kinin B_1 receptor agonist [des-Arg^9]-bradykinin ([des-Arg^9]-BK) on normal (CTR) or cyclophosphamide (CYP, 150 mg/kg i.p., 48 h before)-inflamed urinary bladder in urethane (1.2 g/kg, s.c.)-anaesthetized rats (male Wistar, 330-380 g).

After ureters ligation, intravesical pressure was measured through a polyethylene catheter inserted through the proximal urethra. Experiments with [des-Arg^9]-BK challenge were performed in isovolumetric conditions (0.5 ml) and bladder compliance was measured during slow saline infusion (50 µl/min), in animals subjected to acute (1-2 h), bilateral removal of pelvic ganglia. Two-way analysis of variance followed by Fisher LSD (least significant diffrence) test was used for data analysis. The dose-response to [des-Arg^9]-BK (0.5 log unit dose increase at 30 min intervals, 1-300 nmol/kg i.v.) was evaluated in ganglionectomized rats (CTR n=8 and CYP n=8) which were pretreated with DEX (1 mg/kg s.c., 49, 41, 25, 17 and 1 h before experiments, n=8) or saline (SAL, n=8).

In all groups, [des-Arg^9]-BK evoked a dose-dependent tonic contraction. In the SAL-CYP group, the amplitude of contractions was greater than in the other groups, starting from the dose of 10 nmol/kg ($P<0.01$ vs SAL-CTR and DEX-CYP), whereas no differences were observed between DEX-CTR and DEX-CYP i.e., DEX prevented the up-regulation of the contractile response to [des-Arg^9]-BK after inflammation. The amplitude of the contractile response to [ßAla^8]NKA(4-10) (3 nmol/kg, i.v.) was similar in all groups. A similar result was found in respect to the bladder weight: inflammation increased the bladder weight (86±7 n=10 vs 154±6 mg n=10, SAL-CTR vs SAL-CYP, $P<0.01$) and DEX reversed this effect without affecting the bladder weight in normal rats (76±5 n=10 vs 87±4 mg n=10, DEX-CTR vs DEX-CYP, ns). Bladder compliance was decreased in the SAL-CYP group (n=8) starting from the volume of 100 µl ($P<0.01$ vs SAL-CTR, n=8), and this effect was partially reverted by DEX pretreatment (n=8, $P<0.05$ at 150 µl and $P<0.01$ at higher volumes DEX-CYP vs SAL-CYP), although the compliance in the DEX-CYP group was lower than that recorded in the DEX-CTR group (n=8) starting from the volume of 200 µl ($P<0.05$ at 200 and 250 µl, $P<0.01$ at higher volumes). On the other hand, the compliance in the SAL-CTR group and in the DEX-CTR never differed, showing that DEX had no effect *per se* on urinary bladder compliance. The selective B_1 receptor antagonist [desArg^{10}]Hoe 140 (1 µmol/kg i.v., 5 min before the infusion, n=6) did not affect bladder compliance, thus excluding a role of B_1 receptors to increase the vesical tone during inflammation.

These results indicate that: 1) DEX pretreatment ameliorates CYP-induced bladder inflammation; 2) DEX pretreatment prevents CYP-induced up-regulation of B_1 receptor-mediated motor response; 3) B_1 receptors do not contribute to the increased vesical tone during inflammation.

Marceau, F. (1995) *Immunopharmacology* 30, 1-26.

78P INFLUENCE OF DEXAMETHASONE, HYDROCORTISONE AND PROGESTERONE ON *XENOPUS* OOCYTE MATURATION

H.L. Sudan, I.C. Kilpatrick, P.L. Needham and D.J. Heal. Knoll Pharmaceuticals, Research and Development, Pennyfoot Street, Nottingham, NG1 1GF.

Xenopus oocytes are physiologically arrested at the diplotene stage of prophase I in the late G2 phase of the first meiotic division (Tocque *et al.*, 1995). Mitogenic inducers such as progesterone (Baulieu *et al.*, 1978) or hydrocortisone (Schorderet-Slatkine, 1972) re-initiate the cell cycle towards metaphase II. This resumption of the cell cycle (maturation) is accompanied by germinal vesicle breakdown (GVBD). GVBD occurs in prophase when nuclear chromosomes condense, migrate towards the surface and push the pigment granules away to leave a white spot on the normally pigmented animal pole (Stith *et al.*, 1991). We have studied the effects of dexamethasone (DEX, a synthetic glucocorticoid) on progesterone-induced GVBD (via down-regulation of adenylyl cyclase; Baulieu and Schorderet-Slatkine, 1983) and hydrocortisone-induced GVBD (via nuclear glucocorticoid receptors; Meier, 1997).

Batches of 100, developmental stage VI, *Xenopus* oocytes (each batch defined as n=1) were taken from separate animals and maintained at 22°C to investigate GVBD as judged by the appearance of a white spot under light microscopy. Oocytes incubated in DEX (1 µM, n=10 or 10 µM, n=6) for periods of up to 60 h failed to mature but maturation was induced within 30 h by 10 µM hydrocortisone (n=9, range 18-69%). Since DEX is only slowly accumulated by the oocyte (Bronson and Stumpf, 1991), all co-application studies involving DEX used oocytes pretreated with 1 µM DEX for 24 h. GVBD normally induced by 10 µM hydrocortisone alone was significantly inhibited by DEX (Fig. 1). Exposure to 10 µM progesterone alone also promoted GVBD in oocytes within 30 h (range 14-60%; n=6). In contrast to the findings with hydrocortisone, 1 µM DEX was found to potentiate (n=1) or to have no effect (n=4) on maturation induced by 10 µM progesterone. Co-application of 10 µM hydrocortisone with 10 µM progesterone failed to increase maturation rates (n=4). Perhaps surprisingly, pretreatment for 24 h with 1 µM DEX did not inhibit GVBD observed in oocytes exposed to a 10 µM mixture of both hydrocortisone and progesterone (n=3).

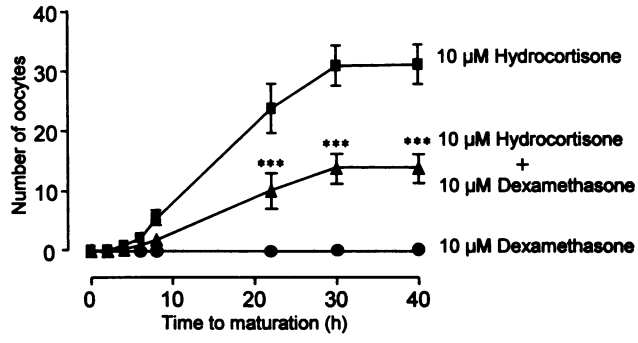


Fig. 1. Inhibition of hydrocortisone-induced GVBD by DEX. Each data point is the mean number (± s.e.mean) of maturing oocytes per 100 oocyte batch (n=8). *** = Significant inhibition ($P<0.001$) by DEX of the hydrocortisone-induced GVBD. Differences between treatments were compared using a mixed linear model followed by the least significant difference test (SAS Software suite).

These data are consistent with the concept that progesterone and hydrocortisone do not re-initiate the cell cycle via a shared signal transduction pathway that involves nuclear glucocorticoid receptors.

Baulieu, E.E., Godeau, F., Schorderet, M. *et al.*, (1978) *Nature* **275**, 593-598

Baulieu, E.E. & Schorderet-Slatkine, S. (1983) *Molecular Biology of Egg Maturation*. Pitman Books, London, pp.137-158

Bronson, D.D. & Stumpf, W.E. (1991) *Prog. Histochem. Cytochem.* **22**, 1-57

Meier, C.A. (1997) *J. Recept. Signal Transduct. Res.* **17**, 319-335

Schorderet-Slatkine, S. (1972) *Cell Different.* **1**, 179-189

Stith, B., Kirkwood, A.J. & Wohnlich, E. (1991) *J. Cell. Physiol.* **149**, 252-259

Tocque, B., Janicot, M. & Kenigsberg, M. (1995) *Methods Enzymol.* **255**, 426-435

79P EFFECT OF PROSTAGLANDIN E_2 AND KETOPROFEN ON *ESCHERICHIA COLI* 0157:H7-INDUCED TUMOUR NECROSIS FACTOR-α PRODUCTION BY HUMAN BLOOD

P. Cameron, D. Rotondo, W.H. Stimson & J. Davidson
Department of Immunology, University of Strathclyde, Glasgow, G4 0NR, Scotland

Escherichia coli O157:H7 can lead to diseases such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) and produces at least five toxins, more commonly termed verotoxins (VTs). The VTs have a cytotoxic effect on vascular endothelium *in vitro*, and induce the release of inflammatory cytokines from human monocytes (Mϕ) (van Setten *et al.*, 1996). These cytokines, particularly tumour necrosis factor-α (TNF-α), upregulate VT receptor expression on endothelial cells resulting in the damage observed in HC and HUS (van de Kar *et al.*, 1993). Prostaglandin E_2 (PGE_2) has been shown to suppress the release of pro-inflammatory cytokines (Davidson *et al.*, 1995) and non steroidal anti-inflammatory drugs (NSAIDS) inhibit the release of PGE_2 from blood Mϕ. The aim of the present study therefore, was to examine whether *E. coli* O157:H7 could induce TNF-α production by human blood and if this could be modulated by PGE_2 and NSAIDS, specifically the cyclooxygenase inhibitor ketoprofen (KP).

Experiments were performed using whole blood incubations in order to mimic the *in vivo* conditions. Blood (obtained from the Glasgow and West of Scotland Blood Transfusion Service) was incubated with either lipopolysaccharide (LPS - positive control), live or heat-killed bacteria (70°C, 30 min) and either PGE_2 or KP. All incubations were carried out in sterile Eppendorf tubes (n = 3) at 37°C, 5% CO_2, 100% humidity for 18 hours. At the end of the incubation period tubes were centrifuged (5,000g x 4 min), plasma transferred to new tubes and stored at -20°C until assayed. TNF-α was estimated using a commercially available ELISA system. All values are expressed as means ± s.d.

LPS (1mg.ml^{-1}), 1x10^6 live and 1x10^6 heat-killed *E. coli* induced TNF-α concentrations of 1496 ± 176 pg.ml^{-1}, 2584 ± 35 pg.ml^{-1} and 3570 ± 146 pg.ml^{-1} respectively. There was no detectable TNF-α in control samples incubated without LPS or bacteria or with KP or PGE_2 alone. PGE_2 (100 nM) reduced levels of TNF-α from 1496 ± 176 pg.ml^{-1} to 553 ± 290 pg.ml^{-1} ($P< 0.05$) in response to 1 mg.ml LPS, while KP (50μM) increased this value to 2,631 ± 107 pg.ml^{-1} ($P< 0.05$). KP increased the TNF-α levels from 2,584 ± 35 pg.ml^{-1} to 4,466 ± 319 pg.ml^{-1} ($P<0.05$), with PGE_2 decreasing this to 356 ± 23 pg.ml^{-1} ($P< 0.05$) when stimulated with live *E. coli*. Heat-killed *E. coli* caused a similar effect, with PGE_2 decreasing the amount of TNF-α from 3,570 ± 146 pg.ml^{-1} to 623 ± 66 pg.ml^{-1} ($P< 0.05$) and KP increasing this value to 5,539 ± 278 pg.ml^{-1} ($P< 0.05$). The results are from a single donor and are typical of at least four separate experiments.

The data obtained show that TNF-α is produced in human blood in the presence of *E. coli* O157:H7. PGE_2 suppressed the *E. coli* -induced production of TNF-α whereas KP increased the level of TNF-α. This indicates that endogenous PGE_2 is produced in response to the *E. coli* which can subsequently downregulate the production of TNF-α. The data suggest that suppression of PGE_2 by NSAIDS during infection may enhance the pathogenic actions of *E. coli* O157:H7 and hence further promote the cytotoxic actions of VTs.

van Setten, P. A. *et al.*, (1996) *Blood* **88**, 174-183.
van de Kar, N. C. A. J. *et al.*, (1993) *Blood* **80**, 2755-2764.
Davidson, J. *et al.*, (1995) *Br. J. Pharmacol.* **116**, 337.

80P CYCLIC GMP-INDEPENDENT REGULATION OF HUMAN MONOCYTIC U937 CELL PROLIFERATION BY FATTY ACIDS AND SODIUM NITROPRUSSIDE

J. Davidson, P. McAllister, L.A. Little & D. Rotondo,
Department of Immunology, University of Strathclyde, Glasgow, G4 0NR, Scotland

Arachidonic acid (AA) can act as an intracellular regulator as can other C20 fatty acids such as dihomo-γ-linolenic acid (DGLA), in addition to their conversion to a range of oxygenated metabolites such as prostaglandins (PG). PGE_2 has been shown to downregulate a wide variety of monocyte functions such as cytokine release and phagocytosis via the elevation of intracellular cyclic AMP levels. We have also shown that PGE_2-receptor-linked adenylate cyclase can control cell function in the human monocytic cell line, U937 (Loh *et al.*, 1993). AA and DGLA can upregulate U937 cell function in contrast to the actions of PGs without the requirement for conversion to PGs or the subsequent elevation of cyclic AMP (Baldie *et al.*, 1993). The aim of this study was to investigate the role of cyclic GMP in the modulation of U937 cell proliferation by AA, DGLA and sodium nitroprusside (SNP).

U937 cells were cultured as a continuous cell line in RPMI 1640/ 10 % foetal calf serum and prepared for incubations in RPMI 1640 alone. All incubations were carried out in 24-well culture plates in a final volume of 500 μl. Proliferation was estimated by incubating cells for 24 hr then adding 0.5 μCi [^{3}H] thymidine (TdR) for a further 24 hr after which the radioactivity incorporated into the acid-insoluble fraction was measured. Cyclic GMP was measured by radioimmunoassay following extraction with 65 % ethanol. The assay was calibrated using rat aorta which showed a dose-dependent increase in cyclic GMP following incubation with SNP with a maximal at 100 μM. Cells were incubated with either of the the following agents: LPS (1 μg.ml^{-1}), IL-1β (25 ng.ml^{-1}), PGE_2, (1 μM), SNP (1-100 μM), 8-bromo-cyclic GMP (8-Br-cGMP) and AA and DGLA (both 0.1 μM) at 37°C, 5 % CO_2 in air. All values are expressed as means of n = 4 ± s.d.

Initial experiments showed that the basal level of proliferation (32,335 ± 3219 cpm) was not altered in the presence of LPS or IL-1. PGE_2 decreased ($P< 0.01$) the level of TdR incorporated to 65.4 % ± that of the mean control level (100 % ± 9.9) whereas AA and DGLA increased the radioactivity incorporated to 140.2 % ± 4.4 and 136 % ± 5.1 respectively above control levels (both $P< 0.05$). The nitric oxide donor, SNP also increased the level of proliferation in a concentration-dependent manner to 165.6 % ± 5.8 that of control levels with 100 μM. However, the cyclic GMP analogue 8-Br-cGMP induced a concentration-dependent decrease in proliferation to 65.9 % ± 4.1 that of the control level ($P< 0.05$). In addition, neither SNP or the fatty acids affected the level of cyclic GMP in cells, which was below the limit of detection in control and treated incubations. Viability studies using trypan blue uptake, showed that AA and SNP increased the viability to 162.1 % ± 5.8 and 118.3 % ± 2.4 that of control levels (100 % ± 3.9, $P< 0.05$).

The data show that the fatty acids AA and DGLA and the nitric oxide donor SNP can enhance the level of proliferation of U937 cells. This does not appear to involve cyclic GMP, moreover, it appears that the increased survival of these cells in response to SNP and AA occurs via a cyclic GMP-independent mechanism.

Loh, L. *et al.* (1993) Biochim. Biophys. Acta. **1177**, 43-48
Baldie, G. *et al.* (1993) Biochim. Biophys. Acta. **1179**, 125.

81P CYCLIC GMP REGULATION OF HUMAN CD4$^+$ LYMPHOCYTE FUNCTION AND MODULATION BY FATTY ACIDS

J. Davidson, E.C. Liddle, P. Marshall & D. Rotondo, Department of Immunology, University of Strathclyde, Glasgow, G4 0NR, Scotland

A critical component of immune responsiveness is the amplification of T-cell proliferation by cytokines, particularly the stimulation of CD4$^+$ lymphocytes by interleukin-2 (IL-2) or interleukin-1 (IL-1). The fatty acids dihomo-γ-linolenic (DGLA: $C_{20:3}$) and arachidonic acids (AA:$C_{20:4}$), which are the immediate precursors of prostaglandins E_1 and E_2 respectively, have been shown to suppress cytokine-stimulated lymphocyte proliferation. However, this inhibition does not involve conversion to prostaglandins (Rotondo *et al.*, 1994) or the elevation of cyclic AMP as prostaglandin E_2 has been shown to inhibit T-cell proliferation via cyclic AMP (Davidson *et al.*, 1995). The aim of the present study was to investigate whether the suppression of the proliferation of human CD4$^+$ T-lymphocytes involves cyclic GMP.

Human CD4$^+$ lymphocytes were isolated from blood using anti-CD4$^+$ antibody coupled to magnetic Dynabeads. Cyclic GMP was measured by radioimmunoassay following extraction with 65 % ethanol. Proliferation was studied by incubating CD4$^+$ cells in RPMI 1640 medium containing 1 $\mu g.ml^{-1}$ phytohaemagglutinin, 5% (v/v) foetal calf serum. Incubations included either of the following IL-1β (25 $ngml^{-1}$), DGLA and AA (10 μM), the nitric oxide donor/ guanylate cyclase activator; sodium nitroprusside (SNP, 50 μM), the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX), 8-bromo cyclic GMP (8-BrcGMP, 50 μM) and the nitric oxide synthase inhibitor L-NMMA (50 μM). 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. Values are means of n = 4 ± s.d.

The basal level of cyclic GMP in the absence of modulators but including IBMX (100 μM) was 2.50 ± 0.26 pmol/ 1 x 10^7 cells. SNP increased the level of cyclic GMP to 171.1 % ± 12.2 that of the mean basal level of 100 % ± 10.4 ($P < 0.001$). AA and DGLA also increased cyclic GMP levels to 131.2 % ± 8.3 and 140.2 % ± 10.1 respectively (both $P < 0.05$). In the proliferation studies IL-1β enhanced the incorporation of [^{3}H]-TdR into CD4$^+$ cells to 46,773 ± 1,413 cpm from 4,438 ± 121 in controls. SNP inhibited the IL-1-stimulated response to 71.3 % ± 2.9 ($P < 0.05$). AA and DGLA inhibited the response to 20.1 % ± 6.5 and 42.5 % ± 7.5 ($P < 0.01$) respectively of the level with IL-1 alone. IBMX (1 μM) and 8-BrcGMP also inhibited the response to 67.2 % ± 4.3 and 73.1 % ± 12.6 ($P < 0.05$). L-NMMA enhanced the IL-1-induced response to 154.7 % ± 24.1 ($P < 0.05$) and reversed the DGLA-induced inhibition.

The data obtained show that the proliferation of human CD4$^+$ T-cells in response to IL-1β can be inhibited under conditions which increase intracellular levels of cyclic GMP. The effects of L-NMMA also suggests that cyclic GMP may be controlled by endogenous nitric oxide formation within the T-cell population. In addition, as the fatty acids also raise cyclic GMP it is possible that they also downregulate proliferation via cGMP.

Rotondo, D. *et al.*, (1994) Biochem. Biophys. Acta.**1223**, 185.
Davidson, J. *et al.*, (1995) Brit. J. Pharmacol.**116**, 354P.

82P TOWARDS DEFINING THE INVOLVEMENT OF TYPE 1 5-HYDROXYTRYPTAMINE RECEPTORS IN PROLIFERATION OF JURKAT T LYMPHOCYTES

S. Katugampola, I.A. Warner & K. Shaw, Pharmacology Group, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO9 3TU.

5-HT acts as an autocrine modulator of T lymphocyte activity and is essential for interleukin 2 -mediated cell proliferation. 5-HT receptors have been located and partially characterised on both mitogen-activated splenic T lymphocytes and the transformed human T lymphocyte cell line, Jurkat (Aune *et al*, 1993). 5-HT_{1A} receptor agonists such as 8-OH-DPAT can induce T cell proliferation and selective antagonists are able to reverse this effect (Aune *et al*, 1994). In Jurkat cells at least, the 5-HT_{1A} receptor appears to preferentially couple to the activation of PLC and the generation of IP_3 rather than the inhibition of adenylate cylase activity which is the most consistent functional correlate. However, the precise intracellular mechanism by which 5-HT_{1A} receptor agonists promote T cell proliferation is largely unknown. We were interested to see whether the Jurkat cell line is responsive to the novel migraine abortive drug, alniditan. This benzopyran derivative binds with high affinity to 5-$HT_{1A/1B}$ and $_{1D}$ receptors (Leysen *et al*, 1996). Since migraine can in part be characterised as the pain response to inflammation, we were interested to see whether this drug could modulate T lymphocyte activity and therefore provide evidence for a causative association between activation of 5-HT type 1 receptors and the inflammatory response.

Jurkat cells were maintained in suspension culture in RMPI-1640 medium supplemented with 10% foetal bovine serum. They were seeded at a density of 1 x 10^4 cells/ml and incubated at 37 °C in an atmosphere of 95% air and 5% CO_2. Cell growth and viability were assessed over a period of 8 days by counting cells in a haemocytometer following trypan blue exclusion. On day 4, the cells were centrifuged at 1000rpm and resuspended in fresh culture medium. On day 5, the cells were counted and alniditan dihydrochloride was added to the Jurkat cell cultures at 1000 fold the indicated final concentrations in DMSO. Data were calculated as % change in cell number (mean ± s.e. mean; n=3) on days 6 to 8 relative to control (cells incubated with vehicle alone). The effect of alniditan on the rate of cell growth on days 6 to 8 was calculated following linear transformation of the data (n=3). Where appropriate, values were analysed using the Students t-test (unpaired) where $P<0.05$ was considered significant.

Cell viability was retained at 98% during the time frame of the experiment. When compared to control, 0.1nM and 10nM alniditan significantly increased cell number on day 8 (13±1%, $P<0.001$ and 13.5±2.5%, $P<0.01$ respectively). However, 1uM alniditan significantly increased cell number on day 6 (23±1.6%, $P<0.001$), day 7 (25±1.2%, $P<0.001$) and day 8 (28±2.5%, $P<0.001$) compared to control. The rate of cell proliferation was significantly increased in the presence of alniditan provided at a concentration of 0.1nM (22.5±2% per day, $P<0.001$ compared to control), 10nM (20±4% per day, $P< 0.05$ compared to control) and 1uM (48.5±1% per day; $P< 0.001$ compared to control). Based on statistical analysis of the data, we conclude that 1uM alniditan increases both the number of cells and rate of Jurkat cell growth. However, the lack of a classical dose-dependent effect and the requirement for a relatively high concentration of the drug would imply that populations of 5-$HT_{1A/1B/1D}$ receptors which are able to promote proliferation are likely expressed at low levels. We are currently exploring these issues using non-transformed T lymphocytes.

We thank the Janssen Research Foundation, Beerse, Belgium for the kind gift of alniditan dihydrochloride. Pfizer Pharmaceuticals generously donated the Jurkat cell line.
Aune, T.A. *et al* .,(1993). *J. Immunol.*, 151(3), 1175-1183
Aune, T.A. *et al* .,(1994). *J. Immunol.*, 153(2), 489-498
Leysen, J.E. *et al* .,(1996). *Mol. Pharmacol.*, 50, 1567-1580

83P EFFECTS OF GLAUCINE ON HUMAN ISOLATED BRONCHUS AND HUMAN POLYMORPHONUCLEAR LEUKOCYTES

E.J. Morcillo, J. Cortijo, M. Martí-Cabrera, V. Villagrasa, L. Berto, J.L. Ortiz & M. Martinez-Losa. Departamento de Farmacología, Universitat de València, València, Spain.

Phosphodiesterase (PDE) inhibitors, particularly of PDE 4, offer a potential therapeutic approach to asthma. S-(+)-Glaucine is an aporphinoid alkaloid, structurally related to papaverine (a non-selective PDE inhibitor), regarded as a selective inhibitor of soluble PDE 4 in bovine aorta (Ivorra *et al.*, 1992). We examined the effects of glaucine on human bronchus and granulocyte function.

Glaucine (10nM-1mM) produced a concentration-dependent inhibition of spontaneous and histamine (0.1mM)-induced tone of human isolated bronchus (surgical specimens; isometric recording) with $-\log EC_{50}$ values of 4.58±0.04 and 4.49±0.05 (means±s.e.m.; n=12) respectively, and reached a maximal effect similar to that of theophylline (1mM). Cytosolic PDE isoenzymes were isolated from human bronchus by ion exchange chromatography followed by determination of PDE activities with a radioisotopic method. Glaucine preferentially inhibited PDE 4 (presence of 10μM SKF94120; $-\log IC_{50}$=5.47±0.06; n=5) and was about one log unit less active against calmodulin stimulated PDE activity (PDE 1) and cyclic GMP stimulated PDE (PDE 2) with marginal inhibition of other PDE activities (PDE 3 and PDE 5).

Human polymorphonuclear leukocytes (PMNs) were isolated from peripheral blood of healthy volunteers and superoxide generation was measured with a microassay of superoxide dismutase-inhibitable cytochrome c reduction in response to N-formylmethionyl-leucyl-phenylalanine (FMLP; 30nM), phorbol myristate acetate (PMA; 10ng/ml) and opsonized zymosan (OZ; 0.5mg/ml). Glaucine (1μM-1mM) inhibited FMLP-, PMA- and OZ-induced responses with $-\log IC_{50}$ values of 4.76±0.17, 3.87±0.04, and 3.60±0.07 (n=6 per group). Elastase release from PMNs stimulated with FMLP (30nM) was also inhibited by glaucine ($-\log IC_{50}$=3.53±0.03; n=5). FMLP (100nM; +20μM thimerosal) induced leukotriene B_4 (LTB_4) production that was potently inhibited by glaucine ($-\log IC_{50}$=5.85±0.07; n=6). The transient $[Ca^{2+}]_i$ response (measured as $AUC_{0\text{-}5\ min}$) evoked by FMLP (30nM) in fluo-3 (2μM) loaded PMNs was reduced by glaucine ($-\log IC_{50}$=3.93±0.06; n=5). The aggregation of human platelets induced by PMN activation (0.5μM FMLP; + 5μg/ml cytochalasin B) was inhibited by glaucine ($-\log IC_{50}$=3.43±0.05; n=5) but ADP(20μM)-induced aggregation was not affected. Glaucine inhibited cytosolic PDE 4 ($-\log IC_{50}$=5.34±0.06; n=5) and PDE 5 (<50% at 100μM) isolated from human PMNs.

Human eosinophils were purified from blood of healthy donors by a magnetic cell separation (MACS) technique to a purity ≥95%. Glaucine (up to 3mM) produced only weak inhibition of OZ (0.5mg/ml)-stimulated superoxide release (n=5) but reduced the release of eosinophil peroxidase stimulated by FMLP (1μM; + 5μg/ml cytochalasin B) with $-\log IC_{50}$=3.74±0.17 (n=5).

In conclusion, glaucine was effective *in vitro* to relax human bronchus and also inhibited granulocyte functional responses. However, the potency of glaucine in producing these effects was generally lower than would have been expected from the inhibition of PDE 4 activity measured in the cytosol of disrupted cells. Further research is required to assess the contribution of other activities of glaucine as well as the structural basis of the differences found in the pharmacological profile of the aporphines.

Supported by CICYT, MEC and *Generalitat Valenciana.*

Ivorra, M.D., Lugnier, C., Schott, C. *et al.* (1992) *Br. J. Pharmacol.*, **106**, 387-394

84P CAPSAICIN DOES NOT ALTER RAT URINARY BLADDER MOTOR RESPONSES INDUCED BY A B_1 RECEPTOR AGONIST AFTER LIPOPOLYSACCHARIDE TREATMENT

A. Lecci, S. Meini, M. Tramontana, S. Giuliani, M. Criscuoli & C.A. Maggi. Pharmacology Department, Menarini Ricerche, Florence, Italy.

Urinary bladder responses to kinin B_1 receptor agonists consist of local and reflex contractions (Lecci *et al.*, this meeting). The local contraction induced by B_1 receptor agonists is not modified by capsaicin pretreatment (Roslan *et al.*, 1995) but a possible involvement of capsaicin-sensitive afferent fibers in the reflex response cannot be excluded. In this study we investigated the effect of pretreatment with a bacterial lipopolysaccharide (LPS), a classical stimulus for B_1 receptor induction (Marceau *et al.*, 1997), on [Sar^1, D-Phe^8, des-Arg^9]-bradykinin (SDABK)-induced bladder response in urethane (1.2 g/kg s.c.)-anaesthetized rats (male Wistar, 320-380 g) pretreated with capsaicin (CAP, 50 mg/kg s.c., 5 days before) or its vehicle (VEH).

Physiological saline solution (1 ml) containing LPS (1 mg, from *E. Coli* 0.111:B4) was applied intravesically for 1 h, then it was washed out. This treatment was preceeded by 1 h intravesical application of 1 ml protamine sulfate (PS, 10 mg). This procedure has been shown to induce granulocyte infiltration in the submucosa, having a maximum at 4 h after LPS application (Stein *et al.*, 1996). Control procedure (CTR) was performed by applying saline (1 ml) instead of PS or LPS. Intravesical pressure was measured by means of a catheter inserted through the bladder dome, the preputium was clamped during PS and LPS application, and before SDABK administration (30 nmol/kg i.v., 4 h after LPS application). Three and half h after LPS, bladder was filled until the micturition reflex was evoked, then 0.15 ml of saline were withdrawn, in order to avoid reflex contractions. Data were analysed by means of two-way analysis of variance followed by Fisher LSD (least significant difference) test.

In VEH-LPS pretreated rats the threshold volume for micturition was lower than in the VEH-CTR group (248±44 vs 534±112 μl, $P<0.05$ n=15). After capsaicin pretreatment the bladder capacity was increased in both CTR- ($P<0.05$) and LPS-treated ($P<0.01$) groups and LPS-induced hyperreflexia was abolished (threshold volumes: 837±120 vs 901±96 μl, n=15 for CAP-LPS vs CAP-CTR). Administration of the selective B_1 receptor agonist SDABK produced either a local, low amplitude tonic contraction (<15 mmHg) or a tonic contraction with high amplitude (≥15 mmHg) reflex contractions superimposed (in 9, 11, 10 and 12 out of 15 preparations in VEH-CTR, VEH-LPS, CAP-CTR and CAP-LPS groups, respectively) but no differences among groups were observed in the incidence of these responses. The amplitude of the local response was increased by LPS treatment (1.4±0.3 vs 4.0±0.7 mmHg, VEH-CTR vs VEH-LPS, $P<0.01$) but capsaicin pretreatment did not modify this effect (2.3±0.4 vs 4.3±0.6 mmHg, CAP-CTR vs CAP-LPS, $P<0.05$). Likewise, the number of reflex contractions induced by SDABK in 20 min was increased after LPS treatment (1.1±0.4 vs 2.7±0.5, VEH-CTR vs VEH-LPS, $P<0.05$) irrespective of CAP pretreatment (1.3±0.4 vs 2.8±0.6, CAP-CTR vs CAP-LPS, $P<0.05$).

These results indicate that: 1) LPS induces a bladder hyperreflexia that is sensitive to CAP pretreatment; 2) B_1 receptor-mediated motor responses (either reflex or local) are enhanced after LPS treatment; 3) CAP pretreatment does not modify B_1 receptor-mediated motor response (either reflex or local).

Lecci, A., Meini, S., Tramontana, M. *et al.* (1998) This meeting.
Marceau, F., Larrivée, J.-F., Saint-Jacques, E. *et al.* (1997) *Can. J. Physiol. Pharmacol.* 75, 725-730.
Roslan, R., Campbell, E.A. & Dray, A. (1995) *Br. J. Pharmacol.* 114, 228P.
Stein, P.C., Pham, H., Ito, T. *et al.* (1996) *J. Urol.* 155, 1133-1138.

85P CHARACTERIZATION OF KININ B_1 RECEPTOR-MEDIATED MOTOR RESPONSES IN NORMAL OR INFLAMED RAT URINARY BLADDER *IN VIVO*

A. Lecci, S. Meini, M. Tramontana, S. Giuliani, M. Criscuoli & C.A. Maggi. Pharmacology Department, Menarini Ricerche, Florence, Italy.

The rat urinary bladder is one of the first *in vivo* preparations where kinin B_1 receptor-mediated responses have been described (Marceau et al., 1980), but the nature (local or reflex) of these responses has not been characterized. We have investigated the motor effects of i.v. or topical (onto the bladder serosa) administration of the selective kinin B_1 receptor agonist [des-Arg^9]-bradykinin ([des-Arg^9]-BK) on normal or inflamed urinary bladder in urethane (1.2 g/kg, s.c.)-anaesthetized rats (male Wistar, 330-380 g).

Urinary bladder inflammation was induced by cyclophosphamide (150 mg/kg i.p., 48 h before). After ureters ligation, intravesical pressure was measured by means of a polyethylene catheter inserted through the proximal urethra. Experiments were performed in isovolumetric conditions with the bladder filled with a volume of saline subthreshold for elicitation of micturition contractions (normal: 447±55 µl n=25, inflamed: 165±25 µl n=25, P<0.01). Increasing doses (1 log unit) of [des-Arg^9]-BK were administered at 20 min intervals, after a 1 h stabilization period. Some experiments were carried out in animals subjected to acute (1-2 h) bilateral removal of pelvic ganglia. Student's t test or analysis of variance followed by Tukey test were used for data analysis.

[Des-Arg^9]-BK (0.001-100 nmol/kg, i.v.) produced a tonic contraction of low amplitude (<15 mmHg) with phasic contractions of high amplitude (≥15 mmHg) superimposed (micturition reflex contractions, MRC) in both normal (n=10) and inflamed bladders (n=10). However, after inflammation, the response to [des-Arg^9]-BK was more prominent: the number of MRC determined in 20 min at the highest dose of [des-Arg^9]-BK was 3.9±1.2 in normal and 11.7±2.2 in inflamed rats (P<0.05). The ED_{50} (Lichtfield-Wilcoxon) of the incidence of MRC was similar in the two groups. These findings were replicated after the topical administration of [des-Arg^9]-BK (0.05 pmol-5 nmol/rat): the incidence of MRC was similar in the two groups whereas the number of MRC was larger after inflammation (at 5 nmol/rat: 8.9±1.1 n=15 vs 3.3±1.0, n=15, P<0.05). In ganglionectomized rats, the response to [des-Arg^9]-BK (100 nmol/kg, i.v.) or [ßAla^8]NKA(4-10) (3 nmol/kg, i.v.) was evaluated at 30 and 240 min after the set up. In both groups (normal n=6 and inflamed n=6) [des-Arg^9]-BK and [ßAla^8]NKA(4-10) induced a tonic contraction only. The response to [des-Arg^9]-BK was greater after inflammation (at 30 min: 0.3±0.1 vs 8.4±2.2, P<0.01; at 240 min: 2.2±0.4 vs 17.7±2.4 mmHg, P<0.01 normal vs inflamed) although a time-dependent increase was evident in both groups (30 vs 240 min, P<0.05). The response to [ßAla^8]NKA(4-10) was similar in both groups and constant over the time. After inflammation, the tonic contraction induced by [des-Arg^9]-BK (100 nmol/kg i.v.) in ganglionectomized rats was dose-dependently reduced by the selective B_1 receptor antagonist [desArg^{10}]Hoe 140. The % inhibition observed at 10 min from antagonist administration was 59 (n=5, P<0.01), 78 (n=5, P<0.01) and 95% (n=5, P<0.01) of the time-matched control response (n=5) at doses of 0.1, 0.3 and 1 µmol/kg, respectively. [DesArg^{10}]Hoe 140 (0.3 and 1 µmol/kg, i.v.) induced *per se* a small contractile response averaging 15% of the response observed with the agonist. The contractile response (number of MRC) induced by [des-Arg^9]-BK in normal, intact rats 4 h after the set-up was not changed after Hoe 140 (30 nmol/kg i.v., 10 min before, n=6), a selective B_2 receptor antagonist.

These results indicate that stimulation of bladder B_1 receptors evokes a local, tonic-type contraction with reflex contractions superimposed in both normal and inflamed bladders, but in the latter situation the motor responses are magnified.

Marceau, F., Barabé, J. St-Pierre, S. *et al.* (1980) *Can. J. Physiol. Pharmacol.* 58, 536-542.

86P COMPARISON OF THE EFFECTS OF SHORT-TERM ETHANOL CONSUMPTION ON ADIPOSE TISSUE LIPOGENESIS IN TO AND CBA MICE

M. Barton, P. Jelic, M-F. Shih & P.V.Taberner, Department of Pharmacology University of Bristol, University Walk, Bristol BS8 1TD, U.K.

Chronic alcohol consumption (24% v/v in drinking water) has been shown to ameliorate the obesity and diabetes syndrome in mature male CBA mice by reducing body mass, food intake and blood glucose but without decreasing overall energy intake (Al Qatari, Shih & Taberner, 1996). This treatment schedule, which produced diurnal plasma ethanol levels of between 1.5 and 12 mM had no effect in lean littermates. To determine whether the effect of ethanol was dose or strain dependent we have compared the effect of a 7% (v/v) ethanol liquid diet (ELD) on adipose tissue lipid metabolism in normal (lean) CBA and TO mice. This treatment schedule produces higher diurnal plasma ethanol levels; between 5 and 100mM (Jelic, Shih & Taberner, 1998).

Adult male CBA and TO mice, housed 6 per cage, were given isocaloric (0.4 kcal/ml) control liquid diet or ELD for 7 days on a pair-feeding schedule. Body mass and ethanol intake were monitored daily. On day 7 brown (BAT) and white epididymal (WAT) adipose tissue weights and total lipid content were measured and *in vivo* lipogenesis (expressed as μg atoms H h^{-1} mg $tissue^{-1}$) estimated from the incorporation of [^{3}H] into total tissue lipid 1h after administration of [^{3}H]-H_2O (Mercer & Trayhurn, 1983). Data are shown as means ± s.e.mean of (n).

Control fed mice showed no significant weight change over the 7 days, whereas the ELD mice lost weight: CBA -1.92 ± 0.36 g (12) (P< 0.01, paired t-test), TO -5.75 ± 0.37 g (12) (P< 0.001). The pair-feeding meant that overall energy intake was the same between the control and ELD groups (500 kcal kg^{-1} on day one, falling to around 300 kcal by day 7). The daily ethanol consumption ranged between 25 -30 g kg^{-1} in CBA mice and 23 - 27 g kg^{-1} in TO mice.

Table 1	Control CBA	Control TO	ELD CBA	ELD TO
BAT Lipogenic rate	112 ± 18 (12)	74.4 ± 6.9 (12)	89.0 ± 9.0 (6)	*55.6 ±4.0 (6)
% lipid content	33.1± 0.34 (12)	51.8 ±4.2 (12)	29.1 ± 4.1 (6)	§28.2±4.5 (6)
WAT Lipogenic rate	32.9 ± 4.1 (12)	12.4 ± 3.7 (11)	§12.5 ±1.9 (6)	§ 0 (6)
% lipid content	79.5 ± 3.5 (12)	72.8 ± 3.1 (11)	*61.6 ±6.6 (6)	§ 0 (6)

* P < 0.01 vs control, § P < 0.001 vs control, unpaired t test

The effects of ethanol on lipid content and lipogenic rates of BAT and WAT are shown in Table 1. Ethanol suppressed both BAT and WAT lipogenesis in TO mice which appeared more sensitive to ethanol than the CBA. In TO mice WAT was non-existent after ELD. The loss of WAT was consistent with the greater fall in body weight in these mice, although pair-fed controls did not lose body mass or epididymal WAT.

P. Jelic was supported by an MRC studentship

Al Qatari, M., Shih, M-F. & Taberner. P.V. (1996) *Alcohol & Alcoholism*, 31, 89-99
Jelic, P., Shih, M-F. & Taberner, P.V. (1998) *Psychopharmacology*, in press
Mercer, S.W. & Trayhurn, P. (1983) *Biochem. J.* 212, 393-398
Shih, M.F. & Taberner, P.V. (1997) *Br. J. Pharmacol.* 120, 721-727.

87P EFFECTS OF THE IMIDAZOLINE S-22068 ON *IN VIVO* GLUCOSE UTILIZATION AND HEPATIC GLYCOGEN SYNTHESIS IN MICE

M-F. Shih, S. Slough, C.A.Williams & P.V. Taberner, Department of Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD, U.K.

The imidazoline compound S-21663 (PMS 812), which has very low affinity for α-adrenoceptors and both I_1 and I_2 sites, has been shown to improve glucose tolerance and stimulate insulin secretion in streptozotocin-diabetic rats (Wang *et al.*, 1996). We have previously reported that the related compound S-22068 (1,4-di-isopropyl-2-(4,5-dihydro-1-H imidazol-2-yl) piperazine) improves glucose tolerance but without stimulating insulin secretion or producing hypoglycaemia in CBA mice (Shih *et al.*, 1997). In this study we have investigated the possible sites of action of S-22068 by measuring peripheral tissue glucose utilization and hepatic glycogen synthesis after acute S-22068, and body weight and food intake during sub-acute dosing.

Adult male CBA mice (30-34 g), housed 4 to a cage during the sub-acute dose schedule, were given p.o. S-22068 (24 mg kg^{-1}), saline, or gliclazide (2 mg kg^{-1}) as a positive control acutely, or daily for 14 days (sub-acute). During sub-acute dosing body weights and food intake were monitored every 24 or 48h, plasma triglyceride levels were measured on day 15. Glucose utilization: after an overnight fast, drug or saline was given 60 min prior to 2.5 μCi [^{3}H]-2-deoxyglucose (2-DG). Brown and white adipose tissue, liver, and soleus muscle samples were taken 40 min later for estimation of uptake rates (Meszaros *et al.*, 1987). [^{14}C]-glucose incorporation into liver glycogen was estimated similarly following extraction of glycogen from the tissue. Differences between groups (shown as means ± s.e.mean of n) were analysed by Student's unpaired t test or 2-way ANOVA.

Neither S-22068 nor gliclazide given sub-acutely had any effect on the body mass or 48h food intake (kJ kg^{-1}) of the mice compared to saline treated controls (ANOVA). Plasma triglyceride levels (mmol l^{-1}) in fed mice were significantly higher than in fasted (1.47 ± 0.08, control: 0.71 ± 0.04 (n = 13, $P < 0.001$)), but sub-acute S-22068 had no effect under either condition (fed: 1.49 ± 0.12; fasted: 0.70 ± 0.07 (n=8)).

2-DG uptake (nCi g^{-1} tissue) into liver was increased significantly by acute gliclazide (2.07 ± 0.26 (6); control 1.43 ± 0.16 (6), $P < 0.01$) but unaltered by S-22068 (1.50 ± 0.09 (6)). In the same animals, 2-DG uptake into brown and white fat and soleus muscle was not significantly altered by either drug. Hepatic glycogen synthesis *in vivo* (nCi g^{-1} tissue) was increased by S-22068: 0.803 ± 0.216 (8), control: 0.392 ± 0.063 (7), although this was not statistically significant. Glycogen synthesis was significantly increased by gliclazide: 0.758 ± 0.151, $P< 0.05$ (7).

We conclude that S-22068, at an oral dose which is equipotent with the sulphonylurea gliclazide in terms of improving glucose tolerance, is not acting to increase muscle glucose uptake but may increase hepatic glycogen synthesis or gluconeogenesis.

C.A.W. is a Daphne Jackson Fellow. We are grateful to I.R.I.S. for their support for this research and for supplying S-22068.

Meszaros, K. *et al.*, (1987) *J.Biol.Chem.*. 262: 10965-10970
Shih, M-F. *et al.*., (1997) *Br.J.Pharmacol.*. 122: 54P
Wang, X. *et al.*., (1996) *J.Pharmac.Exp.Ther.* 278: 82-89

88P NON-SELECTIVE POTASSIUM CHANNEL INHIBITION REDUCES SECRETORY MORE THAN ABSORPTIVE MECHANISMS IN HUMAN ILEAL MUCOSA

D.E. Burleigh, Department of Pharmacology, Queen Mary and Westfield College, London E1 4NS.

In secretory diarrhoea the primary event driving fluid secretion is a transcellular, electrogenic, serosal to mucosal transport of chloride ions. Such transport requires the maintenance of an electrically negative cell membrane voltage which is achieved through a basolateral outward leakage of potassium ions. It follows that potassium channel inhibitors may provide new opportunities for treatment of acute secretory diarrhoeal disease. However, absorptive mechanisms are also thought to be dependent on a basolateral efflux of potassium ions (Dawson & Richards, 1990). Therefore non-selective blockade of potassium channels may compromise sodium-coupled glucose transport a process which underpins oral rehydration therapy. The aim of this investigation is to determine whether non-selective potassium channel blockade by barium has an anti-secretory effect in human ileum and whether absorptive mechanisms may also be affected. Normal human ileum was obtained from patients undergoing right hemicolectomy operations for bowel cancer. This procedure was approved by the Research Ethics Committee of the City and East London Health Authority. Muscle stripped sheets of ileal mucosa were set up Ussing chambers for recording of short-circuit current (Isc) as previously described (Burleigh & Borman 1993).

Mucosal application of Escherichia Coli heat-stable enterotoxin (STa, 0.15-15 nM, n=4) and D-glucose (1-24 mM, n=5) produced concentration-dependent increases in Isc of 2±1 to 50±8 and 16±3 to 108±11 μA.cm^{-2} respectively. Mannitol (24 mM, n=4) given mucosally did not increase Isc. For evaluation of sensitivity to barium, a non-selective K^+ channel blocker, concentrations of STa (5nM) and glucose (12mM) were chosen which produced maximal responses. Serosal application of barium (30 min preincubation time) reduced responses to both STa and glucose. For STa, barium (0.2-5mM, n=6) caused 33±10 to 94±3% reduction. For glucose, barium (1-25mM, n=6) caused 7±10 to 35±7% reduction. Responses to STa were not significantly reduced by serosal application of glibenclamide (10 μM, n=6, $P>0.05$) or phentolamine (100 μM, n=3, $P>0.05$). Parallel time-matched control experiments showed no significant change in responses to STa (n=6, $P>0.05$) or glucose (n=6, $P>0.05$) after exposure to aqueous vehicle. At one concentration barium (1 mM, n=12, $P<0.05$) significantly increased basal Isc by 20±5 μA.cm^{-2}, however the effect was not concentration-dependent in the range tested. Data expressed as mean ±S.E.M. and analysed using the Mann Whitney U-test for unpaired data.

In conclusion, basolateral efflux of K^+ ions is required for the secretory effect of E. Coli STa in human ileal mucosa. Absorptive mechanisms appear to be far less sensitive to K^+ channel block. Preliminary studies indicate that, unlike acetylcholine induced secretion in rat colon (Darko *et al.*, 1997), K_{ATP} channels are not involved in the STa secretory effect in human ileum.

My thanks to the surgeons for providing specimens of bowel.

Burleigh, D.E. & Borman, R.A. (1993) *Eur. J. Pharmacol.* 241, 125-128.
Darko, S.K.A., Palmerley, N.D. & Burleigh, D.E. (1997) J. Pharm.Pharmacol. 49, 985-987.
Dawson, D.C. & Richards, N.W. (1990) *Am. J. Physiol.* 259, C181-C195.

89P T_{84} CELLS, IN CONTRAST TO HUMAN COLONIC MUCOSA, DO NOT RESPOND TO 5-HYDROXYTRYPTAMINE WITH AN INCREASE IN SHORT-CIRCUIT CURRENT

D.E. Burleigh, Department of Pharmacology, Queen Mary and Westfield College (QMWC), London E1 4NS.

T_{84} cells, in contrast to human colonic mucosa, do not respond to 5-HT with an increase in short-circuit current (Dharmsathaphorn *et al.*, 1984; Borman & Burleigh, 1996). One explanation is that experimental conditions were not optimal, as sensitivity of T_{84} monolayers to 5-HT was assessed only 40-56 hours after seeding the cells on semi-permeable supports (Dharmsathaphorn *et al.*, 1984). The aim of this investigation was to establish whether more mature T_{84} monolayers develop sensitivity to 5-HT. T_{84} cells were grown as monolayers in a 1:1 mixture of DMEM & Ham's F-12 media supplemented with foetal bovine serum (10%) L-glutamine (2 mM) penicillin (100 units ml^{-1}) and streptomycin (100 $\mu g\ ml^{-1}$). Following trypsinisation cells (passage 46) were seeded onto semi-permeable membranes (Costar Snapwell inserts, 0.4 μM pore diameter and 1.1 cm^{-2} surface area) and maintained for a variable length of time. The insert with attached monolayer was then placed into a modified Ussing chamber for continuous recording of short-circuit current (Isc). Drugs were added basolaterally and no monolayer received more than one concentration of a given drug. Statistical analysis used the Mann-Whitney U test, with $P < 0.05$ taken to indicate a significant difference. Data is expressed as mean ± s.e. mean and 'n' represents number of monolayers.

Overall basal Isc was 1.0±0.3 μA and transepithelial resistance was 441±17 Ω (n=54) after an equilibration period of 30 mins. Basal Isc was not affected by duration of culture whereas a maximum resistance of 538±35 Ω was obtained after 7 days (n=12). 5-HT (1-100 μM) had no significant effect on basal Isc (0.3±0.2 to 0.2±0.2 μA, n=6, P>0.05). There was no apparent difference between responsiveness of monolayers cultured for 2, 7 & 14 days. Forskolin (25 μM) tested on the same monolayers produced a rise in Isc of 65±9 μA (n=9) while DMSO vehicle was without effect (n=4). In contrast to 5-HT, acetylcholine (1-100 μM, n=6), tested on 6-9 day old monolayers produced a significant concentration-dependent increase in Isc ranging from 4.0±0.9 to 11.8±1.6 μA (P<0.05).

Despite the fact that T_{84} cells were grown as monolayers for periods up to 14 days, electrogenic responses could not be obtained to 5-HT. This is unexpected when one considers that T_{84} cells are derived from a human colonic mucosal tumour and 5-HT increases Isc of human colonic mucosa by a non-neural, action. As the cells were sensitive to acetylcholine, which like 5-HT_2 receptor stimulation acts via the phosphoinositide second messenger system, it is concluded that 5-HT receptors may not be expressed or if present are not functionally active.

Financial support from QMWC is gratefully acknowledged.

Borman, R.A. & Burleigh, D.E. (1996) *Eur. J. Pharmacol.* 309, 271-274.
Dharmsathaphorn, K., McRoberts, J.A., Mandel, K.G. *et al.* (1984) *Am. J. Physiol.* 246, G204-G208.

90P INVESTIGATION OF THE CONTRACTILE EFFECT OF 2-METHYL-5-HT ON THE TERMINAL REGION OF *SUNCUS MURINUS* INTESTINE

F.A. Javid & R.J. Naylor, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP.

In previous studies (Javid *et al.*, 1996, 1997) we have shown the involvement of 5-HT_2 receptors in mediating the 5-HT induced contraction response in different regions of the *Suncus murinus* intestine. The aim of the present studies is to investigate the involvement of 5-HT3 receptors using 2-methyl-5-HT in the terminal region of the *Suncus murinus* intestine.
Segments (1-1.5 cm length) taken from the intestine (1-2 cm proximal to the anal region) of adult Japanese House Musk shrew, *Suncus murinus* (33-94 g) of either sex were mounted in 10 ml organ baths containing Krebs' solution (37°C, 95% O_2, 5% CO_2). The tissues were allowed to equilibrate for 60 min and washed every 20 min. The resting tension was maintained at 0.5 g and contractions were recorded isometrically. Non-cumulative concentration-response curves to 2-methyl-5-HT (0.1-30μM) were established with a 1 min contact time and 22 min intervals. The procedure was repeated in the presence of ondansetron (0.01, 0.1, 1μM), atropine (1μM), methysergide (1μM) and SB204070 (1nM) (Wardle *et al.*, 1994). The control responses to 2-methyl-5-HT alone and in the presence of antagonist were established using a randomised experimental design. Tension changes were expressed as the mean±s.e.mean of n=6 and analysed using one-way ANOVA followed by Bonferroni-Dunnett's t-test.
2-methyl-5-HT (1-30μM) produced a concentration-dependent contraction curve. Ondansetron at 0.01μM caused a competitive antagonism whereas higher concentrations 0.1 or 1μM caused a non-surmountable reduction in the contractions induced by 2-methyl-5-HT. Atropine (1μM) significantly ($p<0.05$) reduced the response to 2-methyl-5-HT. Methysergide (1μM) and SB204070 (1nM) administered alone or in combinaton failed to signifcantly modify the contractions induced by 2-methyl-5-HT. (Figure 1).

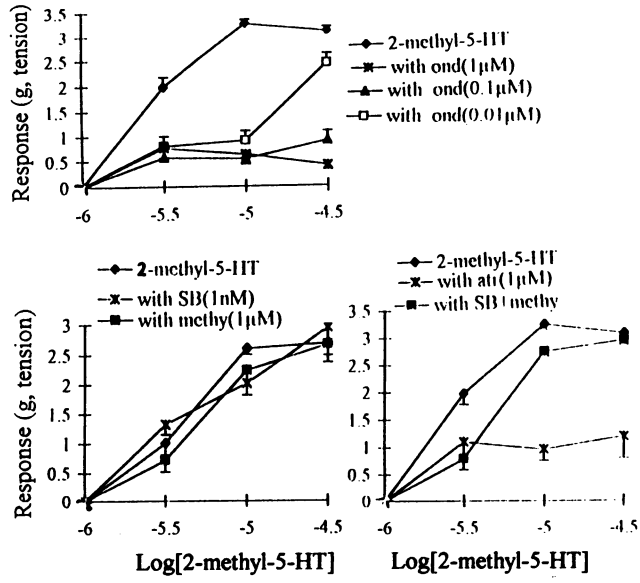


Figure 1. The contractile effect of 2-methyl-5-HT and its antagonism in the terminal region of *Suncus murinus* intestine. The data suggest that 5-HT3 receptors can mediate a contraction response in the terminal region of the *Suncus murinus* intestine and that the responses are mediated via the release of acetylcholine which has been reported in other tissues (Buchheit *et al.*, 1985a).

Buchheit *et al.*, (1985a). J.Pharm. Pharmacy, 37, 664-667.
Javid, F.A. *et al.* (1996) *Br.J.Pharmacol.* 119, 197P.
Javid, F.A. *et al.* (1997) *Br. J. Pharmacol.* 120, 193P.
Wardle, K. A. *et al.* (1994) *Br. J. Pharmacol.* 112, 789-794.

91P ENDOTOXIN-INDUCED INHIBITION OF GASTRIC ACID SECRETION INVOLVES ACTIVATION OF OXYTOCIN RECEPTORS IN THE BRAIN

M.D. BARRACHINA, S. CALATAYUD, B. BELTRÁN, A. CANET, J.V. ESPLUGUES. Dept. of Pharmacology, University of Valencia, Valencia, Spain.

The endogenous release of different peptides is involved in stress-induced modulation of gastrointestinal function. Administration of low doses of endotoxin protects gastric mucosal integrity (Uehara *et al.*, 1990) and inhibits gastric acid secretion (Barrachina *et al.*, 1995), although the mechanisms are still controversial. The aim was to analyze the effects of three peptides released by stress, oxytocin, vasopressin and CRF on distension-stimulated gastric acid secretion and the endogenous role of these mediators in the acute inhibitory effects of low doses of endotoxin on gastric function. Sprague-Dawley (220-280g) rats were anaesthetized with urethane (1.5 g kg^{-1}, i.p.), the stomachs continuously perfused with saline (0.9 ml min^{-1}) and acid output measured. Oxytocin (0.2, 2 or 4 nmol rat^{-1}, i.c.), vasopressin (0.1, 2 or 5 nmol rat^{-1}, i.c.), CRF (0.5, 1 or 2 nmol rat^{-1}, i.c.) or endotoxin (E coli LPS, 5 µg kg^{-1}, i.v.) were administered 60 min after collecting basal secretion and 15 min before distension of the stomach with an intragastric pressure of 20 cm H_2O. In some experiments animals received, 15 min before endotoxin, a single injection of the antagonists of oxytocin [Compound VI(d(CH2)5...)OVT, 1µg rat^{-1}, i.c. or i.v.], CRF [α-Helical CRF (9-41), 20 µg rat^{-1}, i.c.] or vasopressin [ß-mercapto-ßß-cyclopentamethyl-enepropionyl, O-Et-Tyr2, Val4, Arg8)-vasopressin, 20 µg rat^{-1}, i.c.). Net gastric acid secretion (Δ) induced by distension (44.1±10.6 ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=10) was reduced (%) in a dose-dependent manner by oxytocin (25.7±26.6%, n=4 ; 55.1±14.1%*, n=17 and 81.2±3.5%*, n=5) or CRF (65.3±25.8%*, n=3, 77.1±13.0%*, n=6 and 85.9±7.6%*, n=12). However, similar doses of vasopressin (0.1, 2 or 5 nmol rat^{-1}, i.c.) failed to significantly modify acid output (-19.9±16.3%, n=3, 37.9±32.3%, n=3 and 33.6±20.1%, n=6, respectively). Endotoxin-induced inhibition (11.8±1.9*, ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=10) of distension-stimulated gastric acid secretion (27.3±7.3 ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=9) was prevented by i.c. administration of oxytocin antagonist (29.1±9.4[&] ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=5). However, when administered i.v., the antagonist failed to modify the antisecretory effect of endotoxin. Pretreatment with either CRF antagonist (13.2±5.9 ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=7) or vasopressin antagonist (5.2±3.2 ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=5) did not significantly reverse the inhibitory effects of endotoxin (11.8±1.9* ΔµEq H^+ $100g^{-1}$ $90min^{-1}$, n=10). In conclusion, oxytocin or CRF, but no vasopressin act in the brain to inhibit distension-stimulated gastric acid secretion. However, only activation of central oxytocin receptors is involved in endotoxin-induced inhibition of acid output.

*p<0.05 vs the respective vehicle-treated group

[&]p<0.05 vs endotoxin-treated group

Barrachina, M.D., Whittle, B.J.R., Moncada, S. *et al.* (1995) *Br. J. Pharmacol.*, **114**, 8-12.

Uehara, A, Okumura, K, Okamura, Y. *et al.* (1990) *Eur. J. Pharmacol.*, **181**, 141-145.

92P MODULATION BY NSAIDs OF GASTRIC NITRIC OXIDE SYNTHESIS

M.C. RAMÍREZ, S. CALATAYUD, B. BELTRÁN, M.D. BARRACHINA, J.V. ESPLUGUES.
Dept. of Pharmacology, University of Valencia, Spain.

NSAID-related gastroduodenal injury has been classically associated to cyclooxygenase inhibition (Masferrer *et al.*,1994; Hayllar *et al.*, 1995). Recent studies show similarities between Cox and NO synthase and this raise the possibility that NSAIDs could have an effect on NOS activity (Salvemini *et al.*, 1993). The aim was to determine whether NSAIDs modulate the constitutive NO synthase activity (cNOS) in gastric mucosal homogenates of rats. Stomachs were obtained from Sprague-Dawley rats and homogeneized in buffer containing DL-dithiotreitol (1 mM), leupeptin (10 µg.ml^{-1}), soybean trypsin inhibitor (10 µg. ml^{-1}) and aprotinin (2 µg.ml^{-1}). After centrifugation (10 000 g, 20 min, 4° C), the supernatant was aliquoted and frozen (-80° C). Experiments were performed in assay buffer (pH 7.4) containing L-valine (6 mM); NADPH (1 mg.ml^{-1}); $MgCl_2$ (1 mM) and $CaCl_2$ (200 µM) and NSAIDs.The concentrations of NSAIDs were chosen from preliminary dose-response study. Samples were preincubated for 30 min at 37° C and NO-synthase activity was estimated from the conversion of L-(^{14}C)-arginine to NO coproduct citrulline as described previously (Knowles *et al.*,1990).

Constitutive Ca^{2+}- dependent NOS activity was defined as the difference between the activity of gastric homogenate with and without 1mM EGTA in the assay buffer in preliminary experiments.

NSAIDs (min. concent. with action on cNOSactivity)	% CHANGE in cNO- synthase activity vs control
Acetyl salycilic acid (ASA) (0.5 mM)	- 23 ± 6**
Indomethacine (1mM)	- 35 ± 4*
Diclofenac (0.5mM)	- 80 ± 2***
Flurbiprofen (0.1mM)	+ 21 ± 3*
Carprofen (0.1mM)	+ 33 ± 5*
Piroxicam (0.1, 0.5, 1mM)	no effect
Paracetamol (0.1, 0.5, 1mM)	no effect

(means ± S.E.M. of at least 5 independent experiments with 3 replicates in each)).

*$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ vs respective vehicle.*

In conclusion, the NSAIDs evaluated show different pattern of action on constitutive NO synthase activity and this may be related to their ability to induce gastric damage.

Somasundaram, S., Hayllar, J., Rafi, S. et al. (1995). *Scand. J. Gastroenterol.*, **30**, 289-299.

Hayllar, J., Bjarnason, I. (1995). *Lancet*, **346**, 521-522.

Salvemini, D., Misko, T.P., Masferrer, J.L. et al. (1993). *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 7240-7244.

Knowles, R.G., Merret, J., Salter, J. et al. (1990). *Biochem. J.*, **270**, 833-836.

93P PORTAL HYPERTENSION MODULATES ASPIRIN-INDUCED GASTRIC INJURY

S.CALATAYUD, M.C.RAMÍREZ, M.J.SANZ, J.BOSCH, J.M.PIQUÉ, J.V.ESPLUGUES. Dept. of Pharmacology, University of Valencia, Spain.

The development of gastric lesions following aspirin administration is related to a reduction in gastric blood flow (Kauffman et al., 1980). Portal hypertension during cirrhosis results in a marked hyperemia in the gastric mucosa, an effect mediated by nitric oxide (NO) (Casadevall et al., 1993). The aim was to analyse the influence of portal hypertension, and the implication of NO, on the gastrolesive effect of aspirin. Portal hypertension was induced in Sprague-Dawley rats by partial portal vein ligation (PPVL). Sham operated rats (SO) were used as controls. The experiments were carried out 14 days later. Animals received a gastrolesive dose of aspirin (200 mg/kg in HCl 0.2N, p.o.) and were killed three hours later. Gastric emptying of the non absorbable marker phenol red was evaluated. Fifteen minutes before aspirin, some animals were treated with the NO synthesis inhibitor L-NAME (3mg/kg, i.p.) or with the vasopressin analogue terlipressin (0.5 µg/kg, i.p.). Injury was evaluated macroscopically as % of gastric mucosal area damaged. In some experiments, gastric bleeding was assessed by measuring Cr^{51}-labelled erythrocytes in the gastric lumen. Aspirin induced gastric damage (5.7±1.1, n=11) was significantly reduced in portal hypertensive animals (1.4±0.2, n=10, $p<0.05$). There was no difference in gastric emptying between PPVL and SO rats. Pre-treatment with L-NAME restored the damaging effect of aspirin in PPVL rats (5.0±1.1, n=10, $p<0.05$) without modifying the index lesion in control animals (5.4±2.1, n=7). Administration of the vasoconstrictor terlipressin did not modify aspirin induced damage in portal hypertensive rats. Despite the difference in the area of damage, gastric bleeding was similar in PPVL and SO rats and this reflects an increased blood leakage by damaged area unit in PPVL rats (see table)

n=6 both	gastric damage (D) (%total area)	gastric bleeding (B) (µl red blood cells)	B/D
sham	4.6±1.2	4.4±0.6	1.0±0.2
PPVL	1.9±0.3 *	4.1±0.5	2.4±0.4 *

*p<0.05 vs respective sham response

In conclusion, the gastric mucosa of portal hypertensive rats is less sensitive to the damaging actions of aspirin and this effect seems to be related to an increased synthesis of NO. However, despite the reduction in the area of damage the gastric lesions present in these animals show an augmented bleeding per area of injury.

Casadevall, M., Panés, J., Piqué, J.M. et al. (1993) *Hepatology*, **18**, 628-634.
Kauffman, G.L., Aures, D., Grossman, M.I. (1980) *Am. J. Physiol.*, **238**, G131-134.

94P INTESTINAL ANTIINFLAMMATORY ACTIVITY OF UR-12745 ON A RAT MODEL OF COLITIS

Julio Gálvez, Margarita Garrido, [1]Manuel Merlos and Antonio Zarzuelo. Dept. Pharmacology, Univ. Granada, Spain. [1] J. Uriach & C[a] Research Center, Dept. Pharmacology, Barcelona, Spain.

The aim of this study was to evaluate the intestinal antiinflammatory activity of UR-12746 in the acute phase of trinitrobenzene sulfonic acid (TNBS) model of colitis in rats. UR-12746 is a novel locally-acting antiinflammatory compound with PAF antagonist activity.

Colonic inflammation was induced by administration to rats of a single intracolonic dose of 30 mg of TNBS dissolved in 0.25 ml of 50% ethanol (Morris *et al.*, 1989). Animals were divided into five groups, and three of them (n=8) were treated with UR-12746 at different doses: 25, 50 and 100 mg/kg. UR-12746 was suspended in 1% (w/v) methylcellulose and administered orally (vol: 1 ml) and daily for five days before colitis induction as well as one one day after. A TNBS control group (n=12) and a saline group (n=12) were also included for reference; animals from both groups were given 1 ml of 1% (w/v) methylcellulose orally. All animals were sacrificed 48 h after colitis induction with an overdose of urethane and the entire colon was removed, opened and rinsed. The colonic inflammatory status was evaluated both macroscopically and biochemically. Colonic macroscopic damage was scored on a 0 to 10 scale according to the criterion previously proposed by Bell *et al.* (1995) and the weight/length ratio was determined for each specimen. The colonic biochemical parameteres assayed were: myeloperoxidase (MPO) activity, total glutathione (GSH) content and leukotriene B_4 (LTB_4) synthesis.

The results are summarized in the Table. The colon of the TNBS treated animals showed diffuse hemorrhagic necrosis and bowel wall thickening, typically extending 5 to 6 cm along the colon. Pretreatment with UR-12746 to colitic animals was able to significantly reduce the acute inflammatory response induced by the colonic administration of TNBS. In this way, UR-12746 decreased the colonic weight/length ratio, at doses of 50 and 100 mg/kg, compared to control animals. In addition, the highest dose assayed was also able to reduce the area of hemorrhagic necrosis in the colonic mucosa by 20%, as it was shown by the lower macroscopic score compared to control animals. The beneficial effect exerted by UR-12746 was associated with a lower MPO activity, a marker of neutrophil infiltration of the damaged tissue. This acute antiinflammatory effect could be related, at the highest dose assayed, with its ability to reduce colonic glutathione depletion, which resulted in a protection against oxidative insult, and/or with an inhibition in colonic LTB_4 synthesis, since LTB_4 has been considered as an important proinflammatory mediator in colonic inflammation. However, other mechanisms may be involved, since lower doses of UR-12746 (50 mg/kg), which also displayed antiinflammatory activity, showed no significant effect on glutathione depletion or LTB_4 synthesis.

Group	Score (0-10)	Weight (mg/cm)	MPO (U/g)	GSH (nmol/g)	LTB_4 (ng/g)
Saline	0	81.5±2.0	63±8	1350±54	1.0±0.4
Control	8.5 (3)	164.4±7.0	899±97	627±42	6.5±0.6
25 mg/kg	8 (3)	160.9±9.4	967±1.4	613±20	4.8±0.8
50 mg/kg	8.5 (2)	144.3±8.9*	548±105*	715±56	5.3±0.9
100 mg/kg	7.5 (3)*	139.3±8.3*	446±78*	802±59*	4.5±0.6**

All data are expressed as mean ± S.E.M., except score data which are expressed as median (range). Differences among means were tested for statistical significance by one-way ANOVA and *a posteriori* least signifcance tests. Score data were analyzed with the Mann-Whitney *U* test. *p<0.05, **p<0.01 *vs* TNBS control group.

Morris, G.P., Beck, P.L., Herridge, W., Depew, W., Szewczuk, M.R. & Wallace, J.L. (1989). Gastroenterology, **96**, 795-803.
Bell, C.J., Gall, D.G. & Wallace, J.L. (1995). Am. J. Physiol. **268**, G622-G630.

95P THE EFFECT OF PDE1, PDE2, PDE5 SELECTIVE AND NON-SELECTIVE PHOSPHODIESTERASE INHIBITORS ON PERISTALSIS IN THE GUINEA-PIG ISOLATED ILEUM

B.R. Tuladhar[1], N. Cooper[2] & R.J. Naylor[1], [1]Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP, [2]Chiroscience Limited, Cambridge Science Park, Milton Road, Cambridge CB4 4WE.

Isoenzyme-selective inhibitors of the PDE4 family but not the PDE3 family of phosphodiesterase enzymes inhibit peristalsis in the isolated guinea-pig ileum (Tuladhar *et al.*, 1998). However, the concentration of PDE4 inhibitors required to inhibit peristalsis was considerably higher than their reported IC_{50} values to inhibit PDE4 enzymes. In the present study we have examined the effect of non-selective and isoenzyme-selective inhibitors of PDE1, PDE2 and PDE5 families of phosphodiesterase enzymes on peristalsis.

Segments of ileum (taken 5-30 cm from the ileo-caecal junction) were obtained from guinea-pigs (Dunkin Hartley) of either sex (500-1000g) and cannulated at the oral and aboral ends and secured horizontally in a bath containing Krebs-Henseleit solution kept at 37°C and oxygenated with 95% O_2 and 5% CO_2. Peristalsis was measured and recorded using the methodology previously described (Costall *et al.*, 1993). Cumulative concentration response curves to PDE inhibitors (added serosally) were constructed approximately 30 min after the mounting of the tissues as follows: once a regular peristalsis was achieved an increasing concentration of the PDE inhibitor was applied at 3 min intervals. If peristalsis was not abolished with the highest concentration tested, a 100 μM concentration of rolipram was added to abolish peristalsis. The concentration response curves were expressed as a percentage change in peristaltic threshold from the threshold before the addition of the PDE inhibitor taking the maximum pressure generated inside the tissue when peristalsis was abolished as 100%. A comparison of the potency of PDE inhibitors to inhibit peristalsis was made at 50% inhibition.

The non-selective phosphodiesterase inhibitors IBMX (30 μM-1 mM) and papaverine (3-100 μM) inhibited peristalsis with pEC_{50} values of 3.65±0.06 (n=5) and 4.72±0.07 (n=5) respectively. The PDE1 inhibitor vinpocetine (1-10 μM) and the PDE2 inhibitor (erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (10-300 μM) inhibited peristalsis with pEC_{50} values of 5.27±0.01 (n=4) and 4.09±0.13 (n=4) respectively. No significant changes in the threshold for peristalsis were observed with the PDE5 inhibitor zaprinast (1-100 μM) (n=4). Moreover, zaprinast did not alter the ability of rolipram (100 μM) to abolish peristalsis.

The EC_{50} value of EHNA to inhibit peristalsis was much higher than the reported IC_{50} values for the inhibition of PDE2 family of phosphodiesterase enzymes (Beavo, 1995) and zaprinast was ineffective. These results indicate a lack of involvement of the PDE2 and PDE5 families of phosphodiesterase enzymes on peristalsis. Interestingly, the PDE1 selective inhibitor vinpocetine inhibited peristalsis at concentrations suggestive of an inhibition of the PDE1 family of phosphodiesterase enzymes (Beavo, 1995). Thus it is likely that selective inhibition of PDE1 family of phosphodiesterase enzymes inhibits peristalsis in the guinea-pig ileum. The effect of IBMX, papaverine, EHNA or PDE4 inhibitors to inhibit peristalsis may be a non-selective action on the PDE1 family of phosphodiesterase enzymes.

Beavo, J.A. (1995). Physiol. Reviews 75, 725-748.

Costall, B., Naylor, R.J. & Tuladhar, B.R. (1993). Br. J. Pharmacol. 110, 1572-1578.

Tuladhar, B.R., Cooper, N. & Naylor, R.J. (1998). Br. J. Pharmacol. (In press).

96P ENDOTHELIUM-DEPENDENT CONTRACTILE RESPONSE OF MYRICETIN IN RAT ISOLATED VASCULAR SMOOTH MUSCLE

Rosario Jiménez, [1] Emile Andriambeloson, Francisco Pérez-Vizcaino, [1] Ramaroson Andriantsitohaina, José Jiménez, Antonio Zarzuelo, Juan Duarte & [2]Juan Tamargo. Dept. of Pharmacology, Univ. of Granada, Spain; [1] Laboratoire de Pharmacologie et Physiopathologie Cellulaires, Université Louis Pasteur de Strasbourg, France. [2] Dept. of Pharmacology, Univ. Complutense, Madrid, Spain.

Myricetin (3',4',5',3,5,7-hexahydroxyflavone) is a flavonoid that modifies rat aorta contractility in a bifasic manner. It exerts a vasorelaxant effect at high concentrations, while at low concentrations, produces an increase of the maximum contractile responses induced by noradrenaline, high KCl and phorbol 12-myristate 13-acetate in rat aortic rings (Herrera et al., 1996). The aims of the present study were to characterize the contractile effects induced by myricetin on rat aortic rings and the underlying mechanism(s) of action.

Experiments were performed in rat aortic rings incubated in Krebs-Henseleit solution at 37 °C. Contractile force was isometrically recorded as previously described (Herrera et al., 1996). TXB_2 release was measured by an enzymeimmunoassay (EIA) system. Bovine aortic endothelial cells (BAEC) loaded with fura-2/AM were used to measure intracellular calcium concentrations ($[Ca^{2+}]_i$).

KCl (80 mM) produced a control contractile response (831.1 ± 25.9 mg, n = 29). Myricetin (1-100 μM) induced a concentration-dependent contractile response on baseline tension in aortic rings, which was maximal at 50 μM (21.0 ± 2.3% of control KCl contraction). This effect reached the maximal value at 6 min and then decayed. The contraction produced by 50 μM of myricetin was abolished by endothelium removal and after incubation of aortic rings in a Ca^{2+} free medium. However, pretreatment with verapamil (10 μM) or prazosin (1 μM) did not modify the contractile response of myricetin (21.8 ±3.7 %, n = 10, P > 0.05 and 18.0 ± 4.2%, n = 6, P > 0.05, respectively). Nitric oxide (NO) synthase inhibition with L-NAME (100 μM), potentiated by approximately 50% the contractile response induced by myricetin (32.5 ± 3.0%, n = 6, P < 0.01). Furthermore, incubation with superoxide dismutase (SOD 100 u ml^{-1}) plus catalase (100 u ml^{-1}) significantly reduced the endothelium-dependent contractions to myricetin (3.5 ± 1.0%, n = 6, P < 0.01). The inhibitors of the arachidonic acid pathway quinacrine (10 μM), indomethacin (10 μM), dazoxiben (100 μM) or the putative thromboxane A_2/prostaglandin endoperoxide receptor antagonist, ifetroban (0.3 μM or 3 μM), significantly (P < 0.01) reduced myricetin-induced contractions (1.5 ± 1.0%, n = 6, 1.1 ± 0.4%, n = 16, 3.9 ± 1.3%, n = 9, 10.0 ± 4.1%, n = 7 and 1.3 ± 1.0%, n = 5, respectively). The TXB_2 production in non stimulated endothelium-intact and endothelium-denuded aortic rings was 65.9 ± 8.5 and 43.1 ± 3.3 pg mg^{-1} wet weight, n = 6, respectively and it increased after 15 min incubation with myricetin (50 μM) to 131.3 ± 11.0 pg mg^{-1} wet weight, n = 6 and 89.9 ± 4.4 pg mg^{-1} wet weight, n = 6, respectively. Moreover, the presence of SOD (100 u ml^{-1}) plus catalase (100 u ml^{-1}) did not inhibit the TXB_2 production in endothelium-intact aortic rings stimulated by myricetin (138.1 ± 14.3 pg mg^{-1} wet weight, n = 6, P > 0.05). In fura2-loaded BAEC resuspended in HBSS the basal $[Ca^{2+}]_i$ was 225.9 ± 36.7 nM (n = 3). Myricetin, at a concentration at which produced the maximal contractile response (50 μM), increased $[Ca^{2+}]_i$ (maximum increase 94.9 ± 42.2 nM after 40 s incubation period). In a Ca^{2+}-free HBSS containing 0.5 mM EGTA (basal $[Ca^{2+}]_i$ = 101.2 ± 2.3 nM, n = 4) myricetin failed to increase $[Ca^{2+}]_i$ (104.1 ± 0.6 nM, n = 4, P > 0.05).

In conclusion, our results suggest that an activation of PGH_2-TXA_2 receptors on vascular smooth muscle by the TXA_2 released from endothelium by a calcium-sensitive cycloxygenase pathway is the main mechanism involved on the contractile response induced by myricetin on rat aortic rings.

Herrera, M.D., Zarzuelo, A., Jiménez, J., Marhuenda, E. & Duarte, J. (1996). *Gen. Pharmac.*, 27, 273-277.

97P BASAL NITRIC OXIDE IS DEFICIENT IN BOTH MALE AND FEMALE SPONTANEOUSLY HYPERTENSIVE STROKE-PRONE RATS

Fiona J. Dowell, William Martin, Anna F. Dominiczak & Carlene A. Hamilton. CRI & Dept. of Medicine and Therapeutics, University of Glasgow, Glasgow, G12 8QQ.

A previous study demonstrated in aortic rings from spontaneously hypertensive stroke prone rats (SHRSP) a reduced ability of a nitric oxide (NO) synthase inhibitor to elevate tone induced by a single concentration of phenylephrine (PE, EC_{20}) when compared with those from normotensive control Wistar Kyoto rats (WKY) (McIntyre *et al.* 1997). This result suggested a deficiency of basal nitric oxide in the hypertensive strain. We wished to test this possibility by examining the endothelium-dependent depression of contractility across the concentration range for PE in the aorta. Furthermore, to determine whether this was a generalised phenomenon, experiments were also performed in the carotid artery.

Contractile responses to PE (0.1 – 3000 nM) were investigated on endothelium-intact (E+) and -denuded (E-) rings of thoracic aorta and carotid artery from 12 week old male and female SHRSP and WKY rats, killed by pentobarbitone overdose. The rings were mounted under 1 g of tension, bathed in Krebs solution gassed with 95% O_2 / 5% CO_2 and maintained at 37°C.

Blood pressure (B.P.) and responses to PE i.e. pEC_{50} and maximum contraction (Max; expressed as a percentage of contraction to 100 mM KCl) are given in Table 1. Male and female rats were both hypertensive when compared to their respective controls. The presence of the endothelium causes a significant decrease in both sensitivity and maximum response to PE in both aorta and carotid artery irrespective of strain or sex. This depression appears more pronounced in females and perhaps more importantly is reduced in both male and female SHRSP.

Thus, these data support the previous report (McIntyre *et al.* 1997) suggesting that the effectiveness of basal NO is reduced in aortae from SHRSP. Furthermore, this effect is not simply localised to the aorta since it is also observed in the carotid artery. This reduction in basal NO might, therefore, contribute to the increased vascular resistance associated with essential hypertension.

McIntyre, M. *et al.* (1997) *Hypertension*, 30, 1517-1524.

Table 1. Summary of blood pressure and responses to PE in rings of thoracic aorta (TA) and carotid artery (CA).

		WKY				SHRSP			
		male		female		male		female	
B.P. (mmHg)		132 ± 4		120 ± 3*		178 ± 7$		136 ± 4*$	
		E+	E-	E+	E-	E+	E-	E+	E-
TA:	Max	127 ± 9	205 ± 9#	110 ± 9	210 ± 8#	152 ± 6	179 ± 8#	141 ± 9	201 ± 8#
	pEC_{50}	6.99 ± 0.06	7.85 ± 0.04#	6.86 ± 0.06	7.78 ± 0.03#	7.51 ± 0.07$	8.02 ± 0.06#	7.12 ± 0.07*	7.96 ± 0.12#
CA:	Max	131 ± 15	197 ± 11#	117 ± 9	218 ± 10#	148 ± 7	185 ± 9#	171 ± 10$	222 ± 7#
	pEC_{50}	6.87 ± 0.04	7.60 ± 0.09#	6.79 ± 0.06	7.59 ± 0.07#	7.3 ± 0.07$	7.87 ± 0.08#	7.25 ± 0.09$	7.97 ± 0.11#

* denotes a difference due to sex; $ a difference due to strain; # a difference due to endothelial denudation (P<0.05, ANOVA, followed by Bonferroni analysis). Data are mean ± s.e. mean, n≥6 animals.

98P POSSIBLE INFLUENCE OF CHANGES IN CALCIUM LOADING OF THE SARCOPLASMIC RETICULUM ON THE RATE OF BEATING OF GUINEA-PIG ISOLATED ATRIAL PREPARATIONS

Brooks, A.M.S., Rigg, L. & Terrar, D.A. University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT.

Recent evidence supports a role for calcium release from the sarcoplasmic reticulum (SR) in the regulation of heart rate (Rigg & Terrar, 1996; Li *et al.*, 1997). The aim of the study was to investigate the possible influence of changes in calcium loading of the SR on heart rate. Increases in extracellular calcium and addition of isoprenaline were used to enhance loading of the stores.

Male guinea-pigs (400-600 g) were killed by cervical dislocation following stunning. The atria were suspended in a heated organ bath containing balanced salt solution (36°C). The preparation was allowed to stabilise for a period of 1 hour. Heart rate was determined from the interval between action potentials recorded with bipolar platinum electrodes. Extracellular calcium was altered by cumulative additions of $CaCl_2$. Ryanodine (2 μM) was used to inhibit the function of the stores (Rousseau *et al.*, 1987). Means are represented as ± SEM. Statistical significance was tested using paired student's t-test unless otherwise stated.

In six preparations raising calcium from 1 mM to 1.8 mM significantly increased heart rate by 11±3% (P<0.05). A further increase in extracellular calcium to 2.5 mM increased the rate of beating (compared to 1 mM) by 20±6 % (P<0.05). On return to 1 mM calcium heart rate recovered to previously recorded values. Subsequent application of 2 μM ryanodine reduced beating frequency consistently by 12±3 % over a 30 minute period (P<0.05). This reduction was significantly less than that recorded in 2.5 mM calcium (29±2%, n=19; unpaired t-test P<0.05, Rigg & Terrar, 1996). Increase in calcium from 1 to 2.5 mM in the presence of ryanodine produced no significant changes in rate.

Addition of 50 nM isoprenaline in 1 mM calcium increased rate by 103±10% (P<0.05). This increase was significantly greater than that previously recorded in 2.5 mM calcium (36±5%, Rigg & Terrar, 1998; unpaired t-test, P<0.05). Increasing extracellular calcium from 1 mM to 2.5 mM in the presence of 50 nM isoprenaline produced no significant further increase in heart rate.

Our results show that changes in extracellular calcium influence the rate of beating of atrial preparations. This is consistent with the hypothesis that SR loading plays an important role in the determination of heart rate since loading would be expected to be enhanced by increasing extracellular calcium. This interpretation is supported by the observation that ryanodine, a known inhibitor of SR function, prevented the changes in heart rate that accompanied changes in extracellular calcium. In the presence of isoprenaline, when the amount of stores loading would be expected to be near maximal, increasing extracellular calcium had little effect on heart rate, perhaps because the increase in calcium caused little or no further increase in stores loading under these conditions. In summary, the observations are consistent with the suggestion that SR calcium loading might influence heart rate through a mechanism in which changes in calcium released from the SR modify cytosolic calcium and this in turn regulates a variety of ionic currents determining heart rate (see Rigg & Terrar, 1996).

This work was supported by a BPS A.J.Clark Studentship.

Li, J., Qu, J.H. & Nathan, R.D. (1997) *Am. J. Physiol.* 42, H2481-2489.
Rigg, L. & Terrar, D.A. (1996) *Exp. Physiol.*. 81, 877-880.
Rigg, L. & Terrar, D.A. (1998) *Br. J. Pharmacol.* (In Press).
Rousseau, E.,Smith, J.S. & Meissner, G. (1987) *Am. J. Physiol.* 253, C364-368.

99P INCREASES OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE POSITIVE INOTROPISM INDUCED BY ANDROGENS IN ISOLATED LEFT ATRIUM OF RAT

M. Sánchez[1], J.M. Rubín[3], B. Cantabrana[1], A.B. Varona[1], A. Hidalgo[1] and C. Bordallo[2]. [1]Farmacología (Dpto. de Medicina) and [2]Dpto. de Bioquímica y Biología Molecular, Universidad de Oviedo, [3]Servicio de Cardiología. Hospital Central de Asturias, Spain.

Polyamines are mediators of several biological actions (Marton & Pegg, 1995) including some induced by androgens (Koenig *et al.*, 1989). 5α- and 5β-dihydrotestosterone (DHT) induced positive inotropism in the left atrium of rat presumably via genomic mechanisms. The role of polyamine synthesis in the effect of androgens in the left atrium of rat was studied, by means of pharmacological approach. The level of expression of ornithine decarboxylase (ODC) gene and the assay of ODC activity, was measured.

The experiments were performed in isolated left atrium of male Wistar rats (250-300 g) placed in an organ bath in 10 ml of Tyrode solution at 37°C. After 1 h of equilibration under basal tension of 1 g, electrical stimulation (0.5 Hz, 5 ms and a voltage 30-50% above the threshold) was started. To perform the biochemical assay, 100 μM of 5α- or 5β-DHT was added; and 6 min after the induction of positive inotropism the atria were immediately frozen in liquid nitrogen, and stored at -80°C. Northern analysis of ODC and *in vitro* assay of ODC activity were made with these preparations. Northern analysis was assayed using as probe a fragment of 2.15 kb of cDNA. ODC activity was determined essentially by measuring the release of ^{14}C from L-[1-^{14}C]ornithine (Erwin *et al.*, 1983).

5α- and 5β-DHT (0.1 to 100 μM) induced a concentration-dependent positive inotropism which was significantly inhibited by α-diflouromethylornithine (DFMO) 10 mM, an inhibitor of ODC (Figure 1). The northern analysis shown that the androgens did not modify the level of expression of ODC gene.

On the other hand, androgens increased significantly the activity of ODC from 40.2±5.8 (control) to 100.4±12.8 and 95.1±14.4 pmol· h^{-1}·mg^{-1}, respectively for 5α- and 5β-DHT ($P<0.05$, Dunnett's Test, $n=5$).

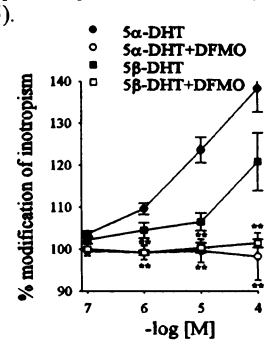


*Figure 1. Effect of DFMO (10 mM) on the positive inotropism elicited by 5α- and 5β-DHT in the left atrium of rats electrically stimulated. **P<0.01 by means of Dunnett's Test.*

Therefore, our results suggest that polyamine synthesis may be involved in the positive inotropism elicited by androgens through the stimulation of ODC activity without changes in the expression of ODC gene.

We thank Dr. P.J. Blackshear (Duke University Medical Center, USA) for providing us with the probe for ODC. This work was supported by a grant from the DGES, PB95-1058.

Erwin B.G., Seely J.E. & Pegg A.E. 1983, *Biochemistry* **22**, 3027-3032.

Koenig H. *et al.*, 1989, *Cir. Res.* **64**, 415-426.

Marton L.J. & Pegg A.E., 1995, *Annu. Rev. Pharmacol. Toxicol.* **35**, 55-91.

100P THE EFFECT OF OESTROGEN ON VASCULAR CONTRACTIONS IN RAT AORTA AND MESENTERIC ARTERY

M. Browne & J.R. Docherty. Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2.

It is well established that pre-menopausal women have a reduced incidence of coronary artery disease, and this is likely to be due to oestrogen, since hormone replacement therapy also reduces the incidence of coronary artery disease. It has been proposed that the vascular actions of 17β-oestradiol may involve activation of adenylate cyclase or calcium antagonistic actions (Farhat et al., 1996; Kitazawa et al., 1997). Hence, the purpose of this study was to examine the acute actions of 17β-oestradiol in rat mesenteric artery and aorta and to answer the following questions: whether the actions are similar against KCl and noradrenaline; whether the actions are oestrogen receptor mediated; whether the actions can be explained by calcium antagonism or activation of adenylate cyclase.

We have examined the effects of 17β-oestradiol on contractions to noradrenaline and KCl (10-120 mM) in mature male Wistar rat (250-350 g) small mesenteric artery (200-300 μm) and aorta. Vessels were set up under 1 g tension in small vessel myographs or organ baths in Krebs-Henseleit solution containing EDTA (30 μM), ascorbic acid (280 μM), cocaine (3 μM), corticosterone (30 μM) and propranolol (3 μM). Two concentration-response curves were carried out, control and following 1 hour exposure to test agents, or vehicle. The maximum response to noradrenaline in the second concentration response curve was 102.5±2.6% (n=20) and 95.5±5.4% (n=19) of control in vehicle experiments in mesenteric artery and aorta, respectively. In male rat mesenteric artery, noradrenaline produced a maximum contraction of 0.83±0.06 g (n=58), and 17β-oestradiol (10 μM) significantly reduced (Analysis of Variance) the maximum contraction to noradrenaline (67.7±5.8% of control, n=30) and KCl (38.8±3.1% of control, n=10) without affecting potency. The adenylate cyclase inhibitor SQ 22,536 (9-(tetrahydro-2-furanyl)-9H-purin-6-amine) (100 μM) did not prevent the inhibitory action of 17β-oestradiol. In male rat aorta, noradrenaline produced contractions with a maximum contraction of 0.62±0.03 g (n=18), and 17β-oestradiol (10 μM) also significantly reduced contractions to noradrenaline (72.4±5.2% of control, n=19), but the effects were prevented by a high dose of the oestrogen receptor antagonist droloxifene (10 μM). In experiments carried out in calcium-free solution (but with all blockers listed above) in which calcium stores were depleted, 17β-oestradiol (10 μM) significantly reduced the contraction to calcium restoration in rat aorta. In female rat aorta, 17β-oestradiol (10 μM) reduced contractions to noradrenaline (46.4±7.3% of control, n=7). Hence, similar effects occur in female rats.

In summary, 17β-oestradiol diminishes the maximum contractile response to noradrenaline in both rat small mesenteric artery and aorta, and this, at least in the, aorta is sensitive to the oestrogen receptor antagonist droloxifene, and may be due to restriction of calcium entry. Such effects may contribute to the beneficial actions of oestrogen in the cardiovascular system.

Supported by RCSI and the Irish Heart Foundation

Farhat, M.Y. et al. (1996). *J. Pharmacol. Exp. Ther.* 276: 652-657.

Kitazawa, T. et al. (1997). *J. Physiol.* 499: 497-511.

101P INDOMETHACIN-INDUCED EFFECTS ON CEREBRAL CIRCULATION IN THE AWAKE RAT ARE UNRELATED TO PROSTACYCLIN, WHICH IS NOT A CEREBRAL VASODILATOR

E. Raczka & A. Quintana, Department of Pharmacology, Basque Country University, 48940 Leioa, Vizcaya, Spain.

Indomethacin reduces resting cerebral blood flow and abolishes hypercapnia-induced cerebral vasodilation (Pickard, 1981). The aim of present work was to determine the possible role of prostacyclin synthesis inhibition in those indomethacin-induced effects on cerebral circulation in awake rats.

The reference sample radioactive microsphere method was applied to perform two determinations of cerebral blood flow and cerebral vascular resistance in each awake undisturbed rat (Quintana et al., 1983).The first microsphere injection was given under basal conditions; fifteen min before the second microsphere injection, indomethacin (5 mg kg^{-1}), indomethacin followed 5 min later by an infusion of prostacyclin (0.3 or 1.0 $\mu g\ kg^{-1}\ min^{-1}$) or

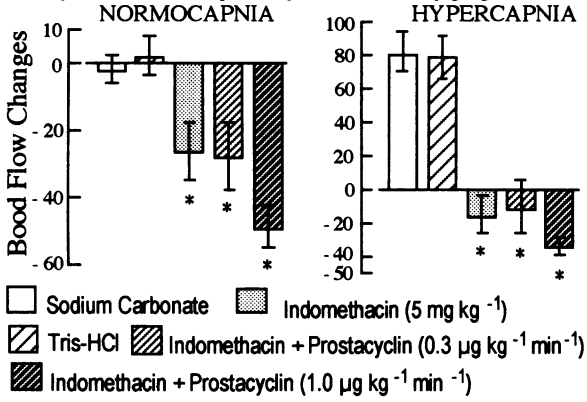


*Figure 1. Changes in cerebral blood flow (% with respect to basal values). Means and their 95 % confidence limits (n=6). * P < 0.001 vs the respective vehicle*

vehicles were administered under normocapnic or hypercapnic (arterial blood PCO_2 62 mmHg) conditions. In other rats, the effects of i.v. infusions of prostacyclin alone were studied under normocapnic conditions. Prostacyclin blood concentrations in the internal carotid at the end of i.v. infusions were determined in additional rats by the *in vitro* inhibition of ADP-induced platelet aggregation of human PRP and were approximately 12 and 50 nM after 0.3 and 1.0 $\mu g\ kg^{-1}\ min^{-1}$, respectively.

Indomethacin-induced cerebral blood circulation changes were not counteracted nor reduced by prostacyclin, as shown in Fig 1. Prostacyclin alone reduced cerebral blood flow while increased the extracerebral flow (Fig 2).

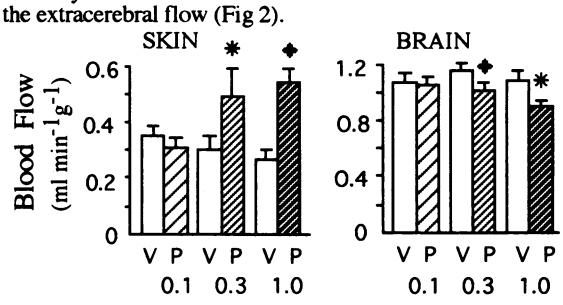


Figure 2. Cerebral and pericranial skin blood flows during vehicle (V) and prostacyclin (P) i.v. infusions. Mean values and SE mean (bars) (n = 6). ✱ P < 0.01 and ✦ P < 0.001

We conclude that in the awake rat, the indomethacin-induced effects on cerebral circulation are not mediated by the inhibition of prostacyclin synthesis and that prostacyclin is not a cerebral vasodilator.

Pickard, J.D. (1981) J. Cereb. Blood Flow Metabol. **1**, 361-384
Quintana, A., Raczka, E., Giralt, M.T. & Quintana, A. (1983) Prostaglandins **25**, 549-556

102P CARDIOPROTECTION AFTER REPEATED DOSING WITH GR79236, AN ADENOSINE A_1 RECEPTOR AGONIST

Travers, A., Middlemiss D. & Louttit, J.B. Systems Biology Unit, GlaxoWellcome Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts. SG1 2NY, U.K.

Stimulation of adenosine A_1 receptors induces both the classic (Lui *et al.*, 1991), and a delayed phase (Baxter & Yellon, 1997), of myocardial protection in the rabbit. Tsuchida *et al.* (1994), showed that myocardial protection was lost after prolonged infusion of the adenosine A_1 receptor agonist 2-chloro-N^6-cyclopentlyadenosine (CCPA). In contrast, Dana *et al.*, (1997), saw no loss of CCPA-induced protection after intermittent administration, and suggested that rabbit myocardium can be maintained in a preconditioned state. We have determined whether once daily dosing with the adenosine A_1 receptor agonist GR79236, (GR; Gurden, *et al.*, 1994), would result in sustained cardioprotection in a rabbit model of myocardial ischaemia and reperfusion.

Myocardial ischaemia was induced in pentobarbitone (45mg kg^{-1} i.v.) anaesthetised, artificially respired, male New Zealand White rabbits (2.4-3.3kg) by occlusion of a branch of the left coronary artery for 30min, followed by 120min reperfusion. A single dose of GR ($3x10^{-8}$mol kg^{-1} i.v.) or vehicle (V; saline, 1ml i.v.) was administered either 10min or 48h prior to the ischaemia and reperfusion protocol. In a third group of rabbits, GR (same dose) or vehicle, was given once a day for 7 days, with ischaemia and reperfusion taking place 24h after the last dose of GR. Infarct (I) was determined by negative tetrazolium staining of 2mm thick slices of myocardium. The area at risk (R) was delineated by fluorescent beads, and the I/R ratio calculated. Body temperature was maintained at 37.0±0.3°C.

Basal mean arterial pressure (73-83mmHg) and heart rate (240-260beats/min) were similar in all groups. GR reduced heart rate and mean arterial pressure by 16.6±5.8% and 18.8±2.4%, respectively, when administered 10min prior to ischaemia. Occlusion of the left coronary artery in vehicle-treated rabbits resulted in an I/R ratio of approximately 39%. GR reduced the I/R ratio by approximately 53% irrespective of whether it was administered 10min or 48h prior to ischaemia, or 24h after the last of 7 daily doses (Table 1). R was approximately 33% of the left ventricle and not significantly different between the groups.

Table 1. Infarct size data

	Single treatment 10min prior to ischaemia		Single treatment 48h prior to ischaemia		7 single doses, ischaemia 24h after last dose	
	V	GR	V	GR	V	GR
I/R %	38.9	17.9*	38.8	18.6*	39.6	18.8*
(s.e.m.)	(4.1)	(4.3)	(3.3)	(5.0)	(3.4)	(3.2)

(All data shown are mean ± (s.e.m.), n=6-7, *P<0.01, Student's t test)

In conclusion, GR induced both acute and delayed protection in the rabbit heart. Once daily dosing for 7 days conferred protection when ischaemia was induced 24h after the last dose of GR. These data show that there is no loss of myocardial protection after repeated administration of an adenosine A_1 receptor agonist.

Baxter G. & Yellon, D.M. (1997) *J. Cardiovasc. Pharmacol.*, **29**, 631-638.
Dana, A., Baxter, G.F., Walker, J.M. *et al.*, (1997) *Circulation*, **96**, (Suppl I) I-257.
Gurden, M.F., Coates, J., Ellis, F. *et al.*, (1994) *Br. J. Pharmacol.*, **109**, 693-698.
Lui, G.S., Thornton, J., Van Winkle, D.M., *et al.*, (1991) *Circulation*, **84**, 350-356.
Tsuchida, A., Thompson, R., Olsson, R.A. *et al.*, (1994) *J. Mol. Cell. Cardiol.*, **26**, 303-311.

103P EFFECT OF XYLAMINE ON ISCHAEMIA AND REPERFUSION ARRHYTHMIA IN ISOLATED PERFUSED RAT HEART

S-A. Boschmans and M.F. Perkin (introduced by I.E. Hughes) Pharmacy Dept., University of Port Elizabeth, P.O. Box 1600, Port Elizabeth, 6000, South Africa.

Under conditions of myocardial ischaemia the uptake$_1$-carrier associated with the re-uptake of noradrenaline has been reported to operate in the reverse direction. This would lead to local release of noradrenaline which could contribute to the genesis of arrhythmias which occur during ischaemia. This study investigated the effect of xylamine, a selective inhibitor of the uptake$_1$-carrier (Fischer and Cho, 1983) on ischaemia and reperfusion-induced arrhythmias in isoloated retrogradely perfused rat hearts.

Hearts from male Long Evans rats (220 g to 310 g ; UPE Animal Unit) were excised under pentobarbital anaesthesia (6 mg per 100g i.p.) 30 seconds after i.v. injection of 200 IU of sodium heparin. Hearts were mounted for Langendorf perfusion (37°C, 100 cm H_20 pressure, pH 7.4) and perfused with modified Krebs-Henseleit buffer (NaCl 118 mM $NaHCO_3$ 25mM, KCl 2.1 mM, KH_2PO_4 1.2mM, $MgSO_4$ 1.2mM, $CaCl_2$ 2.5 mM, with glucose substrate 11 mM). Total K^+ was 3.3mM.

Regional ischaemia was produced, for 20 minutes, by reversible ligation of the left descending coronary artery. This was followed by a reperfusion period of 10 minutes whereafter the experiment was terminated.

Xylamine 1 µM perfusion was commenced at either 4 minutes prior to ischaemia (pre-ligation series - xylamine present in ischaemic zone) or at 1 minute post ligation (post-ligation series - minimal xylamine in the ischaemic zone).

Arrhythmias were defined according to the Lambeth Convention (Walker *et al.*, 1988). ECG traces were analyzed blind to prevent bias. An arrhythmia score similar to that used by Curtis and Hearse (1989) was used to quantify the arrhythmias. Results are reported ± s.e.mean and as incidence data.

Xylamine attenuated the severity of arrhythmias that occurred during ischaemia. There was a reduction in the combined incidence of ventricular fibrillation and ventricular tachycardia (VF+VT) when hearts were exposed to xylamine prior to ligation (3/16 vs 14/33 in controls). Xylamine also significantly decreased the severity of ischaemic arrhythmias (arrhythmia score: xylamine pre-ligation 1.38 ± 0.29, n=16 vs 2.24 ± 0.24 in control hearts, n = 33 : $p < 0.05$; unpaired t-test). Xylamine post-ligation did not significantly lower the arrhythmia score versus control hearts (score = 1.5 ± 0.37; n = 15).

There was no difference in the incidence of VF+VT on reperfusion between xylamine and control (control incidence = 33/33; xylamine pre-ligation incidence = 16/16; xylamine post-ligation incidence = 15/15).

These results indicate that xylamine decreases the incidence and severity of ischaemic arrhythmias. This antiarrhythmic effect of xylamine may be due, in part, to inhibition of noradrenaline release by the uptake$_1$-carrier.

Fischer, J.B. & Cho, A.K. (1983) *J.Pharmacol.Exp.Ther.*, 225, 623-629
Curtis, M.J. & Hearse, D.J. (1989) *J. Mol. Cell. Cardiol.*, 21:21-40
Walker, M.J.A. *et al.*, (1988) *Cardiovasc. Res.*, 22: 447 - 455.

104P THE SELECTIVE EP$_3$-PROSTANOID RECEPTOR AGONIST, ONO-AE-248, REDUCES INFARCT SIZE IN A RAT MODEL OF MYOCARDIAL ISCHAEMIA AND REPERFUSION

Kai Zacharowski, Kigen Kondo & Christoph Thiemermann. The William Harvey Research Institute, St. Bartholomew's & the Royal London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ, UK.

The cardioprotective effects of E-type prostaglandins have been attributed to vasodilatation, inhibition of platelet and neutrophil function (all due to activation of EP_2-receptors) and a 'cytoprotective effect', the mechanism of which is unknown. We have recently reported that the prostanoid-derivative, ONO-AE-248, selectively binds to and activates murine $EP_{3\alpha}$-receptors and reduces the infarct size caused by myocardial ischaemia and reperfusion in the rabbit (Zacharowski et al., 1998). This study was designed to elucidate whether the selective $EP_{3\alpha}$-receptor agonist ONO-AE-248 reduces the infarct size caused by regional ischaemia and reperfusion of the rat heart *in vivo*.

Male Wistar rats (240-350 g) were anaesthetised with thiopentone sodium (120 mg·kg^{-1} i.p.). All animals were tracheotomised and ventilated with room air (tidal volume: 8-10 ml·kg^{-1}; respiration rate: 70 strokes per min). Subdermal platinum electrodes were placed to allow the determination of a lead II electrocardiogram (ECG). The carotid artery was cannulated to measure mean arterial blood pressure (MAP) and the jugular vein was also cannulated for the administration of drugs. The chest was opened by a left sided thoracotomy, the pericardium incised and an atraumatic needle was placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min and subsequently the LAD was occluded for 45 min and then reperfused (for 2 h). At the end of the experiment, the LAD was re-occluded, and 1 ml of Evans Blue dye (2% w/v) was injected into the jugular vein to determine the perfused and the non-perfused (area at risk, AAR) by computer-assisted planimetry (Leica-Quantimed 500). Infarct size (IS) was determined by incubation of the slices with nitro-blue tetrazolium (NBT, 0.5 mg·ml^{-1}). Infusion of ONO-AE-248 (5 µg·kg^{-1}·min^{-1}, n=9) or its vehicle (saline) was commenced at 10 min prior to LAD-occlusion and maintained throughout the experiment. In addition, two separate groups of animals were subjected to the same surgical procedure with the exception that the LAD was not occluded (sham-operated animals). These animals received infusions of either ONO-AE-248 (n=3) or vehicle (n=3). All data are expressed as mean±s.e.mean. Statistical differences between groups were analysed by ANOVA followed by a Bonferoni's test.

Successful occlusion of the LAD was always associated with a substantial increase in the ST-segment of the ECG. The AAR was not different between the two animal groups studied (control: 54±4%, ONO-AE-248: 57±2%). Treatment of rats with ONO-AE-248 resulted in a significant reduction in IS from 78±2% (control, n=11) to 58±4% (n=9, $p<0.05$). Occlusion of the LAD for 45 min resulted in a significant fall in MAP from 119±5 mmHg to 96±5 mmHg ($p<0.05$), while the subsequent reperfusion for 2 h did not result in a recovery in MAP (90±7 mmHg, n=11). ONO-AE-248 attenuated the fall in MAP caused by LAD occlusion/reperfusion (after occlusion: 117±7 mmHg; after reperfusion: 108±5 mmHg; $p<0.05$ when compared to control at the same time point). In sham-operated animals, ONO-AE-248 did not affect any of the parameters measured.

Thus, the selective $EP_{3\alpha}$-receptor agonist ONO-AE-248 attenuates the degree of tissue necrosis caused by regional ischaemia-and reperfusion of the heart of the rat.

KZ is supported by a Research Fellowship of the German Society of Cardiology. CT is a Senior Fellow of the BHF (FS 96/018)

Zacharowski, K., Piper, J., Kondo, K. et al. (1998) Z. Kardiol., 87 (1), 265.

105P THE PARS INHIBITORS 3-AMINOBENZAMIDE, NICOTINAMIDE AND 1,5-DIHYDROXYISOQUINOLINE DO NOT SCAVENGE SUPEROXIDE ANIONS *IN VITRO*

Joanne Bowes, David W. Laight & Christoph Thiemermann The William Harvey Research Institute, Charterhouse Square, London, EC1M 6BQ.

Reactive oxygen species (ROS), such as superoxide anions, contribute to the pathophysiology of ischaemia-reperfusion (I/R) injury. In cultured cells, ROS cause strand breaks in DNA, which activates the nuclear enzyme poly (ADP-ribose) synthetase (PARS). Excessive activation of PARS leads to cell death by ATP depletion (Schraufstatter *et al.*, 1986). We have recently discovered that inhibitiors of the activity of PARS reduces the degree of necrosis caused by I/R of the heart (Thiemermann *et al.*, 1997). In addition, preincubation of cultured rat cardiac myoblasts with PARS inhibitors attenuates the cytotoxic effects of hydrogen peroxide (Bowes *et al.*, 1998). Here we investigate whether PARS inhibitors are scavengers of superoxide anions at physiological pH and temperature.

The ability of the test compounds to scavenge superoxide anions was determined in an *in vitro* microassay as described by Laight *et al.* (1997). The assay mixture consisted of (final concentration): ferricytochrome c (cyt c, 100 μM), xanthine oxidase (XO, 10 mU·ml^{-1}), hypoxanthine (Hx, 100 μM) and catalase (200 U·ml^{-1}) dissolved in phosphate buffered saline (10 mM phosphate, pH=7.4). Changes in absorbance (A) at 550 nm were recorded at 37 °C using a kinetic plate reader over 3 min. The following PARS inhibitors were tested: 3-aminobenzamide (3-AB, 0.1-3 mM, n=4), nicotinamide (Nic, 0.1- 3 mM, n=4) and 1,5-dihydroxyisoquinoline (ISO, 0.01-3 mM, n=4). The established superoxide anion scavengers superoxide dismutase (SOD, 200 U·ml^{-1}) or 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, 1 mM) were used for comparison. Results are expressed as mean±s.e.mean and analysed using one-way ANOVA followed by Dunnett's test (*$p < 0.01$).

As expected TEMPO abolished and SOD significantly depressed the rate of reduction of cyt c by the XO/Hx system, demonstrating the involvement of superoxide anions. In contrast, none of the PARS inhibitors, even at the highest concentrations (figure 1) had any significant effect on the rate.

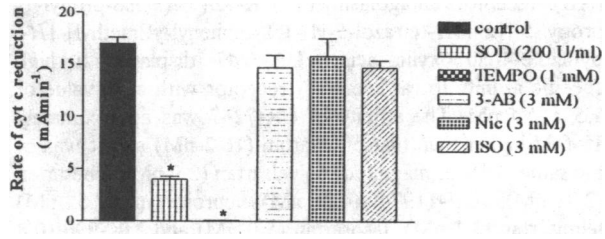


Figure 1 Rate of reduction of cyt c by XO/Hx system: effect of PARS inhibitors (n= 4-12)

In conclusion, under these assay conditions, the PARS inhibitors used, do not scavenge superoxide anions. Thus, these results suggest that the beneficial effects of PARS inhibitors in I/R injury are unlikely to be due to superoxide anion scavenging.

JB is the recipient of a BHF studentship (FS/96015). CT is the recipient of a BHF senior research fellowship (FS/96018).

Bowes, J.,Piper, J & Thiemermann, C. (1998) *Br. J. Pharmacol.*, **123**, 93P

Laight, D.W., Berthelon, J-J., Andrews, T.J. *et al.* (1997) *Br. J. Pharmacol.*, **122**, P130

Schraufstatter, I.U., Hyslop, P.A., Hinshaw, D.B. *et al.* (1986) *Proc. Natl. Acad. Sci.*, **83**, 4908-4912.

Thiemermann, C., Bowes, J., Myint, F. *et al.*, (1997). *Proc. Natl. Acad. Sci.*, **94**, 979-683.

106P TRAPIDIL ACTS AS A POTENT INHIBITOR OF NEOINTIMA FORMATION FOLLOWING BALLOON INJURY: EXAMINATION OF PROLIFERATION AND APOPTOSIS *IN VIVO* AND *IN VITRO*

Kai Zacharowski*, Tilo Großer, Dorien Schneidmüller, Michael Buerke, Jürgen Meyer & Harald Darius (introduced by Timothy D. Warner). *The William Harvey Research Institute, St. Bartholomew's & the Royal London School of Medicine and Dentistry, London, EC1M 6BQ, UK, and II. Med. Klinik, University of Mainz, Germany.

Percutaneous transluminal coronary angioplasty is a well established intervention for the treatment of coronary artery stenosis due to atherosclerosis. In spite of a primary success rate of 90-95%, late restenosis (neointima formation) occurs in 30-50% of patients within 3-6 months of the procedure. The development of successful strategies to prevent restenosis depends upon a better understanding of the underlying mechanisms triggering the proliferative response. Here we have investigated the effect of local administration of the platelet derived growth factor (PDGF) functional antagonist trapidil (Okamoto *et al.*, 1992) in a model of balloon injury in rabbit iliac arteries.

Following balloon angioplasty in anaesthetised (pentobarbitone 30 mg·kg^{-1}) male New Zealand White rabbits (2.8-3.8 kg) trapidil (50, 100 or 200 mg) or its vehicle was injected into the dilated vessel wall of the right iliac artery (left artery as control) using a porous infusion balloon catheter (Boston Scientific). 14 or 21 days later animals were killed and the iliac arteries removed for analysis of intimal hyperplasia (IH). IH was assessed as intima to media area ratio (IMR) by computer-assisted planimetry (Leica-Quantimed 500) and by immunohistological detection of cell proliferation (bromo-deoxyuridine (BrdU)-incorporation, 14 day time point, Wilson *et al.*, 1985). Apoptosis was investigated using Hoechst 33342 staining for chromatin condensation and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling). In addition, the effect of Trapidil (1-300μg·ml^{-1}) on proliferation (Cell Proliferation ELISA, Boehringer Mannheim) and apoptosis (Cell Death Detection ELISA, Boehringer Mannheim) of rabbit aortic smooth muscle cells in culture was investigated, using actinomycin D (250ng·ml^{-1}) as a positive control.

Balloon angioplasty resulted in IH and smooth muscle cell proliferation. Administration of trapidil caused a significant reduction in smooth muscle cell proliferation at 14 days. Values are expressed as % ratio of BrdU-positive to BrdU-negative cells: control (18±3%, n=9) vs trapidil 100mg (5±2%*, n=21), *$p<0.05$. In addition, trapidil (50-200mg) caused a reduction in IMR at 21 days.

table 1

group	n	IMR
control	8	1.67 ± 0.23
T 50 mg	5	1.14 ± 0.04
T 100 mg	9	0.91 ± 0.09*
T 200 mg	7	0.77 ± 0.09*

(* $p < 0.05$ vs control, ANOVA + Bonferoni's post test)

Proliferation of smooth muscle cells in culture was also significantly reduced by trapidil in a concentration-dependent manner. Compared to vehicle treated cells trapidil reduced proliferation by 1μg·ml^{-1}, 12±3.0%; 3μg·ml^{-1}, 13±3.2%, 10μg·ml^{-1}, 22±3.7%, 30μg·ml^{-1}, 34±8.6%, 100μg·ml^{-1}, 44±8.2% and 300μg·ml^{-1}, 66±5.2%), (n=6). Trapidil administration had no effect on apoptosis *in vivo* or *in vitro*. However, apoptosis was induced in smooth muscle cells by actinomycin D.

In conclusion, local administration of trapidil reduces neointima formation in the rabbit iliac artery following balloon angioplasty via a mechanism that is not dependent on apoptosis. This method of administration may contribute a novel approach in the therapy of restenosis using trapidil.

K.Z. is supported by a Research Fellowship of the German Society of Cardiology.

Wilson, G.D., McNally, N.J., Dunphy, E. *et. al.* (1985). *Cytometry*, **6**, 641-647

Okamoto, S., Inden, M., Setsuda, M. *et al.* (1992). *Am Heart J*, **123**, 1439-1444

107P PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL AND HIGHLY POTENT NONPEPTIDE AT1 RECEPTOR ANTAGONIST, UR-7247, *IN VITRO*

Fernández de Arriba A., Gómez L. A., Cavalcanti F. L., Merlos, M., J, Forn J. Dept. of Pharmacology, Research Centre, J. Uriach & Cia S.A., Barcelona, Spain

The aim of the study has been to evaluate the angiotensin II (AII) receptor antagonism of UR-7247 (3-Iso-propyl-1-propyl-5-[[2'-(1*H*-tetrazol-5-yl)-1,1'-biphenyl-yl]methyl]-1*H*-pyrazole-4-carboxylic acid). UR-7247 displayed a high specific affinity to rat liver AT_1 receptor with a Ki value of 1.5 ± 0.4 nM. The affinity of UR-7247 was approximately 10-fold higher than that of losartan (16.2 nM) and it was of the same order of magnitude as valsartan (2.9 nM), irbesartan (2.9 nM), CV-11974 (4.2 nM), eprosartan (7.5 nM) telmisartan (3.1 nM), tasosartan (5.0 nM) and MK-996 (0.8 nM). No effect on binding to cortex of rat adrenal AT_2 subtype has been observed (Ki > 10μM).

UR-7247 displaced the specific binding of [^{3}H]-AII to rat liver microsomes (without pre-incubation) in a monophasic and concentration-dependent manner, suggesting that UR-7247 binds with a high affinity to only one homogeneous type of receptors. Scatchard analysis of saturation revealed a competitive inhibition.

In functional studies in isolated strips of rabbit aortae, UR-7247 behaves as an specific and insurmountable antagonist. The AII-induced contraction curve was shifted to the right with a significant decrease (approximately 30% of control value) in maximal contractile response and with an apparent pKb value of 10.1 ± 0.36, without any effect on the contractile response in isolated rabbit aorta to KCl, noradrenaline and 5-HT at 1 μM UR-7247. The potency of UR-7247 was approximately two orders of magnitude higher than that of losartan (pA_2 = 8.5) and very similar to eprosartan (pKb = 9.6), valsartan (pKb = 10.5) and MK-996 (pKb = 10.5). The apparent pKb fell from the initial value of 10.1 to 7.0, when aortae were pre-incubated in presence of 0.1 % bovine serum albumin, suggesting a possible high interaction between UR-7247 and serum proteins. In both cases insurmountable behaviour was observed in apparent contradiction with a competitive profile.

[^{3}H]-AII binding decreased when the pre-incubation time with UR-7247 increased (0 - 60 min). On the other hand, pre-incubation did not increase the potency of losartan at any of the times tested. The maximal AII-induced contractile force in presence of UR-7247 in aorta strips was restored with losartan. In absense of losartan, the reduction of maximal contraction by UR-7247 (30 nM) was 60%; when losartan (1 and 10μM) was added to the bath, the reductions were 25% and 0% respectively. This fact suggests that UR-7247 and losartan may be interacting with the same binding site, since high concentrations of losartan displaced the UR-7247 from AT_1 receptor and restored the surmontable behaviour.

All these results suggest that UR-7247 has a potent and selective binding to AT_1 receptor and they are consistent with a slow reversible antagonism.

108P STUDY OF THE VASODILATOR EFFECTS OF RESVERATROL, A NATURAL PHENOLIC COMPONENT OF WINES, IN RAT AORTA

F. Orallo & M. Camiña, Departamento de Farmacología. Facultad de Farmacia. Universidad de Santiago de Compostela. España.

The lower incidence of coronary artery disease in the French and other Mediterranean populations, despite a diet rich in saturated fat (the *French paradox*), has been attributed to the high rate and moderate wine consumption by these populations (see, for example, Renaud & de Lorgeril, 1992). In order to explain this so-called *French paradox*, Fitzpatrick *et al.* (1993) have recently reported for certain wines, grape juices and grape skins extracts an endothelium-dependent vasorelaxant activity on rat aortic rings, which appears to be independent of the alcohol content in the wines and probably due to the not yet identified active constituents of grape skins. In view of these reports and in order to determine the possible implication of resveratrol (RESV, trans-3,4',5-trihydroxystilbene) in the protective effects of wine consumption against the incidence of cardiovascular diseases, the potential vasorelaxant activity in rat aorta of this natural compound, mainly derived from grapes of *Vitis vinifera* L. (Vitaceae), was studied in this work.

The contraction and $^{45}Ca^{2+}$ uptake experiments were carried out in aortic rings prepared from aortae of female Wistar-Kyoto rats weighing 250-270 g, essentially as we described elsewhere (Orallo, 1997). Results showed in the text are expressed as means ± s.e.m. Significant differences between two means were estimated by Student's two-tailed *t* test for paired or unpaired data, where appropiate.

RESV (1-10 μM) did not alter the contractile effect induced by noradrenaline (NA, 1 μM) in endothelium-denuded rat aortic rings. However, the cumulative addition of this drug relaxed, in a concentration-dependent fashion, the contractions produced by NA (1 μM) in intact rat aorta (IC_{50} = 3.4 ± 0.32 μM; n = 5). The maximum % of relaxation (R_{max} = 74 ± 6%) was similar to that caused by supramaximal concentrations of superoxide dismutase (300 u ml^{-1}; R_{max} = 78 ± 7%; n = 5; P > 0.05) and less than that produced by high concentrations of acetylcholine (1 μM; R_{max} = 90 ± 9%; n = 5; P < 0.05). These endothelium-dependent vasorelaxant effects of RESV were completely reversed by N^G-nitro-L-arginine (0.1 mM). Furthermore, in the presence of catalase (3000 u ml^{-1}), to prevent the accumulation of hydrogen peroxide, preincubation with RESV (10 μM) for 30 min totally inhibited the augmentation of NA-induced tone (an increase in sustained maximal tension of 424 ± 16; n = 5) caused by hypoxanthine (0.1 mM)/xanthine oxidase (16 mu ml^{-1}) in endothelium-containing rings. At higher concentrations (50 μM-0.1 mM), RESV totally relaxed, in a concentration-dependent manner and with almost equal efectiveness, the endothelium-denuded rat aortic rings pre-contracted with NA (1 μM; IC_{50} = 72 ± 4.2 μM) and phorbol 12-myristate 13-acetate (1 μM; IC_{50} = 78 ± 5.4 μM; n = 5). However, RESV did not modify the contractile response produced by okadaic acid (1 μM) in these rings without endothelium. On the other hand, in the studies with radiolabelled Ca^{2+}, basal ($^{45}Ca^{2+}$ tissue content: 22.4 ± 1.8 nmol kg^{-1}) and NA-induced $^{45}Ca^{2+}$ uptake ($^{45}Ca^{2+}$ tissue content: 37.4 ± 2.7 nmol kg^{-1}) were unaffected by RESV (50 μM-0.1 mM) in endothelium-denuded rat aortic rings [$^{45}Ca^{2+}$ tissue content in presence of RESV (0.1 mM): 23.2 ± 2.0 nmol kg^{-1}(basal); 37.8 ± 3.1 nmol kg^{-1} (NA); P > 0.05; n = 5]. In all the experiments carried out in this work (contractions studies and $^{45}Ca^{2+}$ uptake), the vehicle (dimethyl sulfoxide, 0.14-14 mM) had no significant vascular effects on the control rat aortic rings.

Our results indicate that: a) the characteristic endothelium-dependent vasorelaxant effect of RESV in rat aorta seems to be due to an enhancement of the NO-cyclic GMP pathway through the protection against NO breakdown by superoxide anions, b) the endothelium-independent vasorelaxation caused by RESV appears to be mediated by a inhibition of protein kinase C and not by a blockage of Ca^{2+} influx through transmembrane receptor-operated calcium channels present in smooth muscle cells and/or an increasing myosin light chain dephosphorylation. If RESV exhibited a similar behaviour in human blood vessels, the data obtained in this work could explain, at least in part, the beneficial effects of moderate wine consumption in the prevention of cardiovascular diseases (mainly coronary heart disease).

This study was funded by a grants from CICYT (SAF97-0166) and *Xunta de Galicia* (XUGA20312B97).

Fitzpatrick, D.F., Hirschfield, S.L. & Coffey, R.G. (1993) *Am. J. Physiol.* **65**, 774-778.

Orallo, F. (1997) *Br. J. Pharmacol.* **121**, 1627-1636.

Renaud, S. & de Lorgeril, M. (1992) *Lancet* **339**, 1523-1526.

109P RELEASE OF A STABLE, Ca^{2+}-DEPENDENT ANTIVASOCONSTRICTOR FROM HYPOXIC ENDOTHELIAL CELLS OF THE TRANSFORMED UMBILICAL CELL LINE (ECV304)

N. Buck, A.D. Perris, A.G. Richardson & J.W. Smith. Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET.

Ischaemia is a common clinical event which can lead to both local and remote tissue injury. The tissue damage is associated with factors released from endothelial cells during hypoxia - reperfusion which cause accumulation, activation and migration of neutrophils and may affect contractility of smooth muscle cells directly (Lefer & Lefer, 1993). To investigate this we have examined the effect of release of material from endothelial cells made hypoxic and subsequently restored to normoxic conditions on contractility of rat aorta.

ECV304 cells were grown to confluence in Dulbeccos Minimum Essential Medium (DMEM) supplemented with streptomycin (100 $IU.ml^{-1}$), penicillin ($10^{-4}g.ml^{-1}$) and foetal calf serum (10%) under an atmosphere of 95% O_2, 5% CO_2., at 37^0C. DMEM was removed and replaced with phosphate buffered saline (PBS) containing $6x10^{-4}$M or $2.5x10^{-3}$M Ca^{2+} under an atmosphere of 95% O_2, 5% CO_2 (normoxic) or 95% N_2, 5% CO_2 (hypoxic) at 37^0C for 4 h. In some experiments, cells made hypoxic were reperfused with normoxic PBS at 37^0C for a further 30 min. Male Wistar rats (200-250g) were stunned and killed by cervical dislocation. The thoracic aorta was removed and aortic rings, devoid of endothelium, were suspended in $6x10^{-4}$M or $2.5x10^{-3}$M Ca^{2+} Krebs buffer containing propranolol (10^{-6}M), ascorbic acid ($5x10^{-5}$M) and EDTA (10^{-5}M) at 37^0C, gassed with carbogen, under a resting tension of 2g. Isometric contractures were recorded. After 1h equilibriation, cumulative concentration-response curves were constructed to KCl prior to and either immediately (0 h) or 1 h after superfusion of the rings with normoxic (norm), hypoxic (hyp) or hypoxic-reperfused (hyp/r) supernatant from the ECV304 cells.

In $6x10^{-4}$M Ca^{2+}-Krebs the concentration-response curve to KCl was shifted to the left by norm with no change in maximum response, table 1. This shift was less with hyp and hyp/r supernatants as indicated by the EC_{50} values. The effect of the supernatants was unaffected by incubation time. In $2.5x10^{-3}$M Ca^{2+}-Krebs, EC_{50} values to KCl were reduced by norm and hyp supernatants applied immediately but not after 1 h incubation.

Table 1.	EC_{50} ($x10^{-3}$M)	E_{max} (g)
$6x10^{-4}$M Ca^{2+}:		
Control (n=24)	19.3±0.86	3.3±0.18
Norm.(0/1h) (n=6)	12.5±1.67[1]/12.2±1.25[1]	3.0±0.39/3.5±0.50
Hyp. (0/1h) (n=6)	14.9±1.48[1]/15.7±0.72[1,2]	3.7±0.25/3.9±0.25
Hyp/r (0/1h) (n=6)	15.9±0.97[1]/16.2±0.83[1,2]	3.1±0.42/3.0±0.37
$2.5x10^{-3}$M Ca^{2+}:		
Control (n=24)	16.9±0.91	3.7±0.23
Norm.(0/1h) (n=6)	11.4±1.49[1]/16.5±3.58	3.9±0.48/3.5±0.50
Hyp. (0/1h) (n=6)	11.1±3.04[1]/16.9±1.23	4.4±0.10/4.0±0.24
Hyp/r (0/1h) (n=6)	14.6±2.30/18.0±1.31	4.2±0.96/3.7±0.89

Values are mean±s.e.m. [1] indicates significant difference from control, [2] from norm. $P<0.05$, Students unpaired t-test.

The results indicate release of a stable, Ca^{2+}-dependent, antivasoconstrictor from endothelial cells maintained under hypoxic conditions which is unaffected by reperfusion.

Lefer, A.M. & Lefer, D.J. (1993) *Annu. Rev. Pharmacol. Toxicol.*, 33, 71-90.

110P EFFECTS OF CHANGES IN EXTRACELLULAR pH ON R(+)-BUPIVACAINE-INDUCED BLOCK OF hKv1.5 CHANNELS

M. Longobardo, E. Delpón, R. Caballero, J. Tamargo & C. Valenzuela. Institute of Pharmacology and Toxicology, CSIC/UCM. Universidad Complutense, Madrid, SPAIN.

Bupivacaine (B) is an amide type local anesthetic that blocks, in the same range of concentrations, both cardiac Na^+ and hKv1.5 channels (Valenzuela *et al.*, 1995a,b). B is a weak base (pKa=8.1) and, therefore, at physiological pH, it is predominantly charged. Moreover, B has a chiral carbon and, thus, it can be separated into R(+) and S(-) enantiomers. Block of hKv1.5 channels by B enantiomers has been attributed to the binding of their cationic form to the internal mouth of the ion pore (Franqueza *et al.*, 1997). This requires that the uncharged extracellular B penetrate the membrane and then, the cationic form (B^+) binds to its receptor site. In this study we have analyzed the effects of R(+)-bupivacaine (RB) on hKv1.5 channels at two different extracellular pH values (pH_o) (6.5 and 10.0), thus modifying the extracellular and intracellular concentrations of cationic (RB^+) and uncharged forms of RB.

Cloned human cardiac K^+ channels (hKv1.5) were stably transfected in *Ltk*$^-$ cells and the effects of RB on hKv1.5 currents were studied using the whole-cell configuration of the patch-clamp technique.

Consistent with a channel gating modification induced by H^+, the midpoint (V_h) of the activation curve was shifted to more positive potentials (+2.8±0.7 mV; n=21, $P<0.01$) at pH_o 6.5 and to more negative voltages (-23.1±1.3 mV; n=13, $P<0.01$) at pH_o 10.0 when compared with V_h values obtained at pH_o 7.4 (-14.2±2.1 mV; n=25). Potency of RB block of hKv1.5 channels increased at pH_o 10.0 and decreased at pH_o 6.5 with respect to that observed at pH_o 7.4 (Table 1). At pH_o 10.0, RB will accumulate in its charged form inside the cell with a negligible $[RB^+]_o$. Thus, RB block at pH_o 10.0 mostly represents block induced from the inside of the membrane, with a K_D of 1.7 µM, equivalent to $[RB^+]_i$=13.3 µM (Table 1). On the contrary, at pH_o 6.5 most RB will be present in its cationic form outside the cell, and the $[RB^+]_i$ at the K_D will be only 3.7 µM (Table 1). However, the degree of block induced by such different $[RB^+]_i$ was similar, suggesting that RB can also block hKv1.5 channels from their external side. In order to assess this hypothesis, we studied the effects of a permanent charged RB (RB*) on hKv1.5 channels at pH_o 7.4. Perfusion of the cells with 50 µM RB* induced 28.3±5.4% (n=4) block of hKv1.5 channels, this effect being reversible. At pH_o 6.5 and 10.0, RB block was time-dependent, inducing an exponential fast decline of the current at the beginning of the depolarizing pulse from which the association (k) and dissociation (l) rate constants were derived. Whereas k values increased with the pH_o, l values were similar at pH_o 6.5 and 7.4, and increased 2.5-fold at pH_o 10.0 (Table 1).

Table 1. K_D, k and l values of RB block at different pH_o.

pH_o	K_D (µM)	$[RB^+]_o$	$[RB^+]_i$	k ($\mu M^{-1}s^{-1}$)	l (s^{-1})
6.5	19.1±2.0	18.6 µM	3.7 µM	1.2±0.2	23.5±3.1
7.4	4.1±0.7	3.4 µM	5.4 µM	4.7±0.5	23.6±13.6
10.0	1.7±0.2	0.021 µM	13.3 µM	40.0±3.4	68.0±5.8

These results suggest that hKv1.5 channels exhibit two RB binding sites. Indeed, the effects of RB at pH_o 6.5 and those of RB* indicate that the drug can also block the channel from its external side.

Supported by CICYT SAF96-0042 and SAF98-0058 Grants.

Valenzuela *et al.* (1995a) *Biophys J* **69**, 418-427.
Valenzuela *et al.* (1995b) *Circulation* **92**, 3014-3024.
Franqueza *et al.* (1997) *Circ Res* **81**, 1053-1064.

111P INVOLVEMENT OF Ca^{2+} CHANNEL BLOCKADE IN NICORANDIL-INDUCED VASODILATION

F. Pérez-Vizcaíno, A. Cogolludo, S. Fajardo, F. Zaragozá-Arnáez, M. Ibarra & J. Tamargo. Dept. of Pharmacology. School of Medicine. U.C.M. 28040 Madrid, SPAIN.

Nicorandil (N) is an antianginal agent which causes vasodilation *via* two main mechanisms: it increases K^+ conductance and activates soluble guanylate cyclase (sGC). However, the blockade of Ca^{2+} channels may also be involved (Fujiwara and Angus, 1996). We studied and compared the mechanisms involved in N-induced vasodilation with those of levcromakalim (L), sodium nitroprusside (NP) and combinations with different proportion (1:10, 1:30 and 1:100) of NP:L.

Endothelium-denuded aortic rings from Wistar rats were incubated in Krebs solution (Pérez-Vizcaíno et al., 1993). Rings were contracted with 25mM KCl or noradrenaline (NA, 10^{-6}M) plus nifedipine (10^{-7}M) and concentration-response curves to NP, L, N or their combination were performed.

KCl-induced contractions (1307±37 mg, n=196) were fully relaxed by NP and L with pD_2 values of 8.45±0.13 and 7.12±0.06, respectively. The combinations 1:10, 1:30, 1:100 showed pD_2 values in terms of L of 7.75 ± 0.16, 7.43±0.04 and 7.15±0.04, respectively. The inhibitor of the sGC ODQ (10^{-6}M) markedly shifted the concentration-response curve to NP and N to the right and reduced the maximal relaxant reponse to NP, but it did not affect the response to L, while the K^+_{ATP} channel inhibitor glibenclamide (G, $3x10^{-6}$M) inhibited the relaxant responses to L but not those to NP or N. The shifts induced by these inhibitors were expressed as log dose-ratio (log DR) in Table 1. In the combinations, the log DR values to ODQ raised as the proportion of NP was greater. N presented similar values to those of the 1:30 combination. The log DR values to G were greater as the proportion of L in the combinations was augmented, whereas N presented similar values to those of NP. The log DR values to the combination of ODQ and G for N-induced relaxation were smaller than for any of NP:L combinations, suggesting the existence of an additional mechanism of action for N insensitive to ODQ and G. In rings contracted with NA (1701±93 mg, n=56) and subsecuently treated with nifedipine, which reduced the maximal response by 26 ± 4%, the log DR values to ODQ, G or its combination for N-induced relaxation of the contractions induced by NA were similar to those for the 1:30 combination.

Our results indicate that in the rat aorta N exerts its relaxant effects by activation of the sGC, opening of K^+ channels and, additionally, by blockade of Ca^{2+} channels.

Supported by a CICYT (SAF 96-0042) Grant.

Fujiwara & Angus (1996) *Br. J. Pharmacol.* **119**, 1549-1556.
Pérez-Vizcaíno et al. (1993) *Br. J. Pharmacol.* **110**, 1165-1171.

Table 1. Log DR values of ODQ, G and its combination in KCl and NA+nifedipine contracted rings relaxed by NP, L, 1:10, 1:30, 1:100 combinations and N.

	KCl						NA + nifedipine	
	NP	1:10	1:30	1:100	L	N	1:30	N
ODQ	2.24 ± 0.36	0.96 ± 0.21	0.73 ± 0.28	0.25 ± 0.08	0.17 ± 0.08	0.74 ± 0.19	0.71 ± 0.23	0.78 ± 0.25
G	0.007 ±0.35	0.42 ± 0.29	0.59 ± 0.14	0.88 ± 0.21	1.68 ± 0.16	-0.01 ± 0.29	0.42 ± 0.22	0.23 ± 0.37
ODQ+G	2.09 ± 0.24	2.29 ± 0.21	2.25 ± 0.27	1.85 ± 0.18	1.44 ± 0.10	1.26 ± 0.21	2.04 ± 0.26	2.11 ± 0.21

Dose-ratio (DR) were calculated as the ratio of the concentration of the vasodilator agent inducing a 30% relaxation.

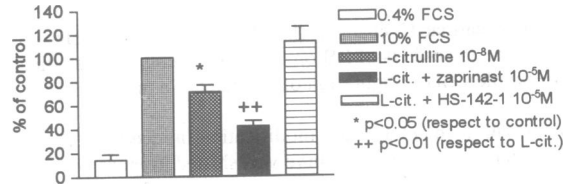
112P L-CITRULLINE, THE BY-PRODUCT OF NO SYNTHESIS, DECREASES VASCULAR SMOOTH MUSCLE CELL PROLIFERATION

Ruiz,E.,DelRio,M.*,Somoza,B.,Ganado,P.,Sanz,M. & Tejerina,T.Dept. of Pharmacology, School of Medicine, Complutense University, 28040 Madrid, Spain.* Present address: Department of Cellular and Molecular Biology, CIEMAT, Madrid, Spain.

Endothelial function is mediated by endothelial factors such as nitric oxide (NO), which is synthetised by the NO synthase through the conversion of L-Arg in NO and L-citrulline. It is well-known that NO inhibits vascular smooth muscle cell (VSMC) proliferation (Garg & Hassid,1989). On the other hand, Atrial Natriuretic Peptides (ANP) is a family of structurally related peptides with a potent vasodilatory and anti-mitogenic actions (Itoh et al.,1990) mediating their biological actions via the binding to a particulate guanylate cyclase and may function in parallel with NO system in the control of cardiovascular function. In a previous work, we demonstrated that L-citrulline relaxed rabbit aorta by increasing cGMP through ANPreceptor particulate guanylate cyclase-linked (pGC) stimulation (Ruiz et al.,1997). In the present study we tested the possibility that L-citrulline could inhibit VSMCs proliferation and whether this effect was related to an action on the particulate guanylate cyclase.

Rat A10 VSMCs (American Type Culture Collection (ATCC; A10 CRL 1476)) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS) supplemented with glutamax I, 100IU/ml penicillin G (sodium salt), 100 mg/ml streptomycin and 0.25 mg/ml amphotericin B (antibiotic-antimycotic solution, Gibco). To follow proliferation in the presence of L-citrulline (10^{-8} M), cells were seeded at $2.0x10^4$ cells/well, detached with trypsin, and counted in a haemocytometer. Throughout the experiments, media were changed daily. DNA synthesis was assayed by the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into nuclei (Campana et al.,1988). After cell attachment, the cells were incubated in DMEM + 0.4%FCS (48h) to obtain quiescent non-dividing cells and then were incubated in DMEM with 10% FCS (18h), containing vehicle alone or L-citrulline (10^{-10}-10^{-8} M) or L-citrulline (10^{-8} M) plus zaprinast (10^{-5} M) (a phosphodiesterase type V inhibitor) or plus HS-142-1 (10^{-5} M) (a pGC inhibitor). Intracellular cGMP measurements were made using the cGMP radioimmunoassay kit of Amershan Int. The results are expressed as the mean±s.e.mean. The statistical analysis of the data was done by Student's t test or by two way ANOVA when necessary.

L-citrulline 10^{-8} M significantly decreased rat aortic (A10 cell line) VSMCs proliferation. The percentage of inhibition exerted by L-citrulline on days 3, 5 and 7 of the growth curve was 20.0±0.5%; 37.5±8.3% and 28.5±7.2% ($F(1,10)=10.19$, $p<0.01$, n=3 duplicated) respectively. In addition, L-citrulline also inhibited serum-induced DNA-synthesis. BrdU incorporation into nuclei of vehicle-treated cells was 40.5±2.4%, while in L-citrulline-treated cells the percentage decreased to 36.0±4.1%; 29.1±2.0% ($p<0.01$,n=4) and 30.5±2.4% ($p<0.05$,n=4) with 10^{-10}, 10^{-9} and 10^{-8} M respectively. Zaprinast (10^{-5} M), enhanced the inhibitory effect of L-citrulline (10^{-8} M) on DNA synthesis. The BrdU incorporation in serum-stimulated cells dropped to 17.0±2.1% ($p<0.01$,n=3) from 30.5±2.4% in the presence of L-citrulline (10^{-8} M) or L-citrulline plus zaprinast. Moreover, L-citrulline inhibition of serum-stimulated DNA synthesis was abolished by HS-142-1 (10^{-5} M) as Figure 1 shows. In another group of experiments, L-citrulline was shown to increase intracellular cGMP levels from 2,1±0,2 pmol of cGMP/mg proteins to 4,1±0,1 L-citrulline 10^{-8} M ($p<0.001$,n=3).



These findings suggest that L-citrulline decreases VSMC proliferation in A10 cell line acting on DNA synthesis by mechanisms which imply the cGMP formation.

Campana, D. et al. (1988). *J. Immunol. Methods* **107**,79-88.
Garg, U.C. & Hassid, A. (1989). *J. Clin. Invest.* **83**,1774-77.
Itoh , V. et al. (1990). *J. Clin. Invest.* **86**,1690-97.
Ruiz, E. et al. (1997). *J. Vet. Pharmacol. Ther.* **20**,247-248.

113P ANTIPROLIFERATIVE EFFECTS OF INDAPAMIDE ON VASCULAR SMOOTH MUSCLE CELLS

Tejerina,T., Ganado,P., Del Rio,M*., Sanz,M. & Ruiz,E. Dept. of Pharmacology, Faculty of Medicine, Complutense University, 28040 Madrid, Spain.* Present address: Department of Cellular and Molecular Biology, CIEMAT, Madrid, Spain.

Abnormal vascular smooth muscle cell (VSMC) proliferation has a fundamental role in the pathogenesis of vascular diseases, such as atherosclerosis (Schwartz et al.,1986). Indapamide (IND), 4-chloro-N-(2-methyl-1-indoline)3-sulfamoylbenzamide, is an oral diuretic antihypertensive drug effective for patients with mild or moderate essential hypertension (Thomas,1985). In a previous study carried out in our group, we found that indapamide decreased the development of atherosclerosis lesions in cholesterol-fed rabbits (Del Rio et al.,1995). In this work, we tested whether indapamide had an antimitogenic effects on VSMC. Finally, we looked into the effects of the drug on the mRNA expression of the transcription factors c-*fos* as part of the early G_0/G_1 transition induced in quiescent VSMCs by mitogenic stimulation.

Rat A10 VSMCs [American Type Culture Collection (ATCC; A10 CRL 1476)] were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS) supplemented with glutamax I, 100IU/ml penicillin G (sodium salt), 100 mg/ml streptomycin and 0.25 mg/ml amphotericin B (antibiotic-antimycotic solution, Gibco). To follow proliferation in the presence of IND (500μM), cells were seeded at $2.0x10^4$ cells/well, detached with trypsin, and counted in a haemocytometer. Throughout the experiments, media were changed daily. DNA synthesis was assayed by the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into nuclei (Campana et al.,1988). After cell attachment, the cells were incubated in DMEM + 0.4% FCS (48h) to obtain quiescent non-dividing cells. The cultures were then incubated in DMEM with 10% FCS (18h), containing vehicle alone or IND (50μM). The results are expressed as the mean±s.e.mean. The statistical analysis of the data was done by Student's t test or by two way ANOVA when necessary.

The expression of c-*fos* mRNA was studied in serum deprived cells that were stimulated with 10% FCS in the presence or absence of IND (500μM) for 30min. Total RNA was extracted from the cells by acid-phenol procedure and was separated in a 6% formaldehyde-1.2% agarose gel, blotted onto hybond N+ membranes in 10x standard saline citrate (SSC) (consisting of 0.15 mM NaCl and 0.015 mM sodium citrate). Blots were hybridised to a random-primed specific DNA probe for a rat c-*fos* and were standardised with a complementary DNA probe for ß-actin.

Exposure to 500mM of IND showed an important stasis of the growth. The percentage of inhibition exerted by IND 500μM on days 4, 6 and 8 of the growth was 44.2±11.0%; 79.0±3.5% and 66.1±9.0% [$(F(1,10)=10.1, P<0.001, n=3$ duplicated] respectively. In addition, IND 50μM inhibited serum-stimulated DNA synthesis, assayed by the incorporation of BrdU into nuclei. BrdU incorporation decreased from 58.1±2.7% (in 10% FCS) to 27.1±7.0% ($P<0.01, n=4$) in IND-treated cells. Accordingly, IND reduced the entrance of cells into S phase as evaluated by flow cytometry. IND decreased the percentage of cells in S phase after 18 h of stimulus (39.2±0.5% in control and 28.2±2.6% ($P<0.05, n=3$) in the presence of IND 500μM. These results suggest that IND may exert an antiproliferative effect on vascular smooth muscle by blocking FCS-induced cell cycle progression in a step near the G_0/G_1 boundary.

Campana et al., (1988). *J. Immunol. Methods* **107**,79-88.
Del Rio et al.,(1995) *J.Cardiovasc. Pharmacol.* **25**,973-978.
Schwartz et al.,(1986). *Circ. Res.* **58**, 427-444.
Thomas, (1985). *Hypertension* **II**, 152-156.

114P DIRECT EFFECTS OF LOSARTAN AND ITS ACTIVE METABOLITE ON A HUMAN CARDIAC CLONED POTASSIUM CHANNEL (Kv1.5)

E. Delpón, R. Caballero, C. Valenzuela, M. Longobardo & J. Tamargo. Department of Pharmacology. School of Medicine. U.C.M. Madrid, Spain.

Losartan (L) is an specific angiotensin II type 1 receptor antagonist. It has been demonstrated that L reduced the mortality in patients with symptomatic heart failure, an effect primarily due to a reduction in sudden cardiac death (ELITE study, 1997). This reduction suggests that L may exert an antiarrhytmic effect. Moreover, L is converted into an active metabolite, E-3174 (E). Information about direct effects of L or E on cardiac ionic channels is scarce. Thus, the present study was undertaken to determine whether L or E block hKv1.5 channels, a *Shaker*-related K^+ channel cloned from human ventricle (Snyders *et al.*, 1993). The current generated by these channels is the counterpart of the ultrarapid delayed rectifier described in native human atrial cells (Wang *et al.*, 1993).

The experiments were performed at room temperature on *Ltk*$^-$ cells stably transfected with the gene encoding the sequence of the hKv1.5 channel. Currents were recorded using the whole-cell configuration of the patch clamp technique (Delpón *et al.*, 1997).

Both L (1 μM) and E (1 μM) initially increased the maximum outward current elicited by depolarizations to +60 mV by 8.2 ± 1.7% and 7.4 ± 1.6%, respectively. Thereafter, both agents inhibited the hKv1.5 currents. L decreased the peak current amplitude by 9.9 ± 1.6% and induced a slow decline (τ= 80.5 ± 10.3 ms) to a new steady state level, so that the reduction at the end of the 500 ms pulses averaged 25.1 ± 3.1%. L-induced block was frequency-dependent, the blockade increasing under conditions of repetitive stimulation (36.5 ± 7.8% at 2 Hz, n=6, P<0.05). E (1 μM) reduced the peak current and the current amplitude at the end of 500 ms-pulses to +60 mV by 13.2 ± 2.6% and 37.2 ± 4.8%, respectively. Similarly to L, E also induced a frequency-dependent block which averaged 48.9 ± 7.5% at a frequency of 2 Hz.

In the absence of L, the midpoint (V_h) and the slope factor (k) values of the activation curve were -22.5 ± 1.6 mV and 5.9 ± 0.4 mV, respectively. In the presence of L, the activation curve exhibited two components, the steeper component (V_h= -26.9 ± 2.2 mV; k=5.8 ± 0.5 mV) was responsible of 75% of the activation process and it was followed by a shallow component (k=52.5 ± 7.5 mV) which did not reach saturation at the voltage range tested (V_h=107.2 ± 12.5 mV). Similar results were obtained in the presence of E, the V_h values for the steeper (k=4.9 ± 1.5 mV) and for the shallow (k= 40.3 ± 4.7 mV) components being -26.3 ± 1.5 mV and 110.8 ± 8.0 mV, respectively. L increased the time constant of decline of tail currents elicited on return to -40 mV after +60 mV depolarizations from 48.7 ± 5.2 ms to 99.2 ± 7.6 ms (n=10, P<0.01). E also slowed the time course of tails deactivation from 56.4 ± 6.4 ms to 111.2 ± 13.5 ms (n=8, P<0.01). Thus, both L and its active metabolite induced the tail current "crossover phenomenon".

The present results demonstrated that both L and E modified hKv1.5 channel gating and blocked these channels in a frequency-, time- and voltage-dependent manner.

Supported by CICYT SAF-96-0042 and SAF 98-0058 Grants.

ELITE study (1997) *Lancet* **349**, 747-752.
Delpón E. *et al.* (1997) *Cardiovasc. Res.* **35**, 341-350.
Snyders D. *et al.* (1993) *J. Gen. Physiol.* **101**, 513-543.
Wang Z. *et al.* (1993) *Circ. Res.* **73**, 1061-1076.

115P EFFECT OF OUABAIN AND 1α,2α-EPOXYSCILLIROSIDIN ON VENTRICULAR FIBRILLATION THRESHOLD IN LANGENDORFF-PERFUSED GUINEA-PIG HEART

M.F.Perkin, S-A Boschmans, G De Jager & [1]G.E. Swan (introduced by I.E. Hughes) Pharmacy Dept, University of Port Elizabeth P.O Box 1600 P.E 6000 & [1]Faculty of Veterinary Science, Onderstepoort, Private Bag X04, Onderstepoort 0110, South Africa.

Poisoining of animals due to ingestion of species of Iridiaceae (*Homeria* and *Moraea* spp:), Liliaceae (*Urginia* spp:) and Crassulaceae (*Tylecodon* spp:) periodically causes substantial livestock loss in South Africa. The compound 1α,2α-epoxyscillirosidin (EPS) and a series of structurally related bufadienolides isolated from these plants (Naude 1977) cause death by cardiac glycoside poisoning in these animals. We examined the hypothesis that EPS may increase susceptibility to fatal ventricular arrhythmia as one of the possible mechanisms of death.

The inotropic and arrhythmogenic activities of EPS and ouabain were compared in Langendorff-perfused hearts dissected from ether-anaesthetised Duncan Hartley guinea pigs (250 - 350 g). Perfusion conditions were oxygenated Krebs-Henseleit buffer (95% O_2, 5% CO_2) at 100 cm H_20, 30° C and pH 7.4. Hearts were paced at 260 beats min^{-1} in all experiments. Cumulative inotropic dose-response curves were performed on EPS and ouabain at a perfusate Ca^{2+} concentration of 1.25 mM. Increases in left ventricular systolic pressure, measured from an intraventricular balloon preloaded to 10 mm Hg diastolic pressure, were taken as an indicator of inotropic activity. Lowering of the ventricular fibrillation threshold (VFT) by EPS and ouabain was used as an indicator of increased susceptibility to electrically induced arrhythmia in hearts perfused at a Ca^{2+} concentration of 2.5 mM. Stimulus parameters were 200 ms trains of 12 pulses (2 ms width) delivered via parallel platinum electrodes placed 10 mm apart into the epicardium of the left ventricle. Stimuli of increasing current strength were delivered 10 ms after the R wave recorded from a two lead ECG. Stimuli were applied at 2 minute intervals. In VFT experiments preload was adjusted to give an initial peak left ventricular systolic pressure of 70 mm Hg prior to administration of drug so that the arrhythmogenic activity of EPS and ouabain could be compared in hearts that were performing the same amount of work.

Results are ± s.e.mean. Inotropic responses are expressed as mean peak left ventricular end systolic pressure (PLVESP) or mean increase in peak left ventricular end systolic pressure (ΔPLVESP) in mm Hg.

EPS (EC_{50} 0.093±0.005μM; n=7) and ouabain (EC_{50} 0.29±0.037μM; n = 10) produced maximum inotropic responses (ΔPLVESP) of 46.57 ± 4.68 vs 52.5±4.64 mm Hg. The difference was not significant (P > 0.4, t-test).

In control hearts (n=6) perfused under identical conditions to glycoside-perfused hearts, VFT remained stable over the duration of the experimental period. Equally effective inotropic doses of EPS (0.2μM - PLVESP 80±7.40 mm Hg) and ouabain (0.4 μM - PLVESP 76.7±5.58 mm Hg), were similarly arrhythmogenic. EPS decreased VFT from 15.63±0.92 to 9.13±0.95 mA (n=8; P<0.001, paired t-test) while ouabain lowered VFT from 15.0±2.25 to 8.25±1.31 mA (n =8; P<0.0001, paired t-test).

These results indicate that increased susceptibility to ventricular arrhythmia may be one of the factors that contribute to death of livestock that graze on plants containing cardioactive bufadienolides in South Africa.

Naude, T.W. (1977) *S Afr Biol Soc* **18**, 7-18

116P ALPHA-PRESSOR RESPONSES IN PITHED RATS FED ON A HIGH-CALCIUM DIET

B. Civantos, V. López-Miranda, A. Ortega & M.A. Aleixandre Dpto. Farmacología. Fac. Medicina, U. Complutense, 28040 Madrid, España.

Studies carried out in our laboratory showed that a high-calcium diet (Ca 2.5%) did not modify the arterial blood pressure (tail cuff method) of normotensive Sprague-Dawley rats (SDR), but caused a decrease in the arterial blood pressure of spontaneously hypertensive rats (SHR), when compared with control animals fed on a similar semi-synthetic casein diet with a normal calcium content (Ca 1%). The maximum difference in the arterial blood pressure between SHR fed on the Ca 1% diet and SHR fed on the Ca 2.5% diet was observed in 14-week-old animals. In this study, after being weaned at 3 weeks, male animals of both strains were randomized with "ad libitum" intake of the Ca 2.5% or the Ca 1% diet, and then we evaluated the pressor responses of the α_1-adrenoceptor agonist, methoxamine (MTX) (10-3000 μg/kg) and the pressor responses of the α_2-adrenoceptor agonist, B-HT 920 (3-1000 μg/kg) in the pithed rat preparation (Shipley and Tilden, 1947), using 9-week-old (adult age) SDR, 14-week-old SHR and 20-week-old SHR. The increases in the systolic (SBP) and the diastolic (DBP) arterial blood pressure were measured using a Panlab 8C Datasystem. Results are expressed as mean values ± s.e. mean for 6-8 rats. α-Adrenoceptor agonist dose-response curves were constructed, and the effect of the dietary calcium content on α-pressor responses was expressed as the area under each dose-response curve (AUC), taking the AUC for the control mean values as 100. For comparison of the areas, Student's t-test was used.

The Ca 2.5% diet decreased the α-adrenoceptor agonist pressor responses in both strains (see table 1). The present results suggest that a high-calcium diet causes an important decrease in the pressor responses mediated by the stimulation of vascular α-adrenoceptors in normotensive and hypertensive rats, even though the increase in dietary calcium may only modify the arterial blood pressure in the hypertensive animals.

Supported by the U.C.M. (PR 156/97-7099) grant.

Shipley, R.E. & Tilden, J.H. (1947) Proc. Soc. Exp. Biol. Med. 64, 453-455.

TABLE 1. AUC of the increases in SBP (AUC-SBP) and DBP (AUC-DBP) caused by α-adrenoceptor agonists in SDR and SHR pithed rats fed on two diets with a different calcium content.

	% Dietary Ca	
	1	2.5
9-week-old SDR		
MTX AUC-SBP	100.0 ± 2.1	61.2 ± 5.5***
MTX AUC-DBP	100.0 ± 8.1	50.3 ± 4.2***
B-HT 920 AUC-SBP	100.0 ± 4.9	64.1 ± 7.2***
B-HT 920 AUC-DBP	100.0 ± 3.0	64.5 ± 6.3***
14-week-old SHR		
MTX AUC-SBP	100.0 ± 11.8	95.9 ± 12.0
MTX AUC-DBP	100.0 ± 10.2	111.2 ± 20.0
B-HT 920 AUC-SBP	100.0 ± 13.0	117.9 ± 16.7
B-HT 920 AUC-DBP	100.0 ± 13.2	105.1 ± 14.6
20-week-old SHR		
MTX AUC-SBP	100.0 ± 2.2	44.4 ± 8.6**
MTX AUC-DBP	100.0 ± 2.4	47.0 ± 5.9***
B-HT 920 AUC-SBP	100.0 ± 15.2	25.4 ± 2.4***
B-HT 920 AUC-DBP	100.0 ± 14.5	25.2 ± 2.0***

The asterisks show significant differences compared with animals fed on the Ca 1% diet (*p<0.05; **p<0.01; ***p<0.001).

117P INVOLVEMENT OF ENDOTHELIN AND ANGIOTENSIN II IN THE PRESSOR EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITION IN CONSCIOUS, HYPERTENSIVE, TRANSGENIC [(mRen-2)27] RATS

Shalynne Ishak, T. Bennett and S.M. Gardiner. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

In rats made hypertensive by introduction of the mouse Ren-2 gene (TG rats; Mullins *et al.*, 1990), the high mean arterial pressure (MAP) is maintained, not only by angiotensin II (AII), but also by endothelin (ET) (Gardiner *et al.*, 1995). In conscious normotensive rats, the acute pressor response to nitric oxide synthase (NOS) inhibition is, in part, mediated by ET, but not AII (Banting *et al.*, 1996). However, in TG rats, chronic treatment with the AT_1 receptor antagonist, losartan, inhibited the pressor response to NOS inhibition (Moriguchi *et al.*, 1994).

In the present study, we have examined the effect of acute, AT_1-receptor antagonism, with losartan (L), ET-receptor antagonism, with SB 209670 (SB), and combined treatment (L + SB), on the pressor response to N^G-nitro-L-arginine methyl esther (L-NAME) in conscious, male, heterozygous, TG rats (400-600 g), instrumented with intravascular catheters 24 h previously (surgery under sodium methohexitone anaesthesia (40-60 mg kg^{-1}, i.p., supplemented as required)). Six h after i.v. pretreatment with saline (0.1 ml; n = 9), L (10 mg kg^{-1}, n = 9), SB (bolus: 300 µg kg^{-1}; infusion 5µg kg^{-1} min^{-1}, n = 9) or both antagonists (doses as above, n = 8), L-NAME (10 mg kg^{-1}, i.v.) was administered, and cardiovascular variables were monitored over 40 min.

There were no differences between resting cardiovascular variables in the 4 groups (Table 1). During treatment with either L or SB, the fall in MAP was similar (-17 ± 4 mm Hg and -21 ± 4 mm Hg, respectively), whereas in rats given both L and SB together, the fall in MAP (-46 ± 6 mm Hg) was significantly greater (Kruskal-Wallis test) than in those given L or SB alone (Table 1). Only in rats treated with L and SB together was the MAP response to L-NAME smaller than that in animals treated with saline (Table 2).

Table 1. Mean arterial pressure (MAP; mm Hg) and heart rate (HR; beats min^{-1}) under resting conditions (control) and 360 m after administration of saline (Sal), losartan (L), SB 209670 (SB) or both (L + SB) in conscious TG rats. Values are mean ± s.e. mean; * P < 0.05 versus control (Friedman's test).

		Sal	L	SB	L + SB
HR	Control	309 ± 11	287 ± 4	304 ± 6	301 ± 9
	360 min	305 ± 9	317 ± 8*	324 ± 9	359 ± 8*
MAP	Control	167 ± 7	160 ± 2	161 ± 6	166 ± 5
	360 min	164 ± 7	143 ± 4*	140 ± 4*	120 ± 6*

Table 2. Integrated (area under (for MAP) or over (for HR) $curves_{0\text{-}40\ min}$) responses to L-NAME (10 mg kg^{-1}) in conscious TG rats, 360 min after treatment. For abbreviations and treatment groups, see Table 1. * P < 0.05 vs Sal (Kruskal-Wallis test). Units for HR = beats; units for MAP = mm Hg min.

	Sal	L	SB	L + SB
HR	943 ± 135	1766 ± 307*	2220 ± 222*	2615 ± 272*
MAP	1704 ± 150	1548 ± 141	1738 ± 137	1226 ± 101*

From these results it appears that neither AII nor ET, independently, contribute significantly to the pressor response to L-NAME in TG rats. It is feasible that the pronounced antihypertensive effect of L and SB together was responsible for the diminution of the subsequent pressor response to L-NAME.

S.I. was supported by Universiti Sains Malaysia.

Banting, J.D. *et al.* (1996). Hypertension, 14, 975-981.
Gardiner, S.M., *et al.* (1995). Br.J.Pharmacol., 116, 2237-2244.
Moriguchi, A. *et al.* (1994). Am.J.Physiol., 266, R1273-R1279.
Mullins, J.J. *et al.* (1990). Nature, 344, 541-544.

118P REGIONAL HAEMODYNAMIC EFFECTS OF SIBUTRAMINE HYDROCHLORIDE IN CONSCIOUS, LONG-EVANS RATS

S.M. Gardiner, P.A. Kemp, J.E. March, K.F. Martin*, D.J. Heal*, and T. Bennett. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, and *Knoll Pharmaceuticals Research & Development, Nottingham NG1 1GF.

Sibutramine hydrochloride monohydrate (BTS 54 524; N-1-{[1-(4-chlorophenyl)-cyclobutyl]-3-methylbutyl}-N,N-dimethylamine hydrochloride monohydrate) is a potent inhibitor of the uptake of serotonin and noradrenaline *in vivo* (Buckett *et al.*, 1988), and causes hypophagia in rats (Fantino & Souquet, 1995). Sibutramine is being developed for the treatment of obesity and, consistent with its activity as a serotonin and noradrenaline reuptake inhibitor, modest and dose-related mean increases in vital signs have been observed (see Lean, 1997 for review). Therefore, we assessed haemodynamic responses to sibutramine in conscious, male, Long-Evans rats (350-450 g, n = 6) instrumented with intravascular catheters and pulsed Doppler probes (to monitor renal, mesenteric and hindquarters flows). All surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1}, supplemented as required). Animals were studied on 3 consecutive days; on day 1 sibutramine was given i.p. at a dose of 3 or 9 mg kg^{-1}, on day 2 saline was given in the same volume (475 µl), and on day 3 sibutramine was administered at the dose not given previously.

Table 1 summarises some of the results, after injection of vehicle or sibutramine at 9 mg kg^{-1} i.p. Prior to injection, resting variables were not different (Heart rate (beats min^{-1}): vehicle, 327 ± 7 (mean ± s.e. mean); sibutramine, 319 ± 11. Mean blood pressure (mm Hg): vehicle, 104 ± 2; sibutramine 105 ± 4. Vascular conductance ([kHz mm Hg^{-1}]10^3): Renal: vehicle, 80 ± 15; sibutramine, 84 ± 16. Mesenteric: vehicle, 54 ± 6; sibutramine, 59 ± 6). Hindquarters: vehicle, 44 ± 7; sibutramine, 48 ± 6). Over the time course shown, vehicle had no significant cardiovascular effects (Table 1). However, sibutramine caused an early, transient

Table 1. Changes in cardiovascular variables following injection of vehicle (V) or sibutramine (S) at a dose of 9 mg kg^{-1}. Values are mean ± s.e. mean, n = 6; *P < 0.05 versus original baseline (Friedman's test).

		Time after injection (min) 5	10	15	20
Δ Heart rate (beats min^{-1})	V	12±3	8±3	10±4	8±7
	S	37±10*	22±11*	5±9	3±11
Δ Mean blood pressure (mm Hg)	V	0±2	-1±1	-1±1	0±1
	S	9±2*	1±3	-2±3	-3±3
Δ Renal conductance (%)	V	3±2	6±2	4±2	4±2
	S	-6±2	-1±2	0±3	-3±2
Δ Mesenteric conductance (%)	V	7±2	7±5	5±4	3±5
	S	14±7*	31±10*	27±6*	16±6*
Δ Hindquarters conductance (%)	V	1±7	1±5	3±6	-2±6
	S	7±7	9±5	9±4	0±4

tachycardia and pressor effect, with no significant changes in renal or hindquarter haemodynamics (Table 1). In contrast, there were marked increases in mesenteric flow and vascular conductance which were significant for 20 min following injection of sibutramine (Table 1). It is feasible that the selective mesenteric hyperaemic vasodilator action of sibutramine contributes to its hypophagic effect.

Buckett, W.R. *et al.* (1988). Prog.Neuro-Psychopharmacol. Biol.Psychiat., **12**, 575-584.
Fantino, M. & Souquet, A.-M. (1995). Int.J.Obes., **19**, 145.
Lean, M.E.J. (1997). Int.J.Obes., **21**, S30-S36.

119P EFFECTS OF ACTIVE IMMUNISATION AGAINST ANGIOTENSIN PEPTIDES ON THE PRESSOR EFFECTS EXOGENOUS ANGIOTENSIN I (AI) IN CONSCIOUS RATS

S.M. Gardiner, T.R. Auton*, M.R. Downham*, H.L. Sharp*, P.A. Kemp, J.E. March, A. Rushton*, T. Bennett and J.F. Glover*. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Proteus Molecular Design Ltd., Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire, SK11 OJL.

Although passive immunisation with antibodies to angiotensin II (AII) has been shown to suppress the pressor effect of exogenous AII (Reilly *et al.*, 1988), the influence of active immunisation has received little attention (Michel *et al.*, 1989). In the present work we assessed the influence of active immunisation with angiotensin peptide analogue immunoconjugate vaccines, on pressor responses to exogenous AI.

Male, Sprague Dawley rats (initially 200-250 g; Harlan Olac; n = 6 in all groups) were injected s.c. (0.5 ml) with saline or immunotherapeutic vaccines as follows: Groups A (saline), B (AII analogue, tetanus toxoid (TT) carrier protein, 5 μg), C (AI analogue, TT, 5 μg), D (AII analogue, diphtheria toxin, 5 μg), E (AII analogue, Keyhole Limpet haemocyanin, 5 μg), F (AII analogue, TT, 5 μg), G (AI analogue and AII analogue, TT, 2 x 2.5 μg each), H (AII analogue, TT, 25 μg), J (AI analogue, TT, 25 μg), K (AII analogue, TT, 5 μg) and L (AI analogue, TT, 5 μg). With the exception of Group F, the vaccine included aluminium hydroxide as an adjuvant; in Group F the adjuvant was diethylaminoethyl cellulose. Groups A-J were injected on days 0, 21 and 42; Groups K and L were injected on days 0, 14 and 28. On day 61, under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p., supplemented as required), catheters were implanted in the distal abdominal aorta (via the ventral caudal artery) and right jugular vein. The following day, conscious rats were given increasing i.v. bolus (0.1 ml) doses of AI (3 - 60 pmol rat^{-1}), while mean systemic arterial blood pressure and heart rate were recorded. At the end of the experiment animals were given i.v. sodium pentobarbitone (100 mg) and a blood sample was taken by cardiac puncture for the measurement of AI antibodies by ELISA.

Table 1. Median AI bolus dose (pmol rat^{-1}) to achieve half-maximal increase in mean blood pressure (ED_{50}) in control (group A) and immunised (groups B-L) rats. Significance probabilities adjusted for multiple comparisons by Dunnett's method (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).

Treatment	Median ED_{50}	Mean treatment-induced dose shift
A	8.9	-
B	39.6	4.5*
C	79.1	8.9***
D	19.6	2.2
E	17.6	2.0
F	15.2	1.7
G	24.5	2.8
H	38.2	4.3*
J	74.7	8.4***
K	13.9	1.6
L	43.0	4.8*

Table 1 summarises some of the results for mean arterial pressure changes. The median ED_{50} for AI was shifted significantly in 5 out of the 10 immunised groups, the biggest reduction in AI sensitivity being almost 9-fold (Group C, Table 1).

With all angiotensin immunotherapeutic formulations, there was a considerable induction of anti-angiotensin antibodies, concomitant with the inhibition of the pressor effects of AI.

The present findings raise the possibility that active immunisation against angiotensin peptides may be a potentially useful, novel approach to treatment of hypertension, and other cardiovascular conditions.

Michel, J.-B. *et al.* (1989). Am.Heart J., **117**, 756-767.
Reilly, T.M. *et al.* (1988). J.Pharmacol.Exp.Ther., **244**, 160-165.

120P EVIDENCE FOR INVOLVEMENT OF CALCITONIN GENE-RELATED PEPTIDE (CGRP), BUT NOT ADRENOMEDULLIN (ADM), IN LIPOPOLYSACCHARIDE (LPS)-INDUCED VASODILATATION IN CONSCIOUS RATS

S.M. Gardiner, P.A. Kemp, J.E. March and T. Bennett. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

In endotoxaemia, increased plasma levels of the vasodilator peptides CGRP and ADM have been reported (eg. Wang *et al.*, 1992; Shoji *et al.*, 1995 for CGRP and ADM, respectively), and there is some functional evidence that CGRP may contribute to the severe hypotension associated with septic shock (Huttemeier *et al.*, 1993). We have previously described the regional haemodynamic profile seen during more modest endotoxaemia in conscious rats, in which a complex interplay between vasodilator and vasoconstrictor mechanisms maintains mean arterial pressure (MAP) within a relatively normal range (Gardiner *et al.*, 1996). However, in that model, inhibition of the expression of inducible nitric oxide synthase (and possibly cyclooxygenase) with dexamethasone, together with antagonism of the cardiovascular actions of endothelin, by primed infusion of the non-selective endothelin antagonist, SB 209670, reveals a marked vasodilatation of unknown mechanism (Gardiner *et al.*, 1996). In the present study we have explored the possible involvement of CGRP or ADM in that vasodilatation, by use of the peptide antagonists, $CGRP_{8-37}$ and ADM_{22-52}, at doses we have shown to be effective against the cardiovascular actions of CGRP (Gardiner *et al.*, 1990) or ADM (Gardiner *et al.*, 1997), respectively. Three groups of male Long Evans rats (350-450 g) were instrumented with miniature pulsed Doppler flow probes for recording renal (R), superior mesenteric (M) and hindquarters (H) blood flows, and, 2-3 weeks later, with intravascular catheters for recording MAP and heart rate (HR). All surgery was performed under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p. supplemented) and experiments began 24 h after catheterization. All rats were treated i.v. with dexamethasone (12.5 μg kg^{-1} h^{-1}) and SB 209670 (300 μg kg^{-1} bolus; 300 μg kg^{-1} h^{-1} infusion) starting 1 h before infusion of LPS (150 μg kg^{-1} h^{-1}; *E coli* serotype 0127 B8, Sigma). Six h after the onset of LPS infusion, an i.v. infusion of either saline (Group a; 0.4 ml h^{-1}; n = 7), $CGRP_{8-37}$ (Group b; 100 nmol kg^{-1} min^{-1}; n = 9) or ADM_{22-52} (Group c; 500 nmol kg^{-1} min^{-1}; n = 7) was given for 20 min.

Prior to any intervention there were no significant differences in the resting cardiovascular variables (Groups a, b and c, respectively: mean ± s.e. mean HR (beats min^{-1}) 358 ± 7, 360 ± 14, 332 ± 11; MAP (mm Hg) 100 ± 1, 101 ± 1, 99 ± 1; vascular conductance ([kHz mm Hg^{-1}]10^3) R: 83 ± 9, 80 ± 9, 68 ± 6; M = 81 ± 4, 84 ± 5, 70 ± 7; H: 49 ± 3, 50 ± 2, 51 ± 3). After 6 h of LPS infusion, in the presence of dexamethasone and SB 209670, there was tachycardia, hypotension and marked vasodilatation in all 3 vascular beds (Groups a, b and c, respectively: Δ HR (beats min^{-1}) 84 ± 5, 86 ± 14, 111 ± 9; Δ MAP (mm Hg) -19 ± 2, -17 ± 3, -13 ± 2; Δ vascular conductance ([kHz mm Hg^{-1}]10^3)R = 55 ± 10, 48 ± 7, 39 ± 5; M = 54 ± 6, 61 ± 7, 42 ± 6; H 23 ± 4, 32 ± 6, 21 ± 3). After 20 min of saline or ADM_{22-52} infusion there were no further changes in any cardiovascular variable, whereas during the 20 min infusion of $CGRP_{8-37}$ there was a significant ($P < 0.05$ Friedman's test) rise in MAP (5 ± 1 mm Hg) associated with significant vasoconstriction in all 3 vascular beds (Δ vascular conductance ([kHz mm Hg^{-1}]10^3) R -17 ± 2, M -26 ± 5, H -5 ± 2). These results indicate that CGRP, but not ADM, may contribute to vasodilatation in this model of endotoxaemia.

This work was supported by the MRC.

Gardiner, S.M., *et al.* (1990). Biochem.Biophys.Res.Comm., 171, 938-943.
Gardiner, S.M., *et al.* (1996). Br.J.Pharmacol., 118, 141-149.
Gardiner, S.M., *et al.* (1997). Br.J.Pharmacol., 122, 115P.
Huttemeier, P.C., *et al.* (1993). Am.J.Physiol., 265, H767-H769.
Shoji, H., *et al.* (1995). Biochem.Biophys.Res.Comm., 215, 531-537.
Wang, X., *et al.* (1992). Circ.Shock, 36, 21-30.

121P CHANGES IN PACKED CELL VOLUME (PCV) AND PLASMA GLUCOSE AND TUMOUR NECROSIS FACTOR (TNF)-α LEVELS DURING INFUSION OF LIPOPOLYSACCHARIDE (LPS) IN CONSCIOUS RATS

S.M. Gardiner, P.A. Kemp, J.E. March, J. Woolley* and T. Bennett. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, and *Therapeutic Antibodies, Charterhouse Square, London, EC1M 6BQ, U.K.

Bolus injection of LPS causes a rapid rise in PCV in rats (e.g., Allcock & Warner, 1997; Filep *et al.*, 1997), and it has been suggested this is due to an increase in capillary hydrostatic pressure, in spite of the fact that systemic arterial blood pressure falls (Allcock & Warner, 1997). The rise in PCV is due, in part, to an action of endothelin on ET_A-receptors (Allcock & Warner, 1997).

It is apparent that bolus injection of large doses of LPS causes cardiovascular effects quite different from those seen during infusion of low doses of LPS (cf Allcock & Warner, 1997; Gardiner *et al.*, 1995a, b), and hence it is feasible that LPS-induced changes in the blood will vary according to the mode of administration of LPS. Therefore, we assessed the effects of LPS infusion on PCV and plasma levels of TNF-α and glucose, since there is evidence that TNF-α influences glucoregulation (e.g., Evans *et al.*, 1989).

Male, Long Evans rats (350-450g) were anaesthetised (sodium methohexitone, 40-60 mg kg^{-1} i.p., supplemented as required) and had intravascular catheters implanted. The following day animals were infused with saline (0.4 ml h^{-1}) or LPS (150 μg kg^{-1} h^{-1}; *E.coli*, serotype 0127:B8, Sigma). PCV was measured (from the microhaematocrit) before and 2, 6 and 24 h after onset of saline or LPS infusion (n = 6 in both groups). In other animals, measurement of plasma glucose (by Reflomat) and TNF-α (by ELISA; Factor-Test-X kit, Genzyme, U.S.A.) was carried out before and 1, 3, 5, 7 and 24 h (n = 8 for TNF-α levels and n = 4 (of the same group) for glucose levels), or 2, 4, 6, 8 and 24 h (n = 9 for TNF-α levels and n = 7 (of the same group) for glucose levels) after onset of LPS infusion. In animals (n = 4) receiving saline, plasma glucose and TNF-α were measured before and 1.5, 3.5, 5.5, 7.5 and 24 h after onset of infusion.

During infusion of LPS, PCV fell (control: 43 ± 0.6; 2 h: 41 ± 0.4; 6 h: 36 ± 0.6; 24 h: 31 ± 0.4%; mean ± s.e. mean, $P < 0.05$ (at 6 and 24 h) (Friedman's test)), but the changes were not different from those in animals receiving saline (control: 43 ± 0.6; 2 h: 40 ± 0.7; 6 h: 38 ± 0.4; 24 h: 34 ± 0.8%). Plasma TNF-α was undetectable before infusion of LPS or saline. There was a peak in plasma TNF-α (10290 ± 2543 ng l^{-1}) 1 h after the onset of LPS infusion; by 2 h, levels had fallen to 2201 ± 539 ng l^{-1}, and by 3 h, plasma TNF-α was not different from that in saline infused rats, in which it was undetectable. Plasma glucose showed no consistent change during LPS infusion (control: 6.3 ± 0.4; 2 h: 5.8 ± 0.3; 4 h: 6.0 ± 0.3; 6 h: 5.5 ± 0.2; 8 h: 6.0 ± 0.2; 24 h: 5.6 ± 0.2 mmol l^{-1}).

The present results indicate that, despite evidence for an important involvement of endothelin in the cardiovascular responses to LPS infusion in conscious rats (Gardiner *et al.*, 1995a), this does not result in a rise in PCV, in contrast to the effects of bolus injection of LPS (Allcock & Warner, 1997). Moreover, although LPS infusion causes a clear increase in plasma TNF-α, this is not reflected in a consistent change in plasma glucose (see also Ou *et al.*, 1996).

Allcock, G.H. & Warner, T.D. (1997). Life Sci., **60**, 271-276.
Evans, D.A. *et al.* (1989). Am.J.Physiol., R1182-R1189.
Filep, J.G. *et al.* (1997). Circ.Res., **81**, 840-847.
Gardiner, S.M. *et al.* (1995a). Br.J.Pharmacol., **116**, 1718-1719.
Gardiner, S.M. *et al.* (1995b). Br.J.Pharmacol., **116**, 2005-2016.
Ou, J. *et al.* (1996). Am.J.Physiol., **271**, G621-G628.

122P POTASSIUM- AND α_1-ADRENOCEPTOR-INDUCED CONTRACTILE ACTIVITY OF SPONTANEOUSLY HYPERTENSIVE RAT AORTA IN THE PRESENCE OF DIFFERENT CALCIUM CONCENTRATIONS

A. Ortega, M.A. Aleixandre, V. López-Miranda, & B. Civantos Dpto. Farmacología. Fac. Medicina, U. Complutense, 28040 Madrid, España.

It has been known for some time that an increase in extracellular Ca^{2+} causes a decrease in the contractile activity of the rat aorta (Weeb et al., 1978). Since hypertensive blood vessel responses are usually abnormal, we studied the contractile responses to KCl and selective α_1-adrenoceptor agonists (methoxamine and phenylephrine) in the spontaneously hypertensive rat (SHR) aorta with different extracellular Ca^{2+} concentrations. Experiments were performed on the aorta rings from 20-week-old SHR. The preparations were suspended in organ baths at 37° C and constantly bubbled with 95% O_2 and 5% CO_2. A low-bicarbonate physiological salt solution (pH 7.3) was used to prevent Ca^{2+} precipitation when using high concentrations of this ion in the experiments. The preparations were mounted with a resting tension of 2 g and allowed to equilibrate with 2.5 mM Ca^{2+} in the medium for a 90-min period before starting the experiments. In a batch of experiments successive contractions were induced by KCl (30 mM and 80 mM) in solutions with increasing Ca^{2+} concentrations (1.25 mM - 10 mM) in which the tissue was incubated for 15 min before the administration of KCl. In another batch of experiments the preparations were first contracted by 80 mM KCl in the 2.5 mM Ca^{2+} solution and washed with the same solution until the basal tension was reestablished. Then the rings were incubated for 15 min in the solution in which the α_1-adrenoceptor agonist responses were to be evaluated and the drug was administered subsequently in increasing cumulative doses. Solutions with 1.25 mM - 10 mM Ca^{2+} were also used for these evaluations and only one dose-response curve was obtained for each ring. The maximal contractile effect (ME), and the PD_2 (-log of the dose producing 50% of the ME) were obtained for each curve. The response to 80 mM KCl in the 2.5 mM Ca^{2+} solution was always taken as the 100 response, serving to quantify the remaining responses. Results are expressed as mean values ± s.e.mean for 5-6 experiments. The data obtained in the 2.5 mM Ca^{2+} solution were used as controls and the statistic analysis used the ANOVA test for comparison of mean values (*$p<0.05$). Both the KCl and the α_1-adrenoceptor agonist responses were augmented when the Ca^{2+} concentration in the bath solution increased (tables 1 and 2). In conclusion, it is likely that the ability of elevated levels of extracellular Ca^{2+} to reduce the contractile activity of the vascular smooth muscle cannot be observed in isolated arteries from hypertensive animals.

Supported by the UCM (PR156/97-7099) grant.

Weeb, R.C. & Bohr, D.F. (1978) Blood Vessels. 15, 198-207.

TABLE 1. KCl contractions in SHR aorta in different Ca^{2+} conditions.

	mM Ca^{2+} in the bath solution			
	1.25	2.5	5	10
KCl				
30 mM	62.1±1.5*	87.2±5.2	92.6±5.5	90.6±5.7
80 mM	68.9±0.9*	100.0±0.0	97.6±6.5	98.7±5.8

TABLE 2. ME and PD_2 values of methoxamine (MTX) and phenylephrine (PHE) in SHR aorta in different Ca^{2+} conditions.

	mM Ca^{2+} in the bath solution			
	1.25	2.5	5	10
MTX				
ME	51.5±8.8	57.3±6.1	84.9±1.9*	83.7±3.3*
PD_2	5.5±0.3	5.4±0.1	5.8±0.3	5.4±0.1
PHE				
ME	74.6±5.3	77.3±1.5	77.9±6.2	82.5±6.9
PD_2	6.9±0.1	7.0±0.1	7.0±0.3	6.9±0.1

123P EFFECT OF DIETARY CALCIUM ON AORTA RESPONSES TO KCl AND METHOXAMINE IN NORMOTENSIVE AND HYPERTENSIVE RATS

M.A. Aleixandre, A. Ortega, B. Civantos & V. López-Miranda Dpto. Farmacología. Fac. Medicina, U. Complutense, 28040 Madrid, España.

The administration of calcium is paradoxically associated with a decrease in arterial blood pressure (ABP) (Hatton & McCarron, 1994). It has been suggested that this effect may be due, among other changes, to variations in vascular reactivity. Nevertheless, the studies with arteries obtained from animals fed on diets with different calcium content showed contradictory results which are usually difficult to interpret. We have studied the contractions induced by KCl and Methoxamine in aorta rings from normotensive Sprague-Dawley (SDR) and spontaneously hypertensive (SHR) rats fed from weaning with three possible diets: control with a normal-calcium content (Ca 1%), low-calcium (Ca 0.1%), and high-calcium (Ca 2.5%). The ABP was measured weekly in the rats by the tail cuff method (Buñag, 1973) from the 6th week of life. Measurements were carried out until the 9th week of life (adult age) in SDR, and until the 20th week of life in SHR, because by this age the animals of this strain fed on normal diets have attained maximum ABP values. Rats were sacrificed for the "in vitro" studies at the moments when maximum differences in the ABP were obtained between animals fed on the control diet and those fed on the low-calcium or the high-calcium diet respectively. For the "in vitro" studies the aorta rings were suspended in organ baths containing Krebs-Henseleit medium kept at 37° C, and constantly gassed with 95% O_2 and 5% CO_2. The preparations were mounted with a resting tension of 2 g, and allowed to equilibrate for a 90 min period. Following this, the preparations were contracted by 80 mM KCl and when the contraction had reached the steady state they were washed until the basal tension was recovered. Then a concentration-response cumulative curve with Methoxamine (10^{-7}-10^{-4} M) was obtained. The stress contraction developed by the rings after KCl administration and those developed after each dose of Methoxamine were measured. The results are expressed as mean values ± s.e. mean for 8-12 rings, and Student's t-test was used for comparison of mean values. The low-calcium diet increased the ABP in SDR and SHR. The maximum difference was obtained in 9-week-old animals in the normotensive strain and in 8-week-old animals in the hypertensive strain. In both strains the aorta responses to KCl and Methoxamine were similar when the animals were fed on the control diet and when the animals were fed on the low-calcium diet. The high-calcium diet did not alter the ABP in SDR but did decrease the ABP in SHR and the maximum difference was obtained in 14-week-old animals. However the responses obtained in the aorta from SHR fed on the high-calcium diet were higher than those obtained in the tissue from SHR fed on the control diet (see table 1). Therefore, the changes observed in the ABP when dietary calcium in the rats is modified cannot be correlated with alterations in their aorta reactivity.

Supported by DGICYT (PB93-0065) and UCM (PR156/97-7099) grants.

Buñag, R.D. (1973). J Appl Physiol. 34, 79.
Hatton, C.D. & McCarron, D.A. (1994). Hypertension. 23, 513-530.

TABLE 1. 80 mM KCl and 10^{-4} M Methoxamine (MTX) contractions (g) in the aorta from SDR and SHR fed on three diets with a different calcium content. (*p < 0.05; Ca 1% control)

	% Dietary Ca		
	0.1	1	2.5
9-week-old SDR			
KCl	2.32±0.17	2.29±0.17	
MTX	2.53±0.19	2.52±0.16	
8-week-old SHR			
KCl	1.29±0.13	1.27±0.06	
MTX	1.33±0.11	1.31±0.09	
14-week-old SHR			
KCl		1.10±0.08	1.71±0.09*
MTX		1.58±0.13	1.85±0.07

124P NADH/NADPH OXIDASE-MEDIATED SUPEROXIDE ANION PRODUCTION BY CORONARY MICROVASCULAR ENDOTHELIAL CELLS IS UPREGULATED IN A GUINEA PIG MODEL OF LEFT VENTRICULAR HYPERTROPHY

S. Mosfer, D. Lang, & M.J. Lewis, Cardiovascular Science Research Group, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN.

Left ventricular hypertrophy (LVH) is associated with endothelial dysfunction, possibly due to changes in the endothelial cell redox state. In the present study we investigated the role of NADH/NADPH oxidase-mediated superoxide anion (O_2^-) production by coronary microvascular endothelial cells (CMVE) in this process. Changes in the activity of these oxidase enzymes were measured in CMVE freshly isolated (Piper *et al.*, 1990) from a guinea pig, supra-renal aortic-banded (band diameter 0.5mm), pressure overload model of LVH (method as used by Linz & Scholkens (1992) for rats), with optimum LVH developing 6 weeks post-banding. Lucigenin-chemiluminescence was used to measure NADH/NADPH (both 1mM)-dependent O_2^- production in the membrane fraction of a CMVE cell lysate (method as used by Greindling *et al.*, 1994 for vascular smooth muscle cells). The integral for the first 10min of the reaction represents the total O_2^- produced over this time, and was normalised to lysate protein content. Plasma angiotensin II (AT II) levels were measured at the time of sacrifice using a commercial radioimmunoassay kit (Nichols Institute Diagnostics Ltd., UK). All data are expressed as mean ± standard error of the mean (n≥5), compared using one way analysis of variance followed by Student-Newman-Keuls test, and considered significant when $p<0.05$. In normal animals (no operation), NADH and NADPH oxidase activity was 581.8±92.3 and 232.2±38.7 V.s/mg protein respectively. The activities of these enzymes were not altered in sham-operated animals (604.14±107.5 and 248.3±23.0 V.s/mg protein for NADH and NADPH oxidase respectively), but were significantly ($p<0.05$) elevated in the aortic banded animals (1324.5±119.7 and 356.9±34.21 V.s/mg for NADH and NADPH oxidase protein respectively). Plasma AT II levels were also unaltered in the sham-operated animals compared to normals (0.63±0.06 cf. 0.68±0.06 µg/l respectively), but were significantly ($p<0.05$) elevated in the aortic-banded animals (1.25±0.12 µg/l). These data demonstrate that the development of LVH in response to pressure overload is associated with increased plasma levels of AT II and concomitant over-production of O_2^- by the CMVE. This increase in oxygen free radical production may be responsible for the endothelial dysfunction associated with this and possibly other cardiovascular diseases.

Greindling, K.K., Minieri, C.A., Ollerenshaw, J.D. *et al.* (1994) *Circ. Res.* 80, 45-51.
Linz, W. & Scholkens, B.A. (1992) *Br. J. Pharmacol.* 105, 771-772.
Piper, H.M., Spahr, R., Mertens, S. *et al.* (1990) in *Cell Culture Techniques in Heart and Vessel Research*, ed. Piper, H.M. pp158-177. Berlin: Springer-Verlag.

125P CHRONIC HYPOXIA DIFFERENTIALLY ALTERS THE VASOCONSTRICTOR RESPONSES TO ENDOTHELIN-1, PHENYLEPHRINE AND U46619 IN RAT ISOLATED PULMONARY BLOOD VESSELS

H. Lal, K.I. Williams & B. Woodward, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY.

Chronic hypoxia (CH)-induced pulmonary hypertension is associated with increased plasma levels of vasoactive agents such as endothelin-1 (ET-1) (Li, *et al.*, 1994). The reactivity of pulmonary vasculature to such agents may be altered in CH. Therefore in the present experiments we have studied the responsiveness of large pulmonary arteries (PA) and pulmonary veins (PV) to ET-1, phenylephrine (PHE) and the thromboxane receptor agonist U46619 in rats after 3 weeks of normobaric hypoxia.

Male Wistar rats (250-270g) designated for CH exposure were housed in a normobaric chamber at PO_2 10% 3 weeks prior to use. Control or CH rats were anaesthetised (Sagatal 60 mg kg^{-1},i.p) and heparinised (500 i.u, i.v.), 5min later heart and lungs were isolated, rings (2-2.5mm) of large pulmonary arteries and veins were then prepared and set up to record isometric contractions in tissue baths as described previously (Lal *et al.*, 1998).

CH increased the ratio of right ventricular weight to total ventricular weight (0.34 ± 0.001, n=20 vs. 0.21 ±.001, n=17, $p< 0.001$) as compared with age-matched control animals. In addition pulmonary artery weight % of body weight ratio was significantly increased in CH vs. control (0.0045 ± 0.0002, n=11 vs. 0.0016 ± 0.0002, n=5, $p< 0.001$).

In control preparations ET-1 produced concentration-dependent contractions of PA (EC_{50} ± s.e.m 4.9 ± 0.58nM, n=10) and PV (EC_{50} 0.79 ± 0.23nM, n=8, $p< 0.01$). The maximal contraction caused by ET-1 in PA (5.3 ± 0.66mN) was significantly greater than that in PV (1.82 ± 0.15mN) $p< 0.05$. In rings from CH animals ET-1-induced vasoconstrictor responses were markedly reduced in PA (EC_{50} 6.6 ±1.2nM, n=10) and PV (EC_{50} 5.8 ± 1.9nM, n=8). The maximal absolute contraction caused by ET-1 in PA (3.1 ± 0.26mN) and in PV (1.1 ± 0.34mN) were significantly reduced ($p< 0.05$).

In the presence of the nitric oxide synthase inhibitor, Nω-nitro-L-arginine (L-NOARG, 100 μM, 30min), basal tone in PA rings from CH animals (1.3± 0.27mN, n=10) was increased more than in control preparations (0.41 ± 0.1 mN, n=6,$p< 0.05$). Whereas L-NOARG had no effect in PV (0.06 ± 0.03 mN vs. 0.08 ± 0.05mN, n= 6-8), the maximal contractions caused by ET-1 in the presence of L-NOARG in PA (2.0 ± 0.51mN) or in PV (1.0 ± 0.16mN) were not altered when compared with CH rings without L-NOARG. The EC_{50} values for ET-1 in the presence of L-NOARG were (PA 1.13 ± 0.26nM, n=8 and PV 2.3 ± 1.06nM, n=5).

In contrast, PHE ($1\text{-}10^6$ pM) contracted PA but not PV in control and CH animals. These responses were potentiated in PA from the CH animals (EC_{50} 2.77 ± 1.25nM, n=8) group compared with control (EC_{50} 12.9 ± 3.1nM, n=10, $p< 0.01$).

The thromboxane receptor agonist U46619 (0.3-1000nM) produced a greater absolute maximal response in PA (3.3 ± 0.38mN) than in PV (1.48 ± 0.09mN). After CH responses to U46619 were not altered, the EC_{50} values in CH vs. control were (in PA 24.7± 3nM, n=5 vs. 9.3 ± 2.36nM, n=7 and in PV 30.8 ± 6.84nM, n=8 vs. 30 ± 7.28nM, n=6).

In summary, CH significantly reduced the vasoconstrictor responses of both PA and PV to ET-1. In CH preparations L-NOARG increased the basal tone in the PA but not in PV suggesting increased basal production of nitric oxide in PA. However, L-NOARG did not potentiate the ET-1-induced contractions in PA or PV. This suggests that reduced ET-1 responses in these vessels are not due to increased production of nitric oxide in CH. PHE produced a selective contraction of PA not PV. Therefore functional α_1-adrenoceptors are located on the arterial side of the pulmonary vascular bed, such findings are consistent with our previous studies in isolated lungs (Lal *et al.*, 1994). In contrast to ET-1, PHE-induced contractions were potentiated in PA after 3wks of CH, whereas U46619-induced responses were not altered. This suggests that vascular responsiveness of the pulmonary vessels to these agonists in CH model is altered differentially. The underlying mechanisms for these changes require further investigation.

Funded by British Heart Foundation.

Lal, H., Woodward, B., & Williams, K.I. (1994). *Pulmonary Pharmacol.*, 7. 271-278.
Lal, H., Williams, K.I. Woodward, B., (1998). *Br. J. Pharmacol.*,123,291P
Li, H., Chen, S.J., Chen, Y.F. et al., (1994). *J. Appl. Physiol.*, 77, 1451-1459.

126P PHENYLEPHRINE MARKEDLY POTENTIATES THE CONTRACTION OF PORCINE ISOLATED PULMONARY ARTERIES TO OTHER CONTRACTILE AGONISTS

F. Plane & [1]K. Stuart-Smith, Departments. of Pharmacology and [1]Anaesthesia, University of Bristol, Bristol, BS8 1TD

The adrenoceptor antagonists phenoxybenzamine and tolazadine alleviate neonatal pulmonary hypertension, suggesting a role for catecholamines in pulmonary arterial contraction. However *in vitro*, application of adrenoceptor agonists causes pulmonary vasoconstriction only at high concentrations (Pepke-Zaba *et al.*,, 1993; Higueras *et al.*, 1997). A recent study has shown that phenylephrine induces intracellular calcium oscillations in pulmonary vascular smooth muscle cells (Hamada *et al.*, 1997). In the present study the effect of phenylephrine on the contractile response of porcine isolated pulmonary resistance arteries to other agonists has been investigated.

Pulmonary resistance arteries (250-300 μm in diameter) were removed from porcine lungs obtained from the abattoir immediately after sacrifice. Arterial segments (2 mm wide) were mounted in a myograph under a previously determined optimal pre-load of 5 mN and were maintained at 37°C in oxygenated Krebs buffer. The endothelial cell layer was removed mechanically by gently rubbing the intima with a hair. All data are expressed as mean ± s.e. mean, and the significance of differences between mean values was calculated using the Students' t-test or ANOVA.

Application of the adrenoceptor agonists phenylephrine, noradrenaline or methoxamine (10-100 μM; n=4-8 in each case) did not constrict isolated segments of porcine pulmonary resistance arteries. In contrast, application of histamine (0.1-100 μM), the thromboxane mimetic U46619 (0.3-100 μM) and potassium chloride (20-100 mM) each evoked concentration-dependent contraction of the tissues with pD_2 values of 2.9 ± 0.5 μM (n=4), 2.6 ± 0.1 μM (n=4) and 37.8 ± 1.3 mM (n=8), respectively.

Pre-addition of phenylephrine (10 μM), which alone had no effect on arterial tone, significantly potentiated the contractile responses to histamine, U46619 and potassium chloride. In the presence of phenylephrine, the pD_2 values for contraction to histamine, U46619 and potassium chloride were reduced to 0.5 ± 0.01 μM (n=4; $P<0.01$), 0.73 ± 0.1 μM (n=4; $P<0.01$) and 27.3 ± 3.0 mM (n=8; $P<0.01$), respectively, and the maximum responses were increased to 117.5 ± 2.4 % (n=4; $P<0.01$), 131.5 ± 6.5 % (n=4; $P<0.01$) and 125 ± 2.5 % (n=8; $P<0.01$) of control values, respectively. The potentiation of the contraction to potassium chloride caused by phenylephrine was unaffected by either the β-adrenoceptor antagonist propanalol (10 μM; n=4 in each case) or the α_2-adrenoceptor antagonist yohimbine (10 μM; n=4 in each case). However, the exposure to either phenoxybenzamine (10 μM) or the α_1-adrenoceptor antagonist prazosin (10 μM), prevented the phenylephrine-induced potentiation of contraction (n=4 in each case).

These data indicate that activation of adrenoceptors in pulmonary resistance arteries does not cause arterial constriction but does potentiate the contractile response of these arteries to other agonists. This potentiation appears to be mediated by the activation of α_1-adrenoceptors as it is inhibited by either prazosin or phenoxybenzamine. Thus, the alleviation of pulmonary hypertension by phenoxybenzamine may be due to an inhibition of this potentiating effect rather than prevention of the direct contractile action of endogenous catecholamines.

F. Plane is a Wellcome Trust Career Development Fellow.
Hamada, H., Damron, D.S., Hong, S.J. *et al.*, (1997) *Circ. Res.*, **81**, 812-823.
Higueras, J., Sarria, B., Ortiz, J.L. *et al.*, (1997) *Eur. J. Pharmacol.*, **326**, 175-181.
Pepke-Zaba, J., Higenbottam, T.W. Dinh-Xuan, A.T. *et al.*, (1993) *Eur. J. Pharmacol.*, **235**, 169-175.

127P THE EFFECTS OF MELATONIN AND OTHER INDOLE-BASED ANALOGUES ON VASCULAR TONE OF THE PORCINE ISOLATED CORONARY ARTERY

K.N.Ting, H.Y.Cheng, D.J.Davies*, B.Guardiola-Lemaitre**, E.Scalbert**, P.Delagrange**, D.Sugden† and V.G.Wilson. Sch, of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH. *Dept. of Chemistry, Univ. College London, London WC1H 0AJ. **IRIS, 6 place des Pleiades, 92415 Courbevoie Cedex, France. †Dept. of Physiology, Univ. London King's College, London W8 7AH.

Recent evidence has shown that near physiological levels of melatonin (nM) can increase vascular tone in cerebral arteries and isolated tail artery of the rats (eg. Geary *et al.*, 1995; Ting *et al.* 1997). In contrast, pharmacological concentrations of melatonin (μM) have been reported to have an inhibitory action on the contractile responses and can enhance nitroglycerin-induced vasorelaxation in several other vascular smooth muscles (eg. Satake *et al.*, 1991). The aims of this study were to (i) investigate the action of melatonin at high concentrations and its effect on sodium nitroprusside-induced vasorelaxation in the porcine isolated coronary artery and (ii) to compare the potency of melatonin with a range of indole-based melatonin analogues.

Sections of the porcine coronary artery (stored overnight at 4°C in 2% Ficoll Krebs-Henseleit (K-H) buffer) were prepared for isometric recording in 10 ml organ baths containing K-H buffer (37°C; 95% O_2/ 5% CO_2). All segments (5mm) were placed under 8-10 g wt resting tension, exposed twice to 60 mM KCl, and then stimulated with U46619 (10-30 nM) to produce a submaximal contraction size of 60-70% of the KCl response. In some vessels, the endothelium was removed by rubbing the luminal layer. Upon attaining a stable contraction with U46619, melatonin or melatonin analogues were added cumulatively. The effect of SNP (0.1nM-3μM) was examined in the presence of melatonin (300μM) in endothelium-denuded arteries. Responses were calculated as a percentage of U46619-induced tone and are shown as the mean±sem of n observations. The negative logarithm of the concentration of drug to produce a 50% relaxation from the initial tone (pIC_{50}) was determined. Differences between mean values have been compared using a Student's t-test ($p < 0.05$).

Melatonin (0.3-300 μM) has no effect on the basal tone of the porcine coronary arteries (n=4) but produced a slow-developing, sustained relaxation of the U46619-induced contractions in a concentration-dependent manner (pIC_{50}=4.16±0.10, maximum relaxation=88.5±7.8%, n=4). The response to melatonin was not affected by removal of the endothelium (pIC_{50}=4.07±0.10, maximum relaxation=80.2±6.3, n=4). SNP caused a concentration-dependent vasorelaxation (pIC_{50}=6.80±0.27, n=8) which was potentiated by the presence of 300μM melatonin (7.42±0.12); the relaxation to melatonin was fully reversed by increasing the concentration of U46619.

The indole-based melatonin analogues, with the exception of N-acetyl-5HT, produced qualitatively similar relaxations in the coronary artery. The rank order of potency of the compounds (pIC_{50}) investigated was as follows: luzindole (5.00±0.14; n=6) > (+)-AMMTC [N-acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4 tetrahydrocarbazole] (4.75±0.17; n=4) ≥ (-)-AMMTC (4.72±0.05; n=4) > 2-iodomelatonin (4.54±0.14; n=6) > melatonin (3.70±0.07; n=10).

Our results show that the inhibitory effect of melatonin on the coronary artery is endothelium-independent and can enhance SNP-induced vasorelaxations. This vasorelaxing action of melatonin is also shared by other indole-based compounds. Luzindole, a putative melatonin receptor antagonist (Dubocovich, 1988), is 20-fold more potent than melatonin. Interestingly, the enantiomer ligands, (+)- and (-)-AMMTC have similar potency, which is in contrast to our earlier observation on the melatonin receptor-mediated action in the rat tail artery; enantiomeric ratio of 400 (Ting *et.al.*, 1997). More studies are warranted to elucidate the mechanisms involved in the pharmacological effect of melatonin.

Dubocovich, M.L. (1988). *J. Pharmacol. Exp.Ther.*, **246**, 902.
Geary,G.G.,Duckles,S.P.&Krause,D.N.(1995).*Soc.Neurosci.Abs.*,**21**, 157.
Satake, N., Oe, H. & Shibata,S. (1991).*Gen.Pharmacol.*, **22**, 1127.
Ting, K.N., Davies, D.J., Scalbert, E., *et al.* (1997). *Bri. J. Pharmacol.*, **122**, 1299.

128P EFFECT OF OVERNIGHT STORAGE ON THE FUNCTIONAL PROPERTIES OF HUMAN ISOLATED, PRESSURIZED, MESENTERIC RESISTANCE ARTERIES

S.J. Wallis, V.G. Wilson and W.R. Dunn. School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Vascular resistance is determined by small arteries (<500μm in diameter) (Mulvany & Aalkjaer, 1990). Thus, the properties of these vessels are important in determining local blood flow and blood pressure. In the present study we have examined the functional properties of mesenteric resistance arteries isolated from humans, set up in a Halpern pressure myograph. In addition, since biopsy samples were frequently not available until late afternoon, we have also examined how vasoconstrictor and vasodilator responses were affected by overnight storage.

Small mesenteric arteries (internal diameter, 310±36μm n=60) were obtained from biopsy specimens, removed from patients undergoing colectomies for colonic cancer and inflammatory bowel disease, as far away from the diseased area as possible. Vessels were then either, mounted on a pressure myograph (Wallis *et al.*, 1996) or, stored overnight at 4°C in physiological salt solution (PSS) containing 2% Ficoll, for study the next day. With both fresh and stored vessels, intraluminal pressure was initially set to 60mmHg and left to equilibrate for 60mins. Thereafter, the myogenic responsiveness of arteries was assessed by determining the pressure-diameter relationship between 10 and 90mmHg (by raising pressure in 10mmHg increments), in calcium-containing and calcium-free PSS (containing 0.5mmol/l EGTA). In separate experiments, concentration-dependent vasoconstrictor responses were obtained to NA and U46619. In others, after reducing vessel diameter by ~50% with U46619, responses to the endothelium-dependent vasodilator bradykinin, and the endothelial-independent vasodilator sodium nitroprusside, were obtained.

Mesenteric resistance arteries isolated from humans did not develop myogenic tone at any pressure within the pressure range 10-90mmHg.

	fresh tissue	stored tissue
	% change in	% change in
agonist	diameter	diameter
NA (0.3μmol/l)	38±9	31±10
U46619 (0.3μmol/l)	52±10	54±9
	log EC-50 value	log EC-50 value
bradykinin	-7.06±0.10	-6.93±0.20
Na nitroprusside	-6.44±0.30	-6.38±0.20

Table 1. Responses to agonists in fresh and overnight stored mesenteric resistance arteries isolated from humans. Each value represents the mean±s.e.mean (n=5). There was no significant difference between fresh and stored vessels in response to any agonist ($p>0.05$, Mann-Whitney U test).

In contrast to human cerebral resistance arteries (Wallis *et al.*, 1996), human mesenteric resistance arteries did not develop myogenic vasoconstriction when exposed to high intraluminal pressures. This may reflect the greater ability of the cerebral circulation to autoregulate blood flow *in vivo*, compared to the mesentery. Human isolated mesenteric resistance arteries did however, constrict in response to both NA and U46619, and responded to both endothelial-dependent and independent vasodilators, indicating their functional viability. Furthermore, these functional responses were not compromised by overnight storage.

Mulvany MJ & Aalkjaer C (1990) *Physiol. Rev* **70** 921-961.
Wallis SJ Firth J & Dunn WR (1996). *Stroke*, **27**, 2287-2291.

129P CHARACTERIZATION OF α_1-ADRENOCEPTOR SUBTYPES IMPLICATED IN INOSITOL PHOSPHATE PRODUCTION IN THE RAT BISECTED VAS DEFERENS

Badia A, Terrades D, Tabernero A & Vivas NM. Departament Farmacologia i Terapèutica. Universitat Autònoma de Barcelona. 08193 Barcelona

The prostatic and the epididymal portions of rat vas deferens differ in their responsiveness to α_1-adrenoceptor stimulation. The more efficient occupancy-response coupling of the epididymal half may account for these differences (Sallés and Badia,1991). α_1-Adrenoceptor stimulation increases inositol phosphates production by coupling to a G-protein. Our objective was to analyse the phosphoinositide hydrolyses linked to α_1-adrenoceptor stimulation, the subtype of α_1-adrenoceptors (α_{1A}, α_{1B} or α_{1D}) implicated in this response and the expression of G proteins in the two portions of rat vas deferens. The accumulation of inositol phosphates was determined) according to Ivorra et al. (1993). Each portion was divided into pieces of 2 mm, labeled with [^{3}H]-inositol and incubated with noradrenaline (0.1 µM- 1 mM). Noradrenaline (10 µM)-stimulated accumulation of inositol phosphates was also inhibited by increasing concentrations of the selective antagonists, 5-methyl-urapidil (α_{1A}), or BMY-7378 (α_{1D}). For binding assays, the epididymal and the prostatic portions from several animals were pooled separately and the membranes were labeled with [^{3}H]-prazosin. Saturation and displacement studies using either 5-methyl-urapidil or BMY-7378 were performed. G proteins linked to phosphoinositide breakdown ($G_{q/11}$) were determined by inmunoblotting assay. The results show no changes in the maximum response but an increase in the sensitivity to noradrenaline in the epididymal half (e) when compared to the prostatic (pEC_{50}=5.97±0.07 (e); 5.47±0.15 (p); $p<0.01$). 5-Methyl-urapidil and BMY-7378 inhibited, in a concentration-dependent manner, the noradrenaline-induced inositol phosphates production in either the epididymal or the prostatic portions of rat vas deferens. The results show a similar percentage of α_1-adrenoceptor subtypes in both halves: (α_{1A}: e=36.6%, p=43.7%; α_{1B}: e=20.3%, p=27.5%; α_{1D}: e=36%, p=35.5%). However, the contribution of each receptor subtype, to the inositol phosphates production, differs between portions (α_{1A}: e=10.8%, p=24.3%; α_{1B}: e= 68%, p=31%; α_{1D}: e=21.1%, p=44.6%). The content of $G_{q/11}$ proteins determined by Western blott, was smaller in the prostatic (-28.91±4.59%) than in the epididymal portion. All these findings suggest that not only α_{1A} and α_{1B} but also α_{1D} adrenoceptors are expressed in the rat vas deferens, being the three subtypes implicated in the accumulation of inositol phosphates. The observed differences in the noradrenaline sensitivity to stimulate inositol phosphates production, could be ascribed to a higher availability of G proteins in the epididymal portion. The different inositol phosphates accumulation in each portion may be related to a dissimilar coupling to $G_{q/11}$ proteins of the several subtypes of α_1-adrenoceptors and/or the role of other G proteins in this response.

Study supported partly by DGCYT PM95-0124.

Sallés J, Badia A (1991) *Br J Pharmacol* 102:439-445
Ivorra D *et al* (1993) *J Cardiovasc Pharmacol* 21: 931-936

130P EFFECTS OF CALCIUM CHANNEL ANTAGONISTS ON ISOPRENALINE-INDUCED HYPERGLYCAEMIA, LIPOLYSIS AND INSULIN SECRETION IN THE RABBIT

M.J. García-Barrado, C. Sancho, M. Martín & J. Moratinos. Department of Pharmacology, School of Medicine, Av/ Campo Charro s/n 37007, Salamanca, Spain.

Extracellular calcium is a main requirement for metabolic responses elicited by α1-adrenoceptor agonists (G^a-Barrado et al., 1997). The role played by calcium channel blockers on β-adrenoceptor mediated effects needs further consideration. We have therefore investigated the effects of verapamil and elgodipine on the in vivo and in vitro metabolic responses induced by isoprenaline. Plasma levels of glucose, lactate and glycerol were measured using conventional kits (Boehringer-Mannhein), and insulin release was estimated by radioimmunoassay (CIS-Radioquimica).

In conscious fasted male rabbits the i-v. infusion of verapamil (0.17 µg.kg^{-1}.min^{-1}, 30 min) but not elgodipine (35 ng.kg^{-1}.min^{-1}, 30 min) enhanced the weak hyperglycaemic effect of isoprenaline (ISO: 0.3 µg.kg^{-1}.min^{-1}, 30 min; Δ at 15 min = 0.41 ± 0.15 mmol/l, n=10, vs, 1.56 ± 0.14 mmol/l, n=5; $p<0.001$). Verapamil did not reduce the ability of ISO to increase arterial insulin (Δ at 45 min = 112.35 ± 24.30 %, n=6, vs 1.27 ± 6.7 %, n=7, $p<0.001$ for agonist and saline treated rabbits) though elgodipine suppressed the response (Δ at 45 min = 11.45 ± 9.40 %, n=5, $p<0.001$). Pre-infusion absolute values of arterial plasma glucose and circulating insulin for saline and drug treated animals oscillated between 5.40 ± 0.22 and 5.90 ± 0.19 mmol/l; 8.45 ± 1.60, 11.1 ± 1.26 µIU/ml respectively. The increase in plasma lactate induced by ISO (Δ at 30 min = 1.60 ± 0.70mmol/l, n=8) was significantly potentiated by both Ca^{2+} antagonists, (Δ=3.02 ± 0.45 mmol/l, n=8; 3.24 ± 0.57 mmol/l, n=7, $p<0.05$ for verapamil and elgodipine pre-treated rabbits respectively). Only elgodipine enhanced the effect of ISO on plasma glycerol (Δ at 45 min =. -0.35 ± 0.20 mmol/l, n=8, vs, 0.58 ± 0.14 mmol/l, n=7, $p<0.001$). Plasma lactate preinfusion values fluctuated between 1.05 ± 0.19 and 1.77 ± 0.34 mmol/l, and those for glycerol ranged between 0.26 ± 0.01 and 0.84 ± 0.29 mmol/l. In rabbit fat cells of different origin obtained by collagenase digestion of adipose tissue fragments (Maroto et al., 1992) the lipolytic effect of ISO expressed as glycerol release was tested. Elgodipine (10^{-6}M) enhanced the effect of ISO in perirenal adipocytes (ISO 10^{-6}M, Δ in the absence and presence of the drug was: 171 ± 21.44 %, n=8; 265.6 ± 44.5%, n=7, $p<0.05$) and epididymal fat cells (ISO 10^{-6}M, Δ in the absence and presence of the antagonist = 197.8 ± 55.6%, n=5; 282.4 ± 76.3 %, n=5, $p<0.01$, being basal release = 0.20 ± 0.06 µmol glycerol/100 mg lipids/90 min). In rabbit isolated islets incubated under static conditions (García-Barrado et al 1996) 10^{-4}M forskolin evoked insulin release (107.11 ± 38.55%, n=14, $p<0.05$, vs, glucose 15 mM, being basal release = 19.14 ± 3.5 µIU/ml/islet/h). Elgodipine (10^{-6}M) but not verapamil reduced the response (20.12 ± 9.25%, n=12, $p<0.01$). Therefore in rabbits ISO mediated insulin release, but not lipolysis seems to require extracellular calcium. The effects of both antagonists on the glycaemic response to ISO could result from alterations in glucose production and insulin independent effects on peripheral glucose utilization.

G^a-Barrado, M.J et al.,(1996). Eur. J. Pharmacol., 298, 279-286.
G^a-Barrado, M.J. et al., (1997). J. Auton. Pharmacol., 17, 293-302.
Maroto, R. et al., (1992). J.Auton. Pharmacol., 12, 277-290.

131P DISCRIMINATION OF WHOLE-CELL CURRENTS MEDIATED BY AMPA- AND KAINATE-TYPE GLUTAMATE RECEPTORS IN RAT CULTURED CEREBELLAR GRANULE CELLS

J.R. Savidge, N.C. Sturgess, D.R. Bristow[1] & E.A. Lock
Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, and [1]School of Biological Sciences, University of Manchester.

Functional discrimination of responses mediated by AMPA- and kainate-types of glutamate receptor is complicated by the fact kainate acts as a non-desensitising agonist at AMPA receptors. However, kainate receptor-mediated increases in intracellular Ca^{2+} concentration have recently been identified in rat cultured cerebellar granule cells (CGCs) by selective blockade of AMPA receptors with the non-competitive antagonist LY300168 (Savidge *et al.*, 1997).

We have made whole-cell voltage clamp recordings (holding potential -60 mV; 20 °C) from CGCs after 6-9 days *in vitro*, to characterise currents mediated by AMPA and kainate receptors. Agonists were applied by pressure ejection (30-500 ms) via a glass pipette close to the cell, while antagonists and other drugs were applied by bath perfusion. 'n' numbers represent total cells tested. Data are expressed as mean ± s.e.mean.

Application of AMPA (10-100 μM) produced small, sustained inward currents (range 10-20 pA) in 8/10 cells tested. A large, rapidly desensitising inward current (range 120-140 pA) was only detected in 2/10 cells. Following bath application of cyclothiazide (CYZ, 100 μM), an inhibitor of AMPA receptor desensitisation, AMPA produced a substantial (>100 pA) non-desensitising current in all 10 cells tested (range 100-2000 pA) which was completely and reversibly blocked by 50 μM LY300168 (n=3).

Kainate (100 μM) evoked inward currents >100 pA (range 120-600 pA) in all 15 cells tested. Their characteristics were typical of responses mediated largely by AMPA receptors, i.e. non-desensitising and markedly potentiated (3.9 ± 0.6 fold) by 100 μM CYZ (n=4). In 6/12 cells, no detectable (<10 pA) kainate evoked current was observed in the presence of 50 μM LY300168. In the other 6 cells, LY300168 produced a partial (78 ± 3 %) block of kainate evoked currents (residual currents 20-60 pA).

Cells pre-treated with Concanavalin A (Con A, 250 μg/ml, 10 min), an inhibitor of kainate receptor desensitisation, showed a substantial (35-140 pA, 40 ± 5 % of control, n=5) residual current to 100 μM kainate in the presence of 50 μM LY300168. Subsequent bath application of 100 μM AMPA failed to desensitise this response.

2S, 4R 4-methyl glutamate (SYM2081) has been shown to be a kainate receptor agonist (Jones *et al.*, 1997). Bath application of 10 μM SYM2081 had no effect on inward currents evoked by 10 μM AMPA/100 μM CYZ (99 ± 2 % of control, n=3), but completely and reversibly abolished inward currents evoked by kainate in the presence of LY300168 (50 μM) in ConA treated cells (n=3).

These data indicate that CGCs display substantial kainate evoked inward currents that are mediated by kainate receptors, particularly following inhibition of kainate receptor desensitisation. LY300168 (50 μM) provides complete blockade of AMPA receptor mediated responses in these cells, since it abolished inward currents evoked by AMPA/CYZ. The fact residual currents evoked by kainate, in the presence of LY300168, are not desensitised by AMPA provides additional evidence these currents are kainate receptor mediated. The ability of SYM2081 to abolish responses to kainate in the presence of LY300168, whilst having no effect on AMPA/CYZ induced currents, is consistent with a selective action of SYM2081 at kainate receptors.

Savidge, J.R. *et al.*, (1997). *J. Neurochem.* 69, 1763-1766.
Jones, K.A. *et al.*, (1997). *Neuropharmacology* 36, 853-863.

132P DISULFIRAM, S-ADENOSYL-L-METHIONINE AND BUSPIRONE REVERTED UP-REGULATED HIPPOCAMPUS 5-HT_{1A} RECEPTORS OF ALCOHOLIZED RATS

Bellido, I., Gomez, A., Fernandez, M. & Sanchez de la Cuesta F. (Tamargo, J.). Dpt of Pharmacology and Clinical Therapeutics. School of Medicine, University of Malaga, 29080 Malaga, Spain.

A negative correlation between the central serotonin concentration and ethanol drinking has been found (Yoshimoto & Komura, 1987). Ethanol inhibit S-adenosyl-L-methionine (SAM) endogeneous synthesis (Corrales et al, 1992). SAM increases serotonin turnover in rats. So, SAM could have a role in ethanol intake by serotoninergic pathway. It has been reported higher density of 5-HT_{1A} receptors in the hippocampus and cerebral cortex of alcohol-preferring P rats (Wong et al, 1990). The 5-HT_{1A} partial agonist buspirone has been reported to diminish human ethanol intakes (Bruno, 1989). Our aims were: 1) to compare disulfiram acute effect vs SAM or buspirone chronic effects on rat ethanol intake. 2) to determine hippocampus 5-HT_{1A} receptor binding characteristics of alcoholized and nonalcoholized rats. 3) to determine the posible changes in hippocampus 5-HT_{1A} receptor binding characteristic from alcoholized rats in response to disulfiram, SAM and buspirone treatment.

Male wistar rats were alcoholized according to the method of Gatto (Gatto et al, 1990). Animals were given 10% ethanol-polycose solution (v/v) by liquid diet for 3 weeks prior to their inclusion in the assay. Male wistar rats were given water ad lib (n=30). Alcoholized rats were injected with saline (n=30), disulfiram (10 mg/kg i.p., x 4 days)(n=10), SAM (50 mg/kg i.p., x 20 days)(n=10) and buspirone (1.25 mg/kg i.p., x 20 days)(n=10), respectively. Intakes of alcohol and food were measured daily. 5-HT_{1A} receptors were characterized with [^{3}H]8OH-DPAT saturation experiment according to the method of Pazos (Pazos et al, 1985) in hippocampus membranes (n=6 in all groups). Binding data were analyzed using Ligand software. Protein was assayed by the Bradford method. Data are mean ± s.e.m. of n values. Statistic analysis used ANOVA test followed by Bonferroni post-test.

Ethanol intakes (mL/kg/day) were 77.25±3, 35.92±5, 61.54±2 and 62.86±1.5 for alcoholized nontreated group, disulfiram, SAM and buspirone, respectively. Disulfiram and SAM significantly reduced ethanol intake respect to alcoholized nontreated group (-53.5% and -20.33%, respectively, $P<0.05$). Bmax was almost 2.3-fold greater in alcoholized nontreated rats (659.75±71 fmol/mg) compared with control rats (285.74±52 fmol/mg, $P<0.01$). All the treatments significantly reduced [^{3}H]8OH-DPAT binding to rat hippocampus membranes. Bmax and percentage of reduction respect to alcoholized nontreated group were disulfiram: 284.99±40 fmol/mg, –56.8%, SAM: 332.02±60 fmol/mg, –49.67% and buspirone: 397.13±24 fmol/mg, –39.81%, respectively ($P<0.05$). The affinity (K_D) of [^{3}H]8OH-DPAT for 5-HT_{1A} receptors was in a similar range in all groups: control 2,37±0.3 nM, alcoholized nontreated 2.40±0.2 nM, disulfiram 2.47±0.4 nM, SAM 2.48±0.5 nM and buspirone 2.97±0.3 nM.

In conclusion, chronic alcoholism up regulated 5-HT_{1A} receptors in rat hippocampus membranes. SAM chronic treatment reduced ethanol intake by 20.33% with respect to alcoholized nontreated group values. Disulfiram reversed hippocampus 5-HT_{1A} receptors up regulation of alcoholized rats. SAM and buspirone chronic treatment reversed hippocampus 5-HT_{1A} receptors up regulation to a lesser extend than disulfiram acute treatment.

Bruno F. (1989) Psychopathology 22:49-59.
Corrales F. et al. (1992) Drug invest 4 (Suppl 4):8-13.
Gatto G.J. et al. (1990) Alcohol 7:531-536.
Pazos A. et al. (1985) Eur J Pharmacol 106: 531-546.
Wong D.T. et al. (1990) Life Sci 46:231-235.
Yoshimoto K. & Komura S. (1987) Pharmacol Biochem Behav 27:317-322.

133P SUPRANIGRAL GRAFTING OF BDNF-PRODUCING FIBROBLASTS PREVENTS THE DOPAMINERGIC AND 5-HTergic NEUROTOXICITY INDUCED BY INTRASTRIATAL INJECTION OF MPP^+ IN RATS

P. Ruiz-Golvano, D. Frechilla, A. García-Osta, M.P. Rubio[1], J.M. Almendral[1] & J. Del Río. Department of Pharmacology, University of Navarra Medical School, Pamplona and [1]Center for Molecular Biology, UAM-CSIC, Madrid, Spain.

Brain-derived neurotrophic factor (BDNF) promotes survival and differentiation in various neuronal systems and may also modulate synaptic transmission (e.g. Altar *et al.*, 1997). The effects of BDNF on dopaminergic neurons have been frequently reported (e.g. Levivier *et al.*, 1995) but neuroprotection studies on other lesioned monoaminergic systems are scarce. Cells transduced with the BDNF gene are a good option for constant intracerebral delivery of this factor and were used to study the protective effect of BDNF in male Wistar rats lesioned with 1-methyl-4-phenylpyridinium (MPP^+).

Human cDNA encoding the full-length prepro-BDNF was obtained by PCR, subcloned and sequenced. The NIH 3T3 rat fibroblast cell line was infected with the retroviral vector pLXSN containing the coding region of h-BDNF. A similar construct containing the E.coli β-galactosidase gene (LacZ) was also generated and used as control. The retroviral vectors were transfected into the cell line and co-cultures with fetal mesencephalic neurons confirmed that the fibroblasts produced biologically active BDNF, which increased the number of tyrosine-hydroxylase-immunoreactive neurons by c. 50% after 3 days in culture. Cells were suspended in PBS (10^5 cells/μl) and 2 μl of fibroblast suspension were stereotaxically implanted into the dorsal tegmentum of mesencephalon (AP -5.3, ML 2.4, DV 6 mm from bregma). Unilateral lesions were made 7 days later, in rats anaesthetized with ketamine, by intrastriatal infusion (AP -0.5, ML 3, DV 4.5 mm) of MPP^+ (28 μg/rat in 4 μl). Animals were killed one week after lesioning and the striatal levels of dopamine (DA) and 5-HT in the striatum were quantified by HPLC. The results obtained are shown in Table 1.

Table 1. Effect of fibroblast grafting on striatal monoamine levels in control and MPP^+-lesioned rats

	DA	5-HT
Sham	9810±880	857±67
Sham + MPP^+	2830±368	243±36
LacZ	11350±914	780±61
LacZ + MPP^+	284±30*	95±9*
BDNF	8670±694	770±66
BDNF + MPP^+	3604±666†	496±51*†

Values (pg/mg wet tissue) are means±s.e.m. (n=4-8). *P< 0.05 or better vs. sham/MPP^+; †P< 0.01 vs. LacZ/MPP^+ (ANOVA followed by Student's t test).

Intrastriatal injection of MPP^+ markedly reduced DA and 5-HT content. Fibroblast grafting, either BDNF(+) or control (LacZ) did not modify monoamine content. When control fibroblasts were implanted, the lesion induced by MPP^+ was still more severe. This was not the case with grafted BDNF(+) fibroblasts, which significantly attenuated the monoaminergic lesion, as compared to the corresponding LacZ-controls, notably on the 5-HT system, suggesting the interest of this approach at the time of modulating brain 5-HTergic neurotransmission.

Supported by EEC (BIO4 CT96-0752) and JALS Fdn. (Spain)

Altar,C.A., Cai,N., Bliven,T. *et al.* (1997) *Nature* 389, 856-860.
Levivier,M., Przedborski,S., Bencsics,C. *et al.* (1995) *J.Neurosci.* 15, 7810-7820.

134P THE Y1 RECEPTOR ANTAGONISTS BW1229U91 AND BIBP3226 RECOGNISE DIFFERENT [^{125}I]PYY BINDING SITES

P. King, P.S. Widdowson, H. Doods[1] & G. Williams, Dept. of Medicine, University of Liverpool, L69 3GA and [1]Boehringer Ingelheim Pharma KG, D-88397 Biberach an der Riss, Germany.

There is evidence to support both Y1 and Y5 receptors in the orexigenic response following neuropeptide Y (NPY) injections in the brain (Gerald et al, 1996; Kanatani et al, 1996). The Y1 antagonist, BW1229U91 (Kanatani et al, 1996) inhibits NPY-induced hyperphagia, but the selective Y1 antagonist, BIBP3226 does not show activity in this paradigm (Haynes et al, 1997). We have therefore compared NPY receptor populations that are sensitive to BW1229U91 and BIBP3226 using quantitative receptor autoradiography, to determine whether BW1229U91 uncovers additional sites that may explain why only this compound inhibits NPY-induced feeding. Seven male Sprague-Dawley rats (300g) were killed by CO_2 inhalation. Quantitative receptor autoradiography was performed on 20 μm coronal cryostat sections of snap frozen brains as described previously (Widdowson, 1997) using 30 pM [^{125}I]peptide YY ([^{125}I]PYY) and 1 μM BIBP3226 and 1 μM BW1229U91, concentrations at which Y1 receptors in rat cerebral cortex are masked (Widdowson, 1997). Non-specific binding was determined using 1 μM porcine NPY. Specific [^{125}I]PYY binding was highest in the hippocampal CA3 field, superficial cerebral cortex, lateral septum and in medial thalamic nuclei, whilst there was moderate binding to striatum, lateral hypothalamic area (Hypo-LH) along with other hypothalamic regions. In most brain regions, BIBP3226 and BW1229U91 both reduced [^{125}I]PYY binding by comparable amounts. For example, BIBP3226 and BW1229U91 both reduced [^{125}I]PYY binding by 80% in the cerebral cortex and by 45% in the striatum. However, BW1229U91 reduced [^{125}I]PYY binding by significantly greater amounts in selective brain regions (Table 1) that include the thalamic reuniens (Thal-RE), centromedial (-CM) and interanteromedial (-IAM) nuclei and also the hippocampal CA3 field.

Table 1. Specific binding (fmol/mg tissue; mean ± s.e.m.)

Region	Specific	+BIBP3226	+BW1229U91
Cortex	5.07 ± 0.39	0.90 ± 0.15	0.86 ± 0.07
Striatum	2.16 ± 0.16	1.22 ± 0.06	1.33 ± 0.06
Hypo-LH	5.17 ± 0.50	4.09 ± 0.22	3.69 ± 0.18
CA3	6.34 ± 0.62	5.84 ± 0.38	4.87 ± 0.22*
Thal-RE	6.40 ± 0.71	4.33 ± 0.44	2.78 ± 0.19*
Thal-CM	5.50 ± 0.65	4.39 ± 0.25	2.97 ± 0.27*
Thal-IAM	5.35 ± 0.51	1.97 ± 0.16	1.30 ± 0.07*
Lat Septal	5.87 ± 0.70	5.54 ± 0.64	4.08 ± 0.48

*P <0.05, BW1229U91 vs BIBP3226 (ANOVA with Bonferroni-modified t-tests).

In conclusion, masking subpopulations of NPY receptors with BW1229U91 reveals additional [^{125}I]PYY binding sites to those masked with the selective Y1 antagonist, BIBP3226, and hence they cannot be Y1 receptors. The distribution of these additional sites closely overlaps those of Y5 receptors, but additional Y1-like sites cannot be excluded.

Gerald, C., et al,(1996) *Nature* 382, 168-171.
Haynes, A.C., Arch, J., et al, (1997) *Regul. Peptides* 71, 221
Widdowson, P. (1997) *Brain Res* 758, 17-25.
Kanatani, A., et al, (1996) *Endocrinology* 137, 3177-3182.

135P PHARMACOLOGICAL CHARACTERIZATION OF THE PROTEASE-ACTIVATED RECEPTORS IN HUMAN ERYTHROLEUKAEMIC CELLS

S.M. Poucher, S. Freeman, C.G. Heapy, S.M. Daniels, R Cotton* and M.B. Giles*. Cardiovascular, Metabolism and Musculoskeletal Research Department; *Lead Discovery Department, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG, UK.

To date, three different protease activated receptors (PAR) have been identified (Ishihara *et al*, 1997). PAR1 is activated by thrombin and the actions are reproduced by peptide sequences mimicking the new N-terminal sequence produced by thrombin (Grand *et al*, 1996); PAR2 is activated by trypsin and tryptase (Blackhart *et al*, 1996); whilst the PAR3 is activated by thrombin only (Ishihara *et al*, 1997). We have used a pharmacological approach to determine the subtype of protease receptor expressed in human erythroleukaemic (HEL) cells.

HEL cells were cultured in Delbucco's Modified Eagle Medium (containing 10% foetal calf serum, 100 units ml^{-1} penicillin, 100μg ml^{-1} streptomycin and 2 mM L-glutamine) in humidified, 5% CO_2 atmosphere at 37°C. 24 hr prior to each experiment the cells were divided to 2 x 10^5 cells ml^{-1}. Intracellular calcium release was measured in HEL cells (3 x 10^5 ml^{-1}) loaded with FURA-2 / AM re-suspended in HBSS containing 1mM $MgCl_2$ and 2mM $CaCl_2$. Intracellular calcium release in response to thrombin, R*para*(f)FR.ChA.hRY-NH_2, SFLLRN-NH_2, SFLLRNPNDKYEPF-OH, (TRAP$_{1-14}$), SFLLRN-OH, SFFLRN-OH, SLIGRL-OH and YFLLRNP-OH were measured. In addition, the effect of the peptide antagonist *trans*-Cinnamoyl-(f)F-(Gn)FLROrn-NH_2 (ZM345906) upon SFLLRN-OH induced calcium release was also investigated.

Thrombin (EC_{50} 0.16u ml^{-1}, 95% confidence limits, 0.02 - 0.97 u ml^{-1}, n = 3) and all peptides resulted in a concentration-dependent, transient increase of the intracellular calcium. The potency of the peptide agonists is given in Table 1. ZM345906 antagonized the responses of SFLLRN-OH, giving a -log (CDR_2) of 6.27 ± 0.08, n = 4). The effects of thrombin were reproduced by the peptide agonists, consistent with the presence of the PAR1 receptor subtype. The order of potency of the PAR1 peptides are similar to that observed in the platelet (Ahn *et al*, 1997). The low potency of SLIGRL relative to its reported EC_{50} for the PAR2 receptor (0.5 μM, Blackhart *et al*, 1996) suggests that the HEL cell contains few, if any, functional PAR2 receptors. In summary, this data suggests that the predominant subtype of protease activated receptor expressed in the HEL cell line is the PAR1 subtype.

Ahn, H-S, Foster, C, Boyhow G *et al*(1997). Mol. Pharm. 51, 350-356.
Blackhart, BD, Emilsson, K, Nguyen, D et al, (1996) J. Biol. Chem. 271:16466-16471.
Grand, RJA, Turnell, AS & Grabham, PW (1996) Biochem J. 313, 353-368.
Ishihara, H, Connoly, AJ, Zeng D *et al*(1997). Nature 386, 502-506.

Table 1. Potency of protease receptor peptides on calcium release in HEL cells.

	R*para*(f)FR.ChA.hRY-NH_2	SFLLRN-NH_2	TRAP$_{1-14}$	SFLLRN-OH	SFFLRN-OH	SLIGRL-OH	YFLLRNP-OH
EC_{50} (uM)	0.24	1.45	4.2	5.3	7.8	>30	>200
95% c.l.	0.08-0.69	0.53-3.90	2.5-7.0	2.9-9.6	2.9-20		
(n)	7	5	5	3	4		

136P THE STEREOCHEMISTRY, LIPOPHILICITY AND STIMULATORY EFFECTS OF NADOLOL ON PKC ACTIVITY IN HUMAN LYMPHOCYTES

AA. Almotrefi*, A. Moorji, C. Basco, HY. Aboul-Enein, N. Dzimiri. Biological & Medical Research Dept, King Faisal Specialist Hospital & Research Centre and *Pharmacology Dept, King Saud University, Riyadh, KSA.

We (Dzimiri *et al.*, 1997) found recently that nadolol stimulates protein kinase C (PKC) in a concentration dependent fashion. This is in contrast to the inhibitory actions of other β-adrenergic receptor blockers (β-blockers), especially those derived from propranolol. Nadolol (NDL) is a mixture of two racemates, racemate A (SQ12181) and racemate B (SQ12182) consisting of four enantiomers (+)SQ12148 (RSR-nadolol), (+)SQ12149 (RRS-nadolol), (-)SQ12150 (SRS-nadolol) and (-)SQ12151 (SSR-nadolol). In this study we wanted to find out whether the difference in the actions of nadolol and other β-blockers is related to the stereochemistry or lipophilic properties of nadolol. We compared its effects with those of its two racemates and four enantiomers on PKC activity in lymphocytes of healthy blood donors. The PKC activity was measured as its ability to phosphorylate a synthetic peptide from myelin basic protein (Ac-MBP), using its pseudosubstrate inhibitor peptide as a specificity control. The results show that nadolol, its racemates and enantiomers stimulate PKC activity in a concentration dependent fashion (Table 1). However, at any given concentration, there is no difference in the stimulatory activities of the drugs. The results therefore indicate the fact that the spatial arrangement of the groups at the three chiral carbon centres in nadolol absolute configuration of the individual enantiomers does not play a significant role in its interaction with the enzyme. Since the lipophilicity of the drugs is similar, the equipotency in their stimulatory effects suggests that this property is a determinant component, and that the structure of nadolol per se may be primarily responsible for its stimulatory actions on the membrane-bound enzymes. This supports our previous suggestion that the difference between nadolol and propranolol actions is probably determined by their membrane stabilizing activity and lipophilicity. This can be envisaged as a result of the propranolol derivatives being able to penetrate the lipophilic membrane compartments to access the embedded PKC regulatory domain in its activated state, as opposed to nadolol interacting as a result of being exposed only to its hydrophilic catalytic domain.

	100 μM	500 μM	1000 μM
NADOLOL	2.4 ± 0.6	16.4 ± 2.2**	32.8 ± 3.4**
SQ12181	2.1 ± 0.4	12.0 ± 2.8**	34.4 ± 5.2**
SQ12182	4.6 ± 1.7	12.3 ± 3.5**	17.4 ± 4.3**
(+)SQ12148	3.0 ± 2.3	12.1 ± 2.4**	31.0 ± 5.6**
(+)SQ12149	6.1 ± 4.3	11.4 ± 6.3	30.0 ± 6.0**
(-)SQ12150	3.6 ± 1.5	12.4 ± 4.9*	27.8 ± 5.2**
(-)SQ12151	4.1 ± 1.6	9.3 ± 1.5**	27.7 ± 1.3**

*p < 0.05, **p < 0.01 vs. max. inhibitable PKC baseline activity in absence of drug; (n = 7).

It should also be noted that these actions occur at concentrations relatively higher than those required for their β-adrenoceptor antagonist effects, pointing to the difference in the underlying mechanisms for their interactions with lipophilic membrane-bound proteins and their specific binding to the β-adrenoceptors.

Dzimiri N, Basco C and Moorji A. FASEB J. 11, 1879, 1997.

137P KINETIC CHARACTERIZATION OF [^{3}H]ATP BINDING TO RAT LIVER PLASMA MEMBRANES

G. Yegutkin & G. Burnstock, Autonomic Neuroscience Institute, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF

Extracellular ATP and ADP are widely accepted as effectors of signal transduction processes mediating signalling events in various tissues via metabotropic and ionotropic P2 purinoceptors (Burnstock, 1997). Studies of kinetic characteristics of P2 purinoceptors are always complicated by rapid enzymatic hydrolysis of ATP and its analogues. The chelating agent EDTA was examined in the present work as non-specific inhibitor of ecto-ATPase activity to aid kinetic and competitive analysis of ATP-binding sites on liver surface.

Plasma membranes were isolated from liver of male Spraque Dawley rats (Dorling & Le Page, 1973). Binding studies were carried out by high-speed filtration technique using [^{3}H]ATP as radioligand under complete inhibition of ecto-ATPase activity by 30 mM EDTA. Steady-state analysis revealed a single receptor site for ATP with binding capacity (B_{max}) of 14.96 $\pm$ 0.85 pmol/mg protein and K_d of 88.20 $\pm$ 9.44 nM. The most potent P2Y purinoceptor agonists 2-MeSATP and ADP were able to compete with [^{3}H]ATP with IC_{50s} of 60 and 280 nM, respectively. Moreover, a two-fold increase of K_d value for [^{3}H]ATP-receptor interaction was observed in the presence of 2-MeSATP (60 nM) or ADP (250 nM) as alternative ligands without any modulation of B_{max} value, clearly indicating that displacement effects of all these ATP analogues are caused by true competition for a common binding domain. Overall, the displacement studies yielded the following rank order of inhibitory potency for [^{3}H]ATP binding sites: 2-MeSATP > ATP > ADP >> Reactive Blue 2 > suramin >> Ap_4A > $\alpha\beta$-MeATP = $\beta\gamma$-MeATP. Excess AMP, adenosine, Ap_5A, PPADS, β-glycerophosphate as well as equimolar concentrations of UTP, GTP and CTP did not exert any effect on the measured binding.

In conclusion, these results /a/ provide accurate parameters for the binding of native ATP to cellular surface in the absence of complications arising from the further metabolism of the ligand; /b/ demonstrate that both ATP and its analogues 2-MeSATP and ADP are able to interact with a common binding domain on liver plasma membranes; /c/ reasonably suggest that the described binding sites are linked to $P2Y_1$ purinoceptors, mediating, in particular, ATP/ADP-induced activation of liver glycogen-phosphorylase and mobilization of intracellular Ca^{2+} (Charest et al., 1985).

The research was supported by The Wellcome Trust.

Burnstock, G. (1997). *Neuropharmacology* 36, 1127-1139.

Dorling, P.R. & Le Page, R.N. (1973) *Biochim. Biophys. Acta* 318, 33-44.

Charest, R., Blackmore, P.F. & Exton, J.H. (1985) *J. Biol. Chem.* 260, 15789-15794.

138P MCC-555 TREATMENT TO YOUNG ZDF RATS ATTENUATES THE DEVELOPMENT OF OVERT DIABETES

L. Pickavance, P.S. Widdowson, P. King, S. Ishii[1], H. Tanaka[1] & G. Williams, Dept. of Medicine, University of Liverpool, L69 3GA and [1]Mitsubishi Chemical Corp., Yokohama 227, Japan.

Thiazolidinediones are a new class of antidiabetic drugs which improve insulin sensitivity in type-2 diabetes (NIDDM) and impaired glucose tolerance (IGT), but have little insulin secretagogue activity (Whitcomb and Saltiel, 1995). We have investigated the ability of a novel thiazolidinedione, MCC-555 (Upton et al., 1997) to attenuate the development of hyperglycaemia in a genetic model of NIDDM, the Zucker Diabetic Fatty (ZDF) rat. These rats display IGT by 6 weeks and rapidly progress to overt diabetes by week 12 because of pancreatic β-cell failure (Peterson, 1994). Groups (n = 9) of male 6-week old ZDF rats (240g) were orally dosed with either MCC-555 (10 mg/kg/day in 0.5% carboxymethylcellulose vehicle) or vehicle for 28 days, while control non-diabetic ZDF rats (200g) received vehicle. Body weight, food and water intake were measured daily and tail-vein blood glucose concentrations were measured every 4 days. After 28 days of MCC-555 treatment, insulin sensitivity was measured using the hyperinsulinaemic euglycaemic clamp technique, as described previously (Upton et al., 1997). MCC-555-treated pre-diabetic and vehicle-treated non-diabetic rats displayed a continuous rise in body weight over the 28 day period whilst the body weight gain in vehicle-treated pre-diabetic rats was curbed by day 17. Daily water intake in pre-diabetic rats became significantly higher than that of non-diabetic rats after 7 days of the study, but the rise in polydipsia was significantly attenuated in MCC-555 treated IGT rats, as compared to vehicle-treated IGT rats (daily water intake at day 28, vehicle-treated IGT rats = 160 $\pm$ 10 ml/day vs MCC-treated IGT rats = 80 $\pm$ 5 ml/day, $P<0.01$ ANOVA followed by Bonferonni modified t-test, mean $\pm$ s.e.m.). Daily food intake in MCC-555-treated and vehicle-treated IGT rats were not significantly different from one another throughout the study period, but were significantly greater than that of non-diabetic rats. Pre-diabetic rats developed severe hyperglycaemia over the 28 day study period, as compared to non-diabetic rats, but the rate of increase and magnitude of hyperglycaemia was attenuated in MCC-555-treated rats (blood glucose [mmol.l^{-1}] at day 18; non-diabetic rats = 4.5 $\pm$ 0.3, MCC-555-treated diabetic rats = 16.9 $\pm$ 1.8, vehicle-treated diabetic rats = 23.7 $\pm$ 0.7 * $P<0.01$ as compared to MCC-555-treated rats. Clamping studies at day 28 of the study revealed a significant improvement in insulin sensitivity (+10.6%, $P<0.05$) in MCC-555-treated rats as compared to vehicle-treated diabetic rats, as demonstrated previously (Upton et al., 1997). In conclusion, we have demonstrated that MCC-555 treatment to young non-diabetic IGT ZDF rats can attenuate the later development of overt diabetes (polydipsia, hyperglycaemia, impaired body weight gain). This antidiabetic action is probably mediated via a partial restoration of insulin sensitivity.

Peterson, R.G. (1994) In: Lessons from animal diabetes V. Ed. E. Shafrir, Smith-Gordon (London)
Upton, R., et al. (1997) *Br. J. Pharmacol.* 122, 149P
Whitcomb, R.W. & Saltiel, A.R. (1995) *Exp. Opin. Invest. Drugs* 4, 1299-1309.

S.L. Smith, C.J. Sadler, *G. Edwards, S.A. Ward & W.G. McLean, Department of Pharmacology and Therapeutics, University of Liverpool, PO Box 147, Liverpool L69 3BX. *Division of Parasite and Vector Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA

Artemisinin and its derivatives are potent antimalarial drugs which show neurotoxicity in animal studies (Brewer *et al.*, 1994) and in neuronal cells in culture (Fishwick *et al.*, 1995). Their activity/toxicity may be due to an interaction with iron to produce toxic free radicals (Meshnick *et al.*, 1994). In this study, we have investigated the effects of free radical scavengers on the neurotoxicity of artemether in the presence of iron (haemin) in the neuroblastoma NB2a cell line.

NB2a cells were plated on to 48-well plates and after 24 h, the cells were preincubated for 2 h with the following antioxidants at concentrations which had no significant effect on their own: ascorbate (100 μM), Vitamin E (1 μM), catalase (100 U/ml), superoxide dismutase (300 U/ml), catalase/superoxide dismutase (75 U/ml + 200 U/ml), L-cysteine (1 mM), N-acetyl cysteine (2 mM), or glutathione (100 μM). The cells were induced to differentiate and grow neurites by addition of serum-free medium plus 0.5 mM dibutyryl cyclic AMP in the presence or absence of 300 nM artemether/2 μM haemin with or without antioxidant. After a further 24 h, the cells were fixed with 4% (w/v) formaldehyde, stained with Coomassie Blue and neurite length measured by light microscopy with automated image analysis.

Haemin significantly potentiated the neurotoxicity (measured as inhibition of neurite outgrowth) of artemether to 11 ± 11.4 (SD) % of control values ($p<0.001$, n=3, two way ANOVA & Bonferroni-modified t-test). Vitamin E and catalase/superoxide dismutase did not protect against the neurotoxicity produced by artemether/haemin, possibly because the antioxidant concentrations that were not toxic in themselves were too low to afford protection. However, artemether/haemin-induced neurotoxicity was protected against by ascorbate (104 ± 12.7 % of control), catalase (107 ± 29.3 %), superoxide dismutase (110 ± 47.8 %), L-cysteine (88 ± 6.3 %), N-acetyl cysteine (107 ± 14.9 %), and glutathione (123 ± 12.4 %; all $p<0.001$, n=4).

These results further support the hypothesis of iron-mediated free radical formation in the neurotoxicity of artemisinin derivatives.

Supported by the Wellcome Trust.

Brewer, T.G., Peggins, J.O., Grate, S.J. *et al.* (1994) *Trans. R. Soc. Trop. Med. Hyg.* 88, 33-36.
Fishwick, J., McLean, W.G., Edwards, G. *et al.* (1995) *Chem.-Biol. Interact.* 96, 263-271.
Meshnick, S.R. Yang, Y-L., Lima, V. *et al.* (1993) *Antimocrob. Agents Chemother.* 37, 1108-1114.

140P CHEMOSENSITIVITY STUDIES USING NOVEL TAMOXIFEN ANALOGUES AND A DOXORUBICIN-TAMOXIFEN PRODRUG AGAINS CULTURED HUMAN BREAST TUMOURS

A. Grondin, M.C. Bibby[1], J.E. Brown (Introduced by D.Wood) Pharmaceutical Chemistry and [1]Clinical Oncology Unit, University of Bradford, Bradford, BD7 1DP, United Kingdom

In this study we targeted doxorubicin to oestrogen receptor-positive (ER (+), >10 fmol ER per mg cytosolic protein) cell lines using tamoxifen analogues as carrier (Manns et al., 1993). The chemosensitivity of three ER (+) (MCF-7, T47D, ZR-75-1) and two human ER negative (ER(-), HS-578T, MT-1) breast tumour and mouse adenocarcinoma (MAC15A) cell lines to novel tamoxifen analogues β-amino-ethyltamoxifen (CCRL-1051), γ-aminopropyltamoxifen (CCRL 1052) and CCRL1053 which contained a 12-hydrocarbon spacer group linked to CCRL1051, plus two novel doxorubicin-tamoxifen prodrugs (CCRL1054, CCRL1056) with 12- and 16-hydrocarbon spacer groups were determined. DNA thermo-denaturation studies using calf thymus DNA were also used to assess CCRL1054 and CCRL1056 intercalated between DNA bases.

Cells were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum. Growth inhibition was determined after 96 h drug exposure using the colorimetric MTT assay (Mosmann T., 1983). Concentrations ranged from 0.001 μM to 10 μM.

Table 1: Percentage cell growth inhibition at 10 μM for ER positive cell lines (mean ± SD, n=3)

	MCF-7	T-47D	ZR-75-1
Tamoxifen	34.7 ± 8.5	36.4 ± 9.7	<20
CCRL1054	36 ± 13.2	43.3 ± 16	51 ± 5.2
CCRL1056	76 ± 5.0	-	64 ± 5.13

Table 2: Percentage cell growth inhibition at 10 μM for ER negative cell lines (mean ± SD, n=3)

	HS-578T	MT-1	MAC15A
Tamoxifen	<20	35 ± 6.0	90.2 ± 1.9
CCRL1054	<20	71.8 ± 7.3	89.4 ± 1.2
CCRL1056	<20	29 ± 9.6	82 ± 2.6

All cell lines were sensitive to doxorubicin with IC50 values ranging from 0.04 to 2.5 μM. The triphenylethylene analogues (CCRL1051, CCRL1052, CCRL1053) were inactive against all cell lines tested. CCRL1054 showed either similar or better growth inhibition than tamoxifen against the three ER positive cell lines and MT-1. CCRL 1054 was inactive against HS-578T and was similar to tamoxifen in activity against MAC15A. CCRL1056 was significantly more active ($P<0.01$, n=3) against ER(+) cell lines than against ER(-) human breast tumour cell lines. Thermodenaturation studies showed that the doxorubicin moiety from CCRL1054 or CCRL1056 did not intercalate between DNA bases: the melting temperature (Tm) of DNA in the presence or absence of either compounds was the same (74°C) unlike doxorubicin for which ΔTm=13°C (m.pt.= 87 °C).

Results for CCRL1054 showed similar activity to tamoxifen but was relatively unselective, whereas CCRL1056 was highly significantly selective against ER positive cell lines and hence the aminotamoxifen-doxorubicin prodrug CCRL1056 is a lead compound in the development of new anti-breast cancer agents.

1) Manns J.E., Double J.A., et al. (1993), *Br. J. Cancer*, 67 (suppl XX): 66
2) Mosmann T. (1983), *J. Immunological Methods*, 65: 55-63

141P COMPARISON OF THE EFFECTS OF $PGF_2\alpha$ AND RELATED COMPOUNDS ON THE HUMAN ISOLATED MYOMETRIUM FROM NON-PREGNANT AND PREGNANT DONORS

F. Abbas, J. Clayton, K. Marshall & J. Senior. Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP.

Previous studies in this laboratory have shown that on the human isolated myometrial strips from non-pregnant (NP) and pregnant (P) donors $PGF_2\alpha$ evokes a stimulant response, suggesting the presence of FP- receptors on these tissues (Senior *et al.*, 1992 & 1993). In the absence of a suitable FP-receptor antagonist two selective FP- receptor agonists were used in this study in order to confirm our earlier findings. The agonists used were fluprostenol (Coleman *et al.*, 1984) and the free acid of latanoprost (Stjernschantz & Resul, 1992).

Samples of human myometrium were obtained from NP pre-menopausal patients at hysterectomy (fundus) or from P donors (who had not gone into labour) during Caesarean section (lower uterine segment) (all patients gave written consent). The myometrial strips were set up for superfusion (2g tension) in Krebs' solution with 2.79μM indomethacin (37°C, 95% O_2/5% CO_2) at 2ml min^{-1} as previously described by Senior *et al.* (1991). After equilibration of the tissues bolus doses of $PGF_2\alpha$, fluprostenol or latanoprost were injected directly into the flow of the superfusate. As the profile of the spontaneous activity changed throughout the course of the experiments, comparisons were made between preparations in a non-paired manner. Because of the variations in myogenic activity results have been normalised to take this into account (Senior *et al.*, 1991). Briefly, excitatory potency was expressed as an ED_1 value. ED_1 values were expressed as geometric means (nmol) with 95% confidence limits in parentheses, n=5 in all cases.

On the human myometrium from NP and P donors $PGF_2\alpha$ (0.0001-100nmol), fluprostenol (0.0001-100 nmol) and latanoprost (0.0001-300nmol) all evoked an increase in myometrial tension. In NP tissues the curves to $PGF_2\alpha$ and fluprostenol were bell shaped, with a decline in the contractile responses occurring after 10nmol for $PGF_2\alpha$ and 0.1nmol for fluprostenol. This was not seen in tissues obtained from P donors.

Table 1 Mean ED_1(nmols) values of the prostanoids on NP and P tissues.

	NP	P
Compound	ED_1 value	ED_1 value
$PGF_2\alpha$	0.04 (0.01-0.14)	0.50 (0.28-0.66)
Fluprostenol	0.04 (0.013-0.1)	0.61 (0.50-0.75)
Latanoprost	0.007 (0.005-0.008)	0.60 (0.50-0.80)

As can be seen from table 1 the rank order of potency of the natural and selective prostanoids in the human myometrium from NP donors is as follows: latanoprost > $PGF_2\alpha$ = fluprostenol. However, in tissues from P donors all three agonists were of the same order of potency. There was a marked difference between NP and P tissues in that the agonists were 10-100 times less effective in stimulating P tissue.

The results from the present study confirm our earlier findings that the human myometrium does contain FP- receptors. The FP- receptor population may also be greater in the NP myometrium than in the non-labouring P myometrium.

Coleman, R.A., Humphery, P.P.A. & Kennedy, I. *et al.* (1984) *Trends Pharmacol. Sci.* **5**, 303-306.
Senior, J. Marshall, K., Sangha, R. *et al.* (1991) *Br. J. Pharmacol.* **102**, 747-753.
Senior, J. Marshall, K., Sangha, R. *et al.* (1993) *Br. J. Pharmacol.* **108**, 501-506.
Senior, J., Sangha, R., Baxter, G.S. *et al.* (1992) *Br. J. Pharmacol.* **107**, 215-221.
Stjernschantz, J. & Resul, B. (1992) *Drugs of the Future* **17**, 691-704.

142P SPICE - A SIMPLE PROGRAM IDENTIFYING CURVACEOUS ELEMENTS

S.R.Bailey[1], R.B.Barlow, J.Elliott[1] and G.A.Gray[2] Ash Lea Cottage, Ravenstonedale, Kirkby Stephen, Cumbria CA17 4NG, [1]Dept Veterinary Basic Sciences, Royal College St, London NW1 0TU [2]Dept Pharmacology, Edinburgh University, Edinburgh EH8 9JZ

The most commonly used model for the relation between effect (E) and concentration ([A]) is the equation (Parker & Waud, 1971):

$$E = \frac{M[A]^P}{[A]^P + [A_{50}]^P} \quad \ldots\ldots 1$$

where M is the maximum, $[A_{50}]$ produces a half- maximum effect and P is an exponent which determines the steepness of the curve. This empirical model, often called "logistic", has the same form as the equation used by Hill (1913) to describe the binding of oxygen to haemoglobin and P is the same as the Hill coefficient (n_H). When P=1 the equation describes binding according to the law of mass action, i.e. the relation between agonist concentration and receptor complex. Increasingly curves are appearing in the literature which are "flat", i.e. P<1. This implies that the order of the process(es) occurring after binding is less than one but there may be other reasons, such as that a maximum or baseline which has been incorrectly calculated or that more than one process is contributing to the effect. This program has been written to identify possible causes for flatness and to identify component elements. It offers:

1 a fit to equation 1

2 a fit to equation 1 + calculation of a baseline, which can be deducted before further analysis.

3 a fit to a 2-site binding equation

$$E = M_1 \frac{[A]}{[A] + [A_1]_{50}} + M_2 \frac{[A]}{[A] + [A_2]_{50}} \quad \ldots\ldots 2$$

where M_1 and M_2 are the two maxima and $[A_1]_{50}$ and $[A_2]_{50}$ are the concentrations producing the corresponding half-maximal effects.

4 a fit to the double logistic equation

$$E = M_1 \frac{[A]^{P1}}{[A]^{P1} + [A_1]_{50}^{P1}} + M_2 \frac{[A]^{P2}}{[A]^{P2} + [A_2]_{50}^{P2}} \quad \ldots\ldots 3$$

5 a fit to a 3-site binding equation (eqn 2 with a term for M_3 and $[A_3]_{50}$).

Procedures for fitting data to such equations based on the methods of Parker & Waud (1971) have been available for some time (e.g. Barlow, 1983) but do not appear to be in general use, perhaps because, with four or more parameters to be calculated, it is difficult to make starting guesses which lead to convergence. This program displays the curve corresponding to the starting values so that appropriate guesses can be obtained by inspection before iteration is attempted. Improvement in fit is estimated from the ratio of the sum of $(E_{observed} - E_{calculated})^2$ for equation 1 to the sum for the equation chosen: it is also expressed as the variance ratio and as the ratio of the coefficients of variation. Critical values of F for 5% probability are included and used to indicate a significant improvement. When there is only one result for each concentration, the appropriateness of the equation used is also indicated by the strings of points lying consistently above or below the line.

It has been particularly useful for analysing the effects of agonists on 5HT receptors in arteries and veins in the horse, fitted to the double logistic equation (3) and for the effects of compounds on rat mesenteric vessels and small arteries in patients, where 3 processes appear to be involved. Its scope is also illustrated by a survey of curves appearing in many recent papers in the British Journal of Pharmacology. The program is offerred (free) to anyone interested.

Barlow, R.B. (1983) *Biodata Handling with Microcomputers*, Cambridge, Elsevier Biosoft.
Hill, A.V. (1913) Biochem.J., 7, 471-480.
Parker, R.B., & Waud, D.R. (1971) J.Pharmacol. Exp Ther., 177, 1-12.